Characterization of Branched Chain Aminotransferase 1 in Small Cell Lung Cancer

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A Dissertation Presented to the Graduate Faculty of The University of Virginia in Candidacy for the Degree of Doctor of Philosophy

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July, 2020

Abstract

Small cell lung cancer (SCLC) is a tumor characterized by rapid onset and a high propensity for metastasis. Treatment options are limited, having remained largely unchanged over the past few decades, and are quickly overcome by drug resistance. These factors are major contributors to the poor prognosis and unusually high mortality, which underscore an urgent need to identify novel biomarkers and therapeutic targets in order to improve the detection and treatment of SCLC. Thus, the overarching goal of our group is to identify molecular changes specific to SCLC cells relative to precancerous precursor cells and determine their roles in tumor growth.

In a previously published report, the Park lab demonstrated that the development of SCLC driven by *Mycl* depends on enhanced ribosome biogenesis and protein translation, and is sensitive to inhibition of RNA Polymerase I. Here, we sought to identify genes downstream of *Mycl* that could promote protein translation and be targeted to inhibit the growth of SCLC. While few studies have explored the metabolism of amino acids to target this tumor, we found that comparative profiling of precancerous cells and cells transformed by *Mycl* revealed *branched chain aminotransferase 1* (*Bcat1*) as one of the most up-regulated genes in the transformed cells. Based on this observation, and the fact that branched chain amino acids (BCAAs) can contribute to the synthesis of non-essential amino acids, which are utilized for protein translation, we hypothesized that the overexpression of BCAT1 promotes the growth of SCLC.

Thus, in chapter 2, we confirm that BCAT1 protein levels are elevated in mouse SCLC cells relative to precancerous cells, and that BCAT1 is also expressed in a subset of human SCLC cells. Importantly, we found that BCAT1 promotes the growth of SCLC cells and tumors. We also found that BCAT1 reduces the intracellular concentration of valine, as well as increases the intracellular concentrations of glutamate and aspartate; additionally, we found that BCAT1 increases the catabolism of leucine. These findings are consistent with the concept that BCAT1 enhances the catabolism of BCAAs. As leucine is sensed by the mechanistic target of rapamycin complex 1 (mTORC1), which has been reported to support the growth of tumors by promoting protein translation, we investigated the role of BCAT1 in the mTORC1 pathway and found that the inhibition of BCAT1 results in notable increases in the phosphorylation of RPS6K and MTOR in SCLC cells, which suggests that BCAT1 inhibits mTORC1 in SCLC. Interestingly, we present data that suggest that BCAT1 does not decrease protein translation, but actually increases it under certain conditions. Taken together, these novel findings suggest that the elevated expression of BCAT1 supports the growth of SCLC, potentially by promoting protein translation. How BCAT1 promotes protein translation and inhibits mTORC1 remains elusive, but given the proven safety profile of an existing inhibitor of BCAT1, our findings are significant in supporting the concept of targeting BCAT1 as a novel and particularly valuable therapeutic strategy for SCLC.

Table of Contents

List of Figures

Chapter 1: Introduction

Although cancer cells frequently undergo metabolic rewiring (Hanahan & Weinberg, 2011), our understanding of altered metabolism in cancer is far from comprehensive. Much of the literature on cancer metabolism is focused on relatively few pathways, such as glycolysis (Heiden et al., 2009). However, there is growing evidence that abberant metabolism of amino acids contributes to the growth of recalcitrant cancers, such as small cell lung cancer (SCLC) (Chalishazar et al., 2019). Previous work from the Park lab reported that ectopic expression of *Mycl* induced the expression of *branched-chain aminotrasferase 1* (*Bcat1*) mRNA (Kim et al., 2016). The increased expression of *Bcat1* raised the question of whether branched chain amino acid metabolism contributes to the growth of SCLC. Thus, the primary aim of this thesis was to elucidate the relationship between increased BCAT1 protein expression and the growth of SCLC cells and tumors. Additionally, we sought to assess changes in downstream pathways, such as the mechanistic target of the rapamycin complex 1 (mTORC1) pathway and protein translation, to reveal how BCAT1 promotes the growth of SCLC. First, this chapter provides an introduction to cancer and hallmarks of cancer, followed by a summary of lung cancer, including key statistics, etiology, and treatment options. Following that is a discussion of the genetics and biology of SCLC, including a review of emerging subtypes of SCLC. This is followed by a summary of models of SCLC development and homeostasis that are used to functionally characterize genetic alterations. Next, reviews of cancer metabolism, protein translation, branched chain amino acid (BCAA) metabolism, and mTORC1 signaling serve to bridge BCAAs and

protein translation in cancer. Finally, the chapter provides an outline of the central questions to be addressed by the research compiled in this thesis.

1.1 Hallmarks of Cancer

In the normal physiological state, cells replicate to generate new cells only when the body requires it, especially to form or repair tissues (Krafts, 2010). The cell cycle, which is the process by which cells grow, duplicate and divide, is normally a highly-regulated set of events. In order to replicate, a cell must encounter pro-growth signals (e.g., growth factors) and lose growth-inhibitory signals (e.g., cell-cell contact). In particular, growth factors in the extracellular space bind to receptors on the cell surface and stimulate them to activate a series of downstream effector proteins, which transduce the signal to the nucleus where deoxyribonucleic acid (DNA) resides. Then, transcription factors within the nucleus generate mRNA from the DNA template, which are translated into proteins involved in cell proliferation. To ensure this process progresses properly, the cell cycle pauses at certain steps, called checkpoints, in order to assess DNA damage and make sure the appropriate amount of genetic material is distributed to each daughter cell (Agarwal et al., 1995). Except for stem cells, non-transformed cells can only divide a limited number times before they become too old and/or damaged to continue (Cerella et al., 2016). In this case, the cell either undergoes programmed death and a new cell replaces it or the cell enters a state in which proliferation ceases without death. Cancer, in contrast, is a collection of diseases in which cells continue to divide unchecked even when a healthy tissue is completely

formed or when the proliferating cell is severely damaged (Symonds et al., 1994). Eventually, cancer cells in tissues may form a solid mass, called a tumor, but cancers of the blood, such as leukaemias, generally do not.

There are several hallmarks of cancer cells that set them apart from normal cells, including sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enabling of replicative immortality, inducing angiogenesis, enhanced invasion and metastasis, deregulated cellular energetics and metabolism, and avoidance of immune destruction (Hanahan & Weinberg, 2011). In essence, cancer cells convert transient growth signals to chronically activated signals and can proliferate indefinitely, they inactivate mechanisms that interfere with cell cycle progression and that induce cell death, they promote the formation of blood vessels (angiogenesis) to ensure nutrient supply, they invade adjacent tissues and blood vessels to spread to other parts of the body (metastasis), they alter utilization of nutrients to support their growth, and they evade surveillance by the immune system that would normally destroy aberrant cells. However, different tumors, even within the same tissue, can acquire these hallmarks through diverse assortment of molecular mechanisms. Thus, a better understanding of the underlying mechanisms distinct to each tumor type is critical to developing better treatments for individual patients.

1.2 Lung Cancer

1.2.1 Lung cancer types and overview: key statistics and general etiology

Lung cancer is the general category of tumors that arise in the lungs and is largely divided into histological subtypes, such as non-small cell lung cancer (NSCLC), SCLC, and lung carcinoid cancer, which, in the U.S., represent about 85%, 15%, and 1-2%, respectively (Travis et al., 2015). Excluding skin cancers, lung cancer is one of the most common types in the U.S. for both men and women, second only to prostate cancer in men and breast cancer in women (Siegel et al., 2019). The American Cancer Society (ACS) estimates there will be more than 220,000 new cases and more than 135,000 deaths from lung cancer in the U.S. in 2020; that is nearly 25% of cancer deaths and more than colon, breast, and prostate cancers combined (Siegel et al., 2020). There are several risk factors for lung cancer, with tobacco smoke being the leading factor (Alberg & Samet, 2003; Mccarthy et al., 2012). About 80% of lung cancer deaths are attributed to smoking, and even inhaling the smoke of others, which is called secondhand smoke, can increase the risk. Beyond smoking, radon, which is a natural product from the degradation of uranium in the ground, is the second leading cause of lung cancer in the U.S.. Additionally, workplace exposure to certain agents, such as asbestos, uranium, arsenic, and diesel exhaust can also increase the risk. Lung cancers largely affect older individuals, with the incidence highest in those above the age of 70 (Siegel et al., 2020).

1.2.2 Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma

NSCLC is further categorized into subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (Travis et al., 2015). Although these subtypes originate from different types of lung cells, they are grouped together because their treatment and prognoses are often similar. Adenocarcinomas originate in cells that normally secrete substances such as mucus (DeVita et al., 2018). They are usually found in the outer portions of the lungs and are more likely to be detected before metastasis. While adenocarcinoma largely affects smokers, it is the most common type of lung cancer found in non-smokers. It is also more likely to occur in younger people and it is more common in women than in men. The 5 year survival rate for adenocarcinomas is about 21% (Lu et al., 2019).

Squamous cell carcinoma (SCC) originates in squamous cells, which are flat epithelial cells that line the insides of lung airways (DeVita et al., 2018). These tumors tend to be centrally located near a bronchus and are more likely to metastasize than adenocarcinoma, settling in nearby areas. However, unless it is diagnosed early, it often spreads to different parts of the body, such as bone or the brain. The 5 year survival rate for SCC is about 18% (Lu et al., 2019).

The diagnosis of large cell carcinoma (LCC) is restricted only to resected tumors that lack any clear morphologic or immunohistochemical differentiation (DeVita et al., 2018). It can arise in any part of the lung and tends to grow and metastasize quickly. The five year survival for LCC is about 13% (Lu et al., 2019).

1.2.3 Neuroendocrine cancer in the lung: small cell lung cancer, large cell neuroendocrine carcinoma, and carcinoid tumors

Mutant lung neuroendocrine cells give rise to neuroendocrine cancers of the lung, such as SCLC (Park et al., 2011). Small cell lung cancer is exceptionally linked to tobacco use and is rare in never smokers (only about 5% of cases) (Nicholson et al., 2002). Recently, a research group extracted incidence data from the Surveillance, Epidemiology, and End Results (SEER) database and focused on the three decades between 1983 and 2012 (Wang et al., 2017). They found that the incidence of SCLC in the U.S. is on the decline, with the proportion of new cases falling from 17% of lung cancers in 1986 to 13% in 2002 (Wang et al., 2017). The incidence was highest in individuals 65–79 years of age. While males experienced a higher incidence than females in each decade, the incidence gaps shrank due to the declining incidence in males and relatively stable incidence in females. Although the incidence decreased each decade regardless of socio-economic status, individuals categorized as high poverty experienced a lower incidence than those categorized as medium or low poverty. Additionally, while the incidence in each race decreased each decade, white individuals and black individuals experienced higher incidences than individuals

categorized as other. However, both black and other individuals had similar numbers of patients, which were significantly lower than that of white individuals.

SCLC presents as a high grade tumor, which is typically centrally located, usually in the bronchi (Farago & Keane, 2018). Compared to NSCLC, SCLC is highly proliferative, it is highly metastatic (70% of new cases present with metastases) and for the overwhelming majority of patients, there are no therapies that elicit a durable response. The median survival of SCLC is only about 7 months with a 5-year survival rate of about 6% (Wang et al., 2017). The high mortality associated with SCLC underscores the urgent need for a deeper understanding of the factors contributing to the growth of this recalcitrant cancer, as well as the need for the development of novel therapeutic strategies to induce durable regression.

There are two stages of SCLC, categorized by the extent to which the disease has spread: limited-stage (LS) and extensive-stage (ES) (Farago & Keane, 2018). LS-SCLC is the less common category, comprising about a third of new cases. In this stage, the disease is limited to one side of the chest. In general, tumors are in one lung and may have spread to lymph nodes on the same side of the chest. Most importantly, lesions are in a small enough area to be treated in a single radiotherapy port. ES-SCLC represents about two thirds of new cases and encompasses cancer that has spread beyond a single radiotherapy port. It may be spread widely throughout a single lung or it may have spread to the other lung, to lymph nodes on the other side of the chest, or to other parts of the body. Metastatic tumors commonly spread to the liver, adrenal glands,

bone or brain (Milovanovic et al., 2017). According to the American Cancer Society (ACS), the 5-year survival rate for localized SCLC is significantly better than for regional or distant disease (27% vs. 16% and 3%, respectively). This is likely due to the increased limitations on treatment strategies and the decreased treatment efficacy as the disease spreads.

There are two types of lung carcinoid tumors: typical and atypical (Gosain et al., 2018; Steuer et al., 2015). Typical carcinoids account for about 90% and grow slowly. Atypical carcinoids grow faster and are more likely to metastasize beyond the lungs. Carcinoids can also be classified by their location: central carcinoids are found on the walls of the bronchus, whereas peripheral carcinoids form closer to the edges of the lungs in the smaller airways. According to the ACS, the 5-year survival rates for lung carcinoid tumors are much better than for SCLC: they are 97% , 86%, and 58% for localized, regional, and distant disease, respectively.

1.2.4 Treatment for lung cancer

Surgery can be useful for some types of lung cancer, especially ones less likely to metastesize. For lung carcinoid tumors, surgery is highly effective and is the standard of care, which results in a 5-year survival of 97% for typical tumors and 78% for atypical tumors (Dahabreh et al., 2009; García-Yuste et al., 2007; Naalsund et al., 2011). For SCLC, surgical resection is considered only in rare cases of LS-SCLC (less than 5% of

patients) in which there is just a small tumor that has not yet spread to lymph nodes (Schneider et al., 2011). Consequently, this strategy is rarely employed, especially in consideration of widespread metastases in the majority of new diagnoses (70%) (Farago & Keane, 2018). However, there is evidence that SCLC patients can benefit from radical resection: one recent study with nineteen cases of SCLC reported a 5-year survival rate of about 41% with all stages grouped together (Motas et al., 2018). This was comparable to NSCLC at about 43%. As the standard of care, most stage I and stage II NSCLC are treated with surgery (Lackey & Donington, 2013).

In order to divide rapidly, cancer cells must increase the frequency of DNA replication; however, they also harbor defects in error-correction mechanisms and are therefore more susceptible to DNA-damaging therapy (Berdis, 2017). Chemotherapy employs chemical compounds, which often damage DNA, to kill cancer cells. For SCLC, a combination of chemotherapeutics, often times the topoisomerase II inhibitor etoposide with either cisplatin or carboplatin, is commonly used (Farago & Keane, 2018). This is often termed platinum-based chemotherapy because cisplatin and carboplatin are platinum-containing alkylating agents. Alkylating agents add an alkyl group to the guanine base of DNA, resulting in DNA damage during replication (Lawley, 1980). This is also toxic to normal cells that divide rapidly, such as those in the gastrointestinal tract, bone marrow, testicles, and ovaries, and is carcinogenic in itself. For NSCLC, combination chemotherapy, such as cisplatin or carboplatin plus docetaxel, is also common (Nagasaka & Gadgeel, 2018). Due to the toxicity on normal cells, chemotherapy is often associated with negative side effects (Islam et al., 2019).

Specifically, cisplatin and carboplatin can damage nerve endings, resulting in peripheral neuropathy (Rajeswaran et al., 2008). External beam radiation is the most common type of radiation therapy used for the treatment of lung cancer and employs a machine to aim high-energy X-rays from outside the body into the tumor (Rodrigues et al., 2011). This also induces DNA damage and kills dividing cells, including cancer cells, and results in non-specific toxicity.

For LS-SCLC, the standard treatment is a combination of chemotherapy and radiation therapy (Farago & Keane, 2018). For ES-SCLC, the standard of care is combination chemotherapy; however, if the extensive disease responds well to initial chemotherapy, then radiation to the chest may be employed. Additionally, regardless of stage, patients with complete response to the initial treatment may be given radiation therapy to the brain in order to prevent the spread of cancer to that area. While this strategy usually elicits a robust anti-tumor response at first, most tumors become resistant to the chemotherapy and mount an aggressive recurrence (Farago & Keane, 2018). For recurrent tumors, other chemotherapeutic regimens can be employed, but they are generally less effective. For example, topotecan, which is a topoisomerase I inhibitor, is currently the only FDA-approved second-line chemotherapy for SCLC and is associated with considerable toxicity (Ardizzoni et al., 1997). Furthermore, topotecan only produces a response rate of about 22% and overall survival of 5 months in patients (Hagmann et al., 2015). Due to the inability of the standard of care to cure the majority of SCLC cases, several alternative therapies are being pursued either singly or in combination with standard therapy.

Immunotherapy, which utilizes components of the immune system to destroy or slow the growth of tumor cells, is one strategy that is rapidly gaining traction. Immune checkpoint inhibitors, which block proteins called checkpoints that are made by some types of immune cells and cancer cells, are of great interest for SCLC (Calles et al., 2019). An important facet of the immune system is its ability to refrain from attacking normal cells in the body, which is facilitated by checkpoints on immune cells that need to be turned on or off to initiate an immune response (Chen & Flies, 2013). One of the hallmarks of cancer is the avoidance of immune destruction (Hanahan & Weinberg, 2011); therefore, cancer cells may express checkpoints to evade the immune system. For example, cancer cells sometimes express a protein called programmed cell death 1 ligand 1 (CD274 or PD-L1), which binds to a receptor on T cells called programmed cell death 1 (PDCD1 or PD1) that normally helps to prevent them from attacking normal cells in the body (Dong et al., 2002; Freeman et al., 2000; Iwai et al., 2002).

Nivolumab (Opdivo) and pembrolizumab (Keytruda) target PD-1, while atezolizumab (Tecentriq) and durvalumab (Imfinzi) target PD-L1 (Faiena et al., 2018; Guo et al., 2017; Krishnamurthy & Jimeno, 2017; McDermott & Jimeno, 2015). Whereas nivolumab and pembrolizumab are approved by the FDA to treat recurrent SCLC following at least two previous therapies (third-line), atezolizumab and durvalumab are approved as part of the first-line treatment for ES-SCLC alongside standard chemotherapy. Nivolumab was approved by the FDA in 2018; initial assessment of data from the phase I/II CheckMate-032 trial showed that treatment with nivolumab alone

resulted in an objective response rate (ORR) of 10% with a median progression-free survival (PFS) of 1.4 months and a median overall survival (OS) of 4.4 months (Antonia et al., 2016). The addition of ipilimumab, an immunotherapy that inhibits a checkpoint called cytotoxic T-lymphocyte associated protein 4 (CTLA4), improved the ORR to 23%, the median PFS to 2.6 months, and the median OS to 7.7 months. Later assessment of the trial showed an ORR to nivolumab monotherapy at 11.9% (Ready et al., 2019); however analysis of the phase III trial CheckMate-451 showed that nivolumab, either alone or in combination with ipilimumab, did not significantly improve the median PFS or OS compared to placebo (Owonikoko et al., 2019). In 2019, the FDA approved pembrolizumab for the treatment of metastatic SCLC; data from the phase Ib KEYNOTE-028 study and the phase II KEYNOTE-158 study showed that pembrolizumab produced an ORR of 19.3%, but the median duration of response endpoint was not reached (Chung et al., 2020). However, in a different phase II study, the combination of pembrolizumab and paclitaxel produced an ORR of 23.1%, a median PFS of 5 months, and a median OS of 9.1 months (Kim et al., 2019).

The FDA approved atezolizumab in 2019 following the phase III IMpower133 trial, which was the first to show a significant improvement in survival as first-line treatment of SCLC since the onset of platinum-based drugs (Horn et al., 2018). When used in combination with etoposide and carboplatin, atezolizumab improved the median PFS from 4.3 to 5.2 months and the median OS from 10.3 to 12.3 months compared to chemotherapy with placebo. Additionally, in 2020, the FDA approved durvalumab for the first-line treatment of ES-SCLC. This was based on the phase III CASPIAN trial, which

showed that durvalumab, in combination with standard chemotherapy, improved the median OS to 13 months versus 10.3 months with standard chemotherapy alone (Paz-Ares et al., 2019). Additionally, durvalumab improved the 1 year survival rate from 40% to 50%, and the 1.5 year survival rate from 25% to 34% compared to the standard chemotherapy alone. While immunotherapy continues to gain traction, first-line treatment with atezolizumab or durvalumab only improves patient survival by 2-3 months over chemotherapy alone (Horn et al., 2018; Paz-Ares et al., 2019). This is likely due to mechanisms of resistance such as low expression of PD-L1 (Hamilton & Rath, 2019). Thus, more research is needed to understand the underlying genetic factors that promote SCLC initiation and progression, which is essential to the development of more effective, less toxic therapies. Due to this realization, this chapter now summarizes some of the key genetic lesions and biological aspects contributing to the progression of SCLC.

1.3 Small Cell Lung Cancer

1.3.1 Cellular features of SCLC

SCLC is usually diagnosed by analysis of haematoxylin and eosin stained slides (Travis et al., 2015). SCLC has long been viewed as a homogenous disease characterized by commonly shared pathological features, including distinct morphology of dense sheets of cells that are small with scant cytoplasm, ill-defined cell borders, and

nuclei that are intensely stained with finely granular chromatin (Nicholson et al., 2002). There is also a high degree of Ki67 staining (indicitive of high proliferation), apoptosis, and necrosis (Travis et al., 2015). Biomarkers such as synaptophysin (SYP), chromogranin a (CHGA), neural cell adhesion molecule 1 (NCAM1) and insulinoma-associated 1 (INSM1) can be used to assess neuroendocrine lineage, although a minority of SCLC are negative for all standard neuroendocrine markers (Guinee et al., 1994).

1.3.2 Genetics of SCLC: significance of the RB1 and TP53 tumor suppressors and other features

Loss of function of both retinoblastoma 1 (RB) and tumor protein p53 (p53), which are encoded by the *RB1* and *TP53* genes, respectively, is a nearly universal feature of SCLC, with bi-allelic losses in 93% and 100% of tumors, respectively (George et al., 2015). *RB* was the first tumor suppressor to be identified; it was cloned in the mid-eighties due to recurrent mutation in the pediatric eye tumor, retinoblastoma (Dryja et al., 1986; Friend et al., 1986). Additionally, frequent mutations have been found in other cancers such as osteosarcoma, small cell lung cancer, prostate cancer, breast cancer, and bladder cancer (Bookstein et al., 1990; Friend et al., 1986; Fung et al., 1987; Harbour et al., 1988; Kaye et al., 1990; Lee et al., 1988; Miyamoto et al., 1995; T'Ang et al., 1988). RB is ubiquitously expressed, although its activity is normally inhibited in proliferating cells by cyclin-dependent kinase (CDK)-mediated hyperphosphorylation (Akiyama et al., 1992; Ewen et al., 1993; Hinds et al., 1992; Kato et al., 1993). It is

hypophosphorylated (activated form), in quiescent cells and differentiating cells, which stalls the cell cycle in the G1 phase (Chen et al., 1989). RB-mediated cell cycle arrest has mainly been attributed to interaction with and inhibition of the E2F family of transcription factors (Bremner et al., 1995; Chellappan et al., 1991; Flemington et al., 1993; Helin et al., 1993; Qin et al., 1995; Ross et al., 1999; Weintraub et al., 1995); E2F controls the expression of many genes that facilitate cell cycle progression such as *minichromosome maintenance* components, *DNA polymerase α*, *cell division cycle 6*, *cyclin E*, and *cyclin-dependent kinase 2* (*CDK2*) (Leone et al., 1998; Yan et al., 1998). However, RB can also interact with chromatin remodeling factors, such as histone deacetylases, to promote the condensation of nucleosomes into chromatin, block transcription factors from binding to DNA, and inhibit gene expression (Dahiya et al., 2001; Luo et al., 1998; Magnaghi-Jaulin et al., 1998; Nielsen et al., 2001; Zhang et al., 2000). Importantly, loss of RB in normal cells usually induces a type of cell death called apoptosis, which is mediated by p53 and is partly dependent on E2F. For example, while inactivation of *Rb* in mice is lethal to embryos, additional inactivation of *E2f1* results in significantly reduced rates of apoptosis and down-regulation of the p53 pathway (Clarke et al., 1992; Jacks et al., 1992; Tsai et al., 1998; Lee et al., 1992). Additionally, deletion of *E2f1* reduced apoptosis by 80% in adult mice in which *Rb* was inactivated (Pan et al., 1998). Moreover, while inactivation of *Rb* in adult mice resulted in slowly growing and highly apoptotic tumors, additional inactivation of *p53* accelerated tumor growth and reduced apoptosis by 85% (Symonds et al., 1994).

p53 is one of the most well-known tumor suppressors and functions to maintain genomic stability (Eischen, 2016; Yeo et al., 2016). Under normal conditions, p53 is expressed at a low level and is non-functional; p53 protein level is largely controlled by proteasomal degradation that is mediated by the E3 ubiquitin protein ligase, transformed mouse 3T3 cell double minute 2 (MDM2) (Ashcroft & Vousden, 1999; Freedman et al., 1999; Honda et al., 1997; Li et al., 2004; Ma et al., 2010). In response to DNA damage, p53 acquires post-translational modifications, such as phosphorylation and acetylation, which convert it to an active form (Brooks & Gu, 2003). It then accumulates in the nucleus and binds to DNA in order to stimulate the production of a protein called cyclin dependent kinase inhibitor 1A (CDKN1A or p21), among others, which inhibits CDKs and stalls cell cycle progression in the G1 and G2 phases (Agarwal et al., 1995; Bates et al., 1998; Harper et al., 1995). Arrest induced by activation of p53 permits cells to repair damaged DNA (Cazzalini et al., 2010); when repair is complete, cells re-enter the cycle to continue proliferation. In contrast, when cells suffer catastrophic DNA damage, p53 induces apoptosis, which occurs through mitochondrial dysfunction and release of cytochrome c (Lemasters et al., 1998; Tomita et al., 2006; Yang et al., 1997), mediated by genes such as *BAX* (BCL2-associated X) and *BBC3* (Bcl-2-binding component 3 or *PUMA*).

In addition to *RB1* and *TP53*, SCLC also features recurrent mutations in functional homologs, such as *RBL1* (3-4%), *RBL2* (5-7%) and *TP73* (13%) (George et al., 2015). Also known as p107 and p130, respectively, RBL1 and RBL2 share key functions with RB, including modulating the cell cycle through regulating E2F

transcription factors (Cheng et al., 2000; Kiess et al., 1995). Therefore, they can compensate for loss of RB to some extent; indeed, additional deletion of *Rbl2* increases tumor burden in the *Rb1*/*Trp53*-mutant genetically engineered mouse model (GEMM), which is discussed in section 1.3.3.5 (Schaffer et al., 2010). Tumor protein p73, which is encoded by *TP73,* is one of the three p53 family of transcription factors and is also considered a tumor suppressor; it arrests the cell cycle and induces apoptosis by promoting the expression of related genes (Costanzo et al., 2002; Flores et al., 2002; Kaghad et al., 1997). In addition to loss-of-function mutations, p73 can also suffer N-terminal truncations, which may lack all or part of the transactivation domain and may exert a dominant negative effect on wild-type p73 and p53 (Tannapfel et al., 2008; Venkatanarayan et al., 2015).

1.3.3 Emerging molecular and cellular subtypes

While nearly all SCLC cases exhibit loss of function of p53, RB, or related proteins, there is growing evidence of distinct genetic subtypes of SCLC. Importantly, different subtypes posses different vulnerabilities (Chalishazar et al., 2019; Huang et al., 2018). A better understanding of these subtypes, which are differentiated by expression of four key transcription regulators, and their individual vulnerabilities will be helpful toward stratifying patients in clinical trials. SCLC can generally be categorized into two groups; most are neuroendocrine tumors, but a smaller percentage exhibit very low expression of neuroendocrine markers (Rudin et al., 2019). The neuroendocrine tumors express *insulinoma-associated protein 1* (*INSM1*), which is a zinc-finger transcription

factor implicated as a driver of neuroendocrine differentiation in multiple tissues (Christensen et al., 2014; Fujino et al., 2015; Gierl et al., 2006; Pedersen et al., 2003; Taniwaki et al., 2006; Wildner et al., 2008). They can be further separated into 'classical' and 'variant' subtypes, which are characterized by expression of *achaete-scute family bHLH transcription factor 1* (*ASCL1*) and *neuronal differentiation 1* (*NEUROD1*) (Borromeo et al., 2016; Carney et al., 1985; Gazdar et al., 1985; George et al., 2015; Rudin et al., 2019); both of these transcription factors are key in the development of neuroendocrine cells in the lung (Borges et al., 1997; Neptune et al., 2008). Whereas 'classical' SCLC exhibits a higher amount of *ASCL1* and is therefore also referred to as SCLC-A, the 'variant' subtype exhibits a higher amount of *NEUROD1* and is therefore also referred to as SCLC-N. The SCLC-A subtype also expresses high levels of other neuroendocrine markers such as *SYP* and *calcitonin related polypeptide* (*CALC* or *CGRP*).

The *MYC* family of transcription factors, which are amplified in up to 50% of SCLC, are expressed in a mutually exclusive fashion between SCLC subtypes (Johnson et al., 1996; Kim et al., 2006; Nau et al., 1985), likely due to functional redundancy; MYCs control gene expression by generating RNA messages from DNA templates (Amati et al., 1992; Amin et al., 1993), and can substitute for each other during murine development (Malynn et al., 2000). While both MYC and MYCL were recently implicated as potent oncogenes for SCLC, MYC accelerates the tumor development of SCLC-N, whereas MYCL accelerates the growth of SCLC-A (Kim et al., 2016; Mollaoglu et al., 2017). As the MYC family is not currently a feasible therapeutic

target (Carabet et al., 2019; Clausen et al., 2010; Yu et al., 2016), it is vital to understand which downstream pathways may be targeted in order to attenuate their oncogenic effects. Along that line, there is mounting evidence that indicates the specific subtypes of SCLC may exhibit selective sensitivity to targeted therapies. For example, a recent clinical trial in relapsed SCLC showed that response to alisertib, an Aurora A inhibitor, was stratified based on *MYC* expression (Owonikoko et al., 2020).

There are two other subtypes of SCLC, which exhibit very low expression of neuroendocrine markers such as *INSM1*, *ASCL1* and *NEUROD1*. Interestingly, one of these, termed SCLC-Y, is enriched for expression of *RB* (McColl et al., 2017; Sonkin et al., 2019). Expression profiling and RNA-seq data revealed that this subtype exhibits high expression of *yes1 associate transcriptional regulator* (*YAP1*) (George et al., 2015; McColl et al., 2017), which is a downstream effector of the Hippo (MST1 and MST2 in mammals) signaling pathway. While the functional significance of *YAP1* in this subtype is not well defined, there is evidence that it plays a role in cell morphology; for example, *YAP1* expressing cells are more adherent and *YAP1* knockdown results in a change from an elongated morphology to more round (Horie et al., 2016). More recently, inhibition of *YAP1* was shown to decrease cell growth and sensitize cells to multiple drugs, including cisplatin, etoposide, and adriamycin (Song et al., 2020).

The other non-neuroendocrine subtype, termed SCLC-P, is characterized by high expression of the transcription factor *POU class 2 homeobox 3* (*POU2F3*) (Huang et al., 2018). Interestingly, the facts that *POU2F3* expression is normally restricted to

chemosensory tuft cells of the lung and that the expression profile of SCLC-P is more similar to tuft cells than neuroendocrine cells has raised the possibility of a previously unrecognized cell of origin or a trans-differentiation event in SCLC (Huang et al., 2018; Yamashita et al., 2017). SCLC-P is likely to have different therapeutic vulnerabilities from the rest because of the unique set of dependencies, including the transcription factors SOX9 and ASCL2 as well as the receptor tyrosine kinase (RTK), insulin-like growth factor 1 receptor (Huang et al., 2018). An analysis of publicly available gene expression profiling of 54 human SCLC cell lines and 81 primary tumors clearly distinguishes the four subtypes with SCLC-A, -N, -P and -Y accounting for 70%, 11%, 16% and 2%, respectively (Rudin et al., 2019). Moreover, comprehensive profiling of the SCLC genome has uncovered a list of candidate oncogenes for this tumor (George et al., 2015).

1.3.4 Discovery of the SCLC genome

Cells acquire the hallmarks of cancer through various mechanisms, such as genomic deletion of tumor suppressors and amplification of oncogenes (Hanahan & Weinberg, 2011). Array-based comparative genomic hybridization (aCGH) has greatly facilitated the detection of individual genes that are amplified or deleted in cancer. For SCLC, these analyses accentuated recurrent losses of some regions of the genome, such as those harboring *RB* and *p53* (Levin et al., 1994; Voortman et al., 2010). They also accentuated amplifications of other regions, such as those encoding the MYC family of transcription factors (Johnson et al., 1996; Kim et al., 2006; Nau et al., 1985).

Another major step in characterizing the SCLC genome was the discovery of an extremely high mutational rate. Through array-based single-nucleotide polymorphism (SNP) analysis, groups have uncovered a large number of nonsynonymous mutations that produce changes in amino acid sequence at an average rate of about 8 mutations per million nucleotides (Augert et al., 2017; George et al., 2015; Peifer et al., 2012; Rudin et al., 2012). C:G>A:T transversions, which is a signature of tobacco exposure, represented a significant portion (28%) of mutations, which underscores the tight relationship between tobacco and SCLC (George et al., 2015). Notably, nearly all SCLC tumors exhibited loss-of-function alterations in both copies of the *RB1* and *TP53* genes (George et al., 2015).

The functional significance of a large number of alterations is not well understood, presenting a major obstacle to understanding the mechanisms underlying SCLC development. To help highlight functionally relevant alterations, groups have used bioinformatics to filter candidates through several parameters: ‘significant occurrence’ indicates mutation rates higher than expected, ‘clustering pattern’ indicates enrichment of mutations in protein coding sequences related to tumor suppressor or oncogenic functions, ‘damaging nature’ indicates how likely mutations are to inhibit protein function, and ‘cancer census’ enables screening against lists of genes frequently affected by somatic alterations in human cancers, e.g, the Cancer Gene Census and COSMIC databases (George et al., 2015).

1.4 Models of SCLC development and homeostasis

Due to the standard of care for extensive-stage SCLC, which does not include surgery, resection of SCLC is rare, resulting in a paucity of human tumor samples (Farago & Keane, 2018). Testing the function of candidate genes and translating growth effects to the clinic has therefore been difficult. However, groups are investigating the significance of genetic aberrations in SCLC using various biological model systems, such as established cancer cell lines, patient-derived xenografts, and genetically engineered mice. Strengths and weaknesses of each model are discussed below.

1.4.1 Cell lines

Derived mainly from metastatic SCLC tumors, human cancer cell lines have long been utilized to identify genetic abnormalities, functionally characterize genes and test candidate therapies (Gazdar et al., 1980; Harbour et al., 1988; Johnson et al., 1996; Pettengill et al., 1980). However, after being maintained outside the body for decades, cell lines often adjust to selective pressure and acquire de novo alterations, which may be unrelated to tumor initiation and progression in vivo (Gazdar et al., 2016). Additionally, cells in monoculture or injected subcutaneously into nude mice lack interactions with other types of cells within their native tumor microenvironment (TME), which is the space in which malignant cells and non-transformed cells interact (Hanahan & Coussens, 2012). These interactions can alter the efficacy of therapies and

contribute to the frequent discrepancy between pre-clinical findings and results from human trials (Meyer et al., 2014).

Recently, patient-derived xenograft (PDX) models generated from biopsies, resected tumors and circulating tumor cells (CTX) have facilitated the identification of prognostic biomarkers and functional interrogation of candidate therapeutic targets (Drapkin et al., 2018; Hodgkinson et al., 2014). However, PDXs are more difficult to manipulate and more expensive; they are also grown in mice without intact immune systems (Gazdar et al., 2016). Importantly, while PDX models more accurately recapitulate features of late-stage disease as well as clinical response to standard treatment, it is difficult to systematically characterize the roles of genetic alterations in tumor initiation and development because these models lack key features of early transformation (Drapkin et al., 2018).

1.4.2 Genetically engineered mouse models

The inactivation of RB and p53 in the overwhelming majority of SCLC (over 90%) (George et al., 2015) led to the generation of a GEMM in which *Rb1* and *Trp53* genes are conditionally deleted through intratracheal administration of adenovirus encoding Cre recombinase (Meuwissen et al., 2003); another GEMM additionally deletes *Rbl2*, which is a functional homolog of *Rb1* (Schaffer et al., 2010). Both the *Rb1*/*Trp53*- and the *Rb1*/*Trp53/Rbl2*-mutant mice generate lung tumors, which recapitulate the

pathology and response to chemotherapy observed in human SCLC (Gazdar et al., 2015; Meuwissen et al., 2003). While the *Rb1*/*Trp53*-mutant GEMM exhibits prolonged tumor latency, which is more suited for assessing the ability of candidate alterations to accelerate tumor progression, the *Rb1*/*Trp53/Rbl2*-mutant GEMM develops tumors faster and is more suited for assessing the ability of candidate tumor suppressors to slow tumor progression. These autochthonous models allow for analysis of lungs at any stage of tumor development and facilitate the identification of mechanisms contributing to tumor initiation and progression within the native TME (Mollaoglu et al., 2017; Schaffer et al., 2010). As non-malignant cells of the TME can have profound and varying effects on tumor development at different stages of tumorigenesis (Ricciardi et al., 2015), these GEMMs have the advantage of identifying relevant genes that may not be identified in other models such as monoculture *in vitro* or subcutaneous implantation into nude mice. However, because these GEMMs are generally limited to interrogating one candidate gene at a time, they are not ideal for studying interactions between multiple genes.

1.4.3 Precancerous cells

Rb1/*Trp53/Rbl2*-mutant precancerous pulmonary neuroendocrine cells, which are isolated from early-stage lesions in the GEMM, offer a streamlined approach to functionally characterize genomic alterations. These cells are selected utilizing a neuroendocrine lineage-specific green fluorescent protein (GFP) under the control of gene regulatory elements surrounding *Chga*, which is a pan-neuroendocrine gene (Kim

et al., 2016). Unlike SCLC tumor cells, most of which cluster in loose aggregates or tight spheres and are highly tumorigenic when transplanted into subcutaneous allograft mouse models, mutant premalignant cells grow as adherent monolayers in culture and do not readily form subcutaneous tumors. Although these cells are immortalized, they lack necessary oncogenic factors and are not transformed; hence, they are designated precancerous cells of SCLC (preSC). Their susceptibility to transformation upon induction of oncogenic alterations, alone or in combination, makes preSC a suitable model for systematic characterization of the SCLC genome (Jia et al., 2018; Kim et al., 2016). Recently, there has been significant progress in the field of SCLC, which has been facilitated by advances in high-throughput analytics and the development of more robust disease models (Drapkin et al., 2018; George et al., 2015; Jia et al., 2018; Kim et al., 2016; Meuwissen et al., 2003; Schaffer et al., 2010). Ultimately, to address the complexity of mutational heterogeneity, a multi-omics approach utilizing multiple preclinical models is necessary to facilitate functional characterization of a number of alterations.

1.5 Cancer metabolism

1.5.1 General features of cancer metabolism

One of the hallmarks of cancer is deregulated cell energetics and metabolism (Hanahan & Weinberg, 2011). Over 90 years ago, Otto Warburg observed that cultured

cancer cells exhibit increased glycolysis, which is the pathway that generates energy from glucose, even in the presence of abundant oxygen, a phenomenon referred to as “the Warburg effect” (Warburg, 1956; Warburg et al., 1927). Since glycolysis is much less efficient at producing the main form of cellular energy, adenosine triphosphate (ATP), than mitochondrial oxidative phosphorylation, this observation was unexpected. However, in addition to ATP, aerobic glycolysis generates metabolic intermediates that cells utilize for rapid growth and division (Boros et al., 1997; Chesney et al., 1999; Lee et al., 1995). Also, cancer cells do not switch completely from oxidative phosphorylation to aerobic glycolysis; rather, they utilize variable rates of glycolysis in addition to oxidative phosphorylation (Rodríguez-Enríquez et al., 2000).

Additionally, rapid cell division requires sustained pools of nucleotides used for DNA and RNA synthesis (Cano-Crespo et al., 2019; Kondo et al., 2000; Sigoillot et al., 2003). Cancer cells largely rely on the de novo nucleotide synthesis pathways, which are reliant on amino acid metabolism (Heidelberger et al., 1957; Huang et al., 2018; Labuschagne et al., 2014; Li et al., 2019). Biosynthesis of both purines and pyrimidines is altered in cancer and requires the production of 5-phosphoribose-1-pyrophosphate (PRPP), which is produced through the oxidative and nonoxidative arms of the pentose phosphate pathway (PPP) (Goldthwaitt, 1956). The pyrimidine synthesis pathway utilizes glutamine and aspartate to produce uridine monophosphate (UMP), which is the basis for the pyrimidine deoxyribonucleotides and ribonucleotides necessary for DNA and RNA synthesis, respectively (Jones, 1980). The pyrimidine ring is initially assembled from glutamine, bicarbonate, and aspartate, and is then attached to PRPP

through six reactions. The first three reactions in the de novo pyrimidine synthesis pathway occur in the cytosol and are catalyzed by carbamoyl phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), which produces dihydroorotate (Coleman et al., 1977; Jones, 1980). Dihydroorotate enters mitochondria, where it is oxidized to orotate by dihydroorotate dehydrogenase (DHODH) (Chen & Jones, 1976; Jones, 1980; Knecht et al., 1996). Then, UMP synthase (UMPS) converts orotate into UMP in the cytosol (Jones, 1980). Purine synthesis differs from pyrimidine synthesis in that all of the reactions occur in the cytosol. Additionally, the purine ring is directly built onto PRPP (Pedley & Benkovic, 2017). The purine ring is synthesized from glutamine, glycine, N10-formyl-tetrahydrofolate (THF), bicarbonate, and aspartate. Inosine monophosphate (IMP) is produced following several reactions and is converted to guanosine monophosphate (GMP) by the enzymes inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS). IMP may also be converted to adenosine monophosphate (AMP) through reactions involving adenylosuccinate synthase (ADSS) and adenylosuccinate lyase (ADSL). Once formed, the ribonucleoside triphosphates (NTPs) can be used for RNA synthesis, but DNA synthesis requires the formation of deoxy-NTPs, which is catalyzed by ribonucleotide reductase (RNR) (Brown et al., 1969; Reichard et al., 1961; Thelander & Reichard, 1979). Altered de novo nucleotide synthesis in cancer cells is controlled by metabolic rewiring, such as alterations in the PI3K/AKT/mTORC1 pathway and overexpression of MYC (Ben-Sahra et al., 2016; Chalishazar et al., 2019; Liu et al., 2008).

As discussed above, cancer cells rely on certain amino acids to sustain nucleotide pools. However, amino acids, such as glutamine and the branched chain amino acids can be used for several other processes, such as the tricarboxylic (TCA) cycle, which helps maintain cellular energy homeostasis (Neinast et al., 2019; Yang et al., 2014). Additionally, branched chain amino acids can be used for the production of acetyl-CoA, which can then be used for lipid biosynthesis (Halama et al., 2015). This is very important because lipids make up most of the cell membrane, which controls what gets in and out of the cell (Mondal et al., 2009; Singer & Nicolson, 1972); they are also important signal messengers (Bae et al., 1998). Amino acid metabolism can also alter gene expression. For example, arginine-derived polyamines modulate chromatin structure, altering the accessibility of regions of the genome (Basu et al., 1992; Hobbs et al., 2002; Sato et al., 2003). Glutamate, glycine, and cysteine mediate oxidative stress by contributing to the synthesis of glutathione (Wessner et al., 2003), which helps reduce the levels of reactive oxygen species (McBrayer et al., 2018). Importantly, amino acids are also the major components of proteins, which catalyze numerous reactions in the cell. Indeed, progression through the cell cycle is highly dependent on the synthesis and degradation of pro-growth and growth-restrictive proteins (Evans et al., 1983; Lohka et al., 1988).

1.5.2 Metabolism in SCLC

Consistent with a high rate of glycolysis, SCLC exhibits enhanced glucose uptake as detected by fluorodeoxyglucose positron emission tomography (Lee et al.,

2009; Schumacher et al., 2001). Furthermore, this enhanced uptake is associated with poor prognosis in both LS- and ES-SCLC (Lee et al., 2009). Glycolysis is the culmination of ten enzymatic steps; three are irreversible, such as the pyruvate kinase (PK)-mediated production of pyruvate from phosphoenol-pyruvate. PK is encoded by two genes, *PKM* and *PKLR*, but most cell types predominantly express *PKM*, which produces two splice variants that are translated into distinct isoforms (Harada et al., 1978; Noguchi et al., 1986, 1987): PKM1 is constitutively active, whereas activation of PKM2 is dependent on the concentration of allosteric activators (Chaneton et al., 2012; Keller et al., 2012). Cancer cells abundantly express PKM2 relative to PKM1 (Koss et al., 2004; Reinacher & Eigenbrodt, 1981); however, lung neuroendocrine cancer cells, especially SCLC, express relatively more PKM1 compared to non-neuroendocrine lung cancer cells, such as NSCLC, and others (Morita et al., 2018). Furthermore, ectopic expression of mouse Pkm1, but not Pkm2, enhanced the growth of PKM-depleted human SCLC cell lines *in vitro*, indicating that glycolysis supports the growth of SCLC, which exhibits increased dependence on PKM1.

Following the PK-mediated reaction is the production of lactate from pyruvate, which is catalyzed by lactate dehydrogenase (LDH). Elevated serum level of LDH is one of the most significant predictors of poor prognosis for SCLC (Zhang et al., 2016; Zhou et al., 2018). Furthermore, chemoresistant SCLC exhibits both reduced basal oxygen consumption and increased lactate production (Tripathi et al., 2017), which is consistent with increased dependence on glycolysis; however, excess lactate must be exported from the cell in order to avoid detrimental effects related to intracellular acidification.

Accordingly, pharmacological inhibition of monocarboxylate transporter 1 (MCT1), a lactate transporter, increased the intracellular concentration of lactate and inhibited the growth of a subset of human SCLC cell lines *in vitro*, especially under hypoxic conditions, and in xenografts (Polanski et al., 2014). Together, these studies demonstrate that aberrant glucose metabolism can be targeted to inhibit the growth of SCLC and, importantly, there may be preferences toward specific isoforms of related proteins.

There is emerging evidence that SCLC depends on multiple metabolic pathways, in addition to glycolysis, in order to sustain rapid cell growth and proliferation. For example, the cholesterol biosynthesis pathway has gained interest in recent years (Mahoney et al., 2019). The 3-hydroxy-3methylglutaryl CoA reductase inhibitor simvastatin has been shown to block the proliferation of human SCLC cells *in vitro* and induce apoptosis (Khanzada et al., 2006). Furthermore, simvastatin was shown to sensitize the SCLC cells to etoposide and impair tumor growth *in vivo*. These effects were attributed, at least in part, to disruption of Ras localization to the plasma membrane, and replenishing cholesterol reversed the effect on N-Ras. More recently, another group found that depletion of MEK5 and ERK5, which promote SCLC growth and survival *in vitro*, disrupts several lipid metabolism pathways, such as the mevalonate pathway that controls cholesterol synthesis (Cristea et al., 2020). Furthermore, inhibition of the mevalonate pathway with atorvastatin reduced cell growth in both murine and human cell lines, providing more evidence that the cholesterol biosynthesis pathway is an important mediator of the development of SCLC.

Another recent report employed a CRISPR screen against the “druggable genome”, including over 4900 mouse genes that are known targets of existing drugs or belong to gene categories predicted to be druggable, to identify those that support the growth of cell lines derived from GEMMs of SCLC, lung adenocarcinoma or pancreatic ductal adenocarcinoma (PDAC) (Li et al., 2019). Enzymes involved in pyrimidine biosynthesis, including *Dhodh*, *Umps* or *Cad* were among the top candidate genes identified in SCLC compared to the others. SCLC cells exhibited smaller pools of UMP and a slower rate of UMP synthesis over time compared to the other cancer cells, which correlated with increased sensitivity to brequinar and leflunomide, two inhibitors of Dhodh. Brequinar treatment in an autochthonous model of SCLC resulted in the decreased growth of primary tumors and the delayed onset of liver metastases. Furthermore, brequinar treatment induced tumor regression and extended time to progression in two out of four PDX models of SCLC, demonstrating that at least a subset of human SCLC is sensitive to inhibition of pyrimidine biosynthesis.

There is evidence that SCLC subtypes differ in their dependence on amino acid metabolism. For example, the MYC-driven (SCLC-N) subtype was recently found to be more dependent on arginine than the MYCL-driven (SCLC-A) subtype (Chalishazar et al., 2019). Metabolomic profiling of cells, together with gene set enrichment analysis of publicly available datasets, revealed the upregulation of multiple metabolic intermediates and genes involved in the arginine biosynthesis pathway. Depletion of arginine through amino acid withdrawal decreased the viability of MYC-expressing cells

more than MYCL- or MYCN-expressing cells. Additionally, MYC-expressing cells were also more sensitive to inhibition of polyamine biosynthesis and inhibition of the mTORC1 pathway, which are both downstream of arginine. Notably, while depletion of arginine by ADI-PEG 20 monotherapy significantly improved tumor regression and nearly doubled the overall survival of Myc-driven GEMM over treatment with cisplatin and etoposide, ADI-PEG 20 monotherapy did not increase the survival of MYCL-driven GEMM over control, supporting the concept that arginine metabolism is a limiting factor for the growth of MYC-driven tumors, but not for MYCL-driven tumors. On the other hand, ASCL1-high (SCLC-A subtype) cells are more dependent on methionine metabolism (Huang et al., 2018). They exhibited increased levels of methionine cycle intermediates and were more sensitive to an inhibitor of methionine adenosyltransferase (MAT) in comparison to ASCL1-low cells. Furthermore, knockout of MAT2A selectively reduced the proliferation of ASCL1-high cells, suggesting that SCLC-A has its own amino acid vulnerabilities. Whether branched chain amino acid metabolism is also a vulnerability for SCLC is an interesting question and the ultimate focus of this dissertation.

Comparison of 26 human SCLC cell lines through metabolomic clustering to revealed a set of metabolites that distinguished the majority ASCL1-high subtype (SCLC-A) from the minority ASCL1-low, which included metabolites from pathways involving nucleotide biosynthesis, amino acid metabolism, and the TCA cycle (Huang et al., 2018). The ASCL1-low group exhibited higher levels of purine nucleotides including IMP, GMP, xanthosine 5′-monophosphate (XMP), and AMP, which, like pyrimidines,

contribute to DNA and RNA synthesis as well as intracellular signaling. Consistent with this observation, the ASCL1-low cells had increased expression of multiple genes involved in purine biosynthesis, as did an ASCL1-low subset of patient tumors, and exhibited higher rates of purine biosynthesis. The increased purine synthesis was controlled by MYC, because knockout of MYC resulted in dramatically decreased expression of IMPDH isoforms 1 and 2 as well as decreased production of GMP and AMP. Direct regulation is supported by the observation that ASCL1-low tumors, which arise in the *Rb1*/*Trp53*-mutant GEMM expressing *Myc-T58A* (RPM mice), exhibit increased binding of Myc at the *Impdh1* and *Impdh2* promoters, increased expression of their encoded proteins, and increased concentrations of XMP and GMP. Furthermore, ASCL1-low cells were more sensitive to mycophenolic acid, an inhibitor of IMPDH, than ASCL1-high cells. They also exhibited a reduction in RNA polymerase I (RNA Pol-I) transcripts (pre-ribosomal RNA; pre-rRNA) following treatment. Additionally, treating RPM mice with a combination of cisplatin, etoposide and mizoribine (another IMPDH inhibitor) reduced tumor burden and increased survival over chemotherapy alone. Thus, this study revealed an ASCL1-low subtype-specific dependency on purine synthesis to support ribosome biogenesis (Huang et al., 2018).

However, ASCL1-low tumors are not the only subtype to depend on ribosome biogenesis. Through pathway analysis of differentially expressed genes, a collaboration between our group and others found that, compared to non-transformed preSC, preSC lines transformed with each of the MYC family members separately exhibit enrichment of molecular pathways related to ribosome biogenesis and protein synthesis (Kim et al.,

2016). In particular, ectopic expression of MYCL both enhanced pre-rRNA synthesis and increased protein synthesis. Additionally, treatment with CX-5461, which is a specific inhibitor of RNA Pol-I that was shown to be safe and effective in a phase I study of advanced hematological cancers, significantly reduced the viability of three mouse SCLC cell lines derived from the *Rb1*/*Trp53*-mutant GEMM as well as five out of seven human SCLC lines; two of the seven human lines were less sensitive to the same concentrations of the drug. Further experiments revealed that the mouse cells and the more sensitive human SCLC lines exhibited relatively higher levels of MYCL and MYCN, while the less responsive cells had higher expression of MYC. Furthermore, CX-5461 treatment suppressed SCLC in an autochthonous *Rb1*/*Trp53*-mutant GEMM. These two reports suggest that although SCLC is heavily dependent on increased protein synthesis and is sensitive to RNA Pol-I inhibition, specific subtypes may differ in the mechanism by which the levels of pre-rRNAs are maintained and in their sensitivities to RNA Pol-I inhibitors (Huang et al., 2018; Kim et al., 2016).

1.6 Protein Translation in Cancer

1.6.1 Regulation of protein translation in health

Proteins are macromolecules, which are vital for maintaining cellular homeostasis, regulating cellular structure and relaying signals from the environment into the cell in order to regulate cell growth and proliferation (Sonenberg & Hinnebusch,

2009). Protein translation is a multi-step process that builds upon a mRNA template to assemble single amino acids into peptides and proteins. At the center of this process is the ribosome. The eukaryotic ribosome is a multi-subunit RNA-protein complex composed of the small 40S ribosomal subunit, which contains the 18S rRNA and 33 ribosomal proteins (RPs), and the large 60S ribosomal subunit, which contains the 28S, 5.8S, and 5S rRNAs and 47 RPs (Ben-Shem et al., 2011; Klinge et al., 2011; Rabl et al., 2011). Ribosome biogenesis is one of the most highly regulated and energy consuming processes in the cell (Warner, 1999), involving coordination between three RNA polymerases and more than 200 protein cofactors (Henras et al., 2008; Kressler et al., 1999; Planta & Mager, 1998). This process begins with the RNA Pol-I-mediated synthesis of the 45S pre-rRNA, encoding the 18S, 5.8S, and 28S rRNAs, which occurs in the nucleolus (Bowman et al., 1981). In the nucleoplasm, RNA Pol-III transcribes the 5S rRNA, which is then imported to the nucleolus (Weinmann & Roeder, 1974). These rRNAs are modified through nucleolytic processing and then complex with RPs (e.g., RPS6), which are transcribed by RNA pol II, to form ribosomal precursors. Following further processing steps, pre-40S and pre-60S ribosomes are translocated to the cytoplasm where they are finally processed to form the mature 40S and 60S ribosomal subunits to facilitate protein translation, which is divided into three major steps: initiation, elongation and termination. Ribosomal availability is regulated through ribosome biogenesis and may be adjusted according to the needs of the cell (Jorgensen et al., 2004). For example, multiple ribosomes can attach to the same mRNA and simultaneously translate it into protein; however, whereas a low ribosomal density along mRNA can slow translation elongation, too many ribosomes can interfere with each

other and also slow elongation (Zarai et al., 2016). Therefore, the rate of protein translation is maximized when the ribosomal density is half of the maximum possible density.

1.6.2 Role of protein translation in cancer

In general, cancer cells exhibit uncontrolled cell growth and proliferation, which is dependent on enhanced ribosome biogenesis and protein synthesis. For example, the MYC transcription factors control the expression of multiple genes involved in ribosome biogenesis and the translation process (Boon et al., 2001; Grandori et al., 2005; Kim et al., 2000). However, while MYC-induced tumorigenesis relies on its ability to increase protein synthesis, at least in part, this is not sufficient to induce malignant transformation (Barna et al., 2008; Zindy et al., 1998). The cell must also inactivate tumor suppressors, such as p53, which inhibits RNA Pol-I in order to block rRNA synthesis (Zhai & Comai, 2000). Following inactivation of tumor suppressors, subsequent deregulation of protein translation can promote tumorigenesis. Indeed, many studies have linked deregulation of RPs and ribosome biogenesis factors to cancer initiation and progression (Kim et al., 2004; Naora et al., 1998; Wang et al., 2009); additionally, some reports have shown that aberrant expression of RPs correlates with poor prognosis (Bee et al., 2006; Guimaraes & Zavolan, 2016; Wong et al., 2014). Translation initiation factors are also deregulated in multiple cancer types; for example, eukaryotic initiation factor (EIF) 4E and EIF4G are amplified in breast cancer and squamous cell lung carcinoma (Bauer et al., 2002; Silvera et al., 2009; Sorrells et al., 1998). Additionally, EIF5A2, which is necessary for

the formation of the first peptide bond (Benne et al., 1978; Benne & Hershey, 1978), is overexpressed in several cancers (Cao et al., 2017; Guan et al., 2001; Liu et al., 2015; Yang et al., 2009). Amplification of initiation factors can upregulate the translation of proteins important for cell proliferation and survival. For example, not only does overexpression of EIF3C or EIF3H in immortalized mouse fibroblasts (NIH3T3 cells) result in a shift of *cyclin D1*, *Myc*, and *fibroblast growth factor 2* mRNAs into heavier polysomal fractions, but it also results in malignant transformation (Zhang et al., 2007). Furthermore, while silencing the expression of EIF4G in inflammatory breast cancer only marginally inhibits global protein translation, it significantly inhibits the translation of mRNAs with internal ribosome entry sites, such as p120 catenin, which enhances the cancer cell invasiveness (Silvera et al., 2009).

1.6.3 The necessity to regulate protein translation

Although tumor growth is dependent on protein translation, even cancer cells must regulate this process, particularly because translation and nascent chain folding is not 100% accurate and sometimes produces a protein folded into an unstable and non-native state (Gregersen et al., 2006). While unstable proteins are normally quickly degraded by the ubiquitin-proteasome system (Vembar & Brodsky, 2008), an imbalance toward translation can result in the toxic accumulation of misfolded proteins (Harding et al., 2000). To relieve endoplasmic reticulum (ER) stress induced by increased protein folding load and accumulation of misfolded proteins in the ER lumen, cells expand the ER membrane (Shaffer et al., 2004; Wiest et al., 1990), increase expression of protein-

folding chaperones (Kozutsumi et al., 1988; Lee et al., 2003), and inhibit global protein translation (Harding et al., 1999; Harding et al., 2000) by initiating the unfolded protein response (UPR). Indeed, the UPR contributes to pathogenesis and can be targeted to inhibit the growth of some cancers, such as multiple myeloma (Carrasco et al., 2007; Chauhan et al., 2012; Papandreou et al., 2011); however, prolonged activation of the UPR can also result in cell death. For example, eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3 or PERK) mediates one branch of the UPR. PERK phosphorylates EIF2A, which inactivates the EIF2 complex to inhibit global protein translation and relieve ER stress (Harding et al., 1999; Harding, et al., 2000). However, this preferentially increases the translation of some mRNAs with short open reading frames in their 5′-untranslated regions, such ATF4, which is a transcription factor that upregulates CHOP (transcription factor C/EBP homologous protein) among others (Harding, et al., 2000). CHOP is a transcription factor that promotes apoptosis during ER stress (Zinszner et al., 1998). Thus, the PERK branch of the UPR is cytoprotective at moderate levels of signaling, but prolonged activation can induce cell death. Importantly, hyperactivation of mTORC1 can induce ER stress and enhance the UPR; indeed, phosphorylation of EIF2A was increased in cells lacking tuberous sclerosis complex subunits 1 and 2 (TSC1 and TSC2, respectively) (Ozcan et al., 2008), which inhibit mTORC1 (described in detail in section 1.8.3). In contrast, the phosphorylation of EIF2A was decreased in cells treated with rapamycin or everolimus (Dong et al., 2015; Ito et al., 2011; Ozcan et al., 2008), underscoring the necessity to control the activity of mTORC1, which is partially regulated by availability of amino acids, such as leucine (Han et al., 2012).

1.7 Branched Chain Amino Acid Pathway

1.7.1 Branched chain amino acid metabolism

The branched chain amino acids (BCAAs) are a group of nonpolar, hydrophobic, and essential amino acids consisting of leucine, isoleucine and valine. They have been studied in various disorders, especially liver cirrhosis and renal failure, and they have implications on many bodily processes, such as immune function, neurotransmitter balance, and insulin secretion (Bonvini et al., 2018; Holeček, 2018). BCAAs are not produced by the body in sufficient levels and must be supplemented from the environment. Upon digestion of food, the BCAAs largely pass first round uptake by the liver and are instead taken up by other tissues, especially skeletal muscle (Neinast et al., 2019; Suryawan et al., 1998). BCAA uptake is largely mediated by the system L transporters, solute carrier family 7 member 5 (SLC7A5 or LAT1) and solute carrier family 7 member 8 (SLC7A8 or LAT2) (Oxender & Chritensen, 1963; Verrey, 2003). Solute carrier family 3 member 2 (SLC3A2) is the heavy chain subunit required to tether LAT1 and LAT2 to the cell membrane (Chiduza et al., 2019; Mastroberardino et al., 1998; Yan et al., 2019). LAT1 and LAT2 are obligate amino acid exchangers and regulate the efflux of other neutral amino acids like glutamine during influx of BCAAs and other essential amino acids (Nicklin et al., 2009; Verrey, 2003). Once inside the cell, the BCAAs can serve as direct substrates for protein synthesis or stimulate protein

synthesis indirectly by activating mTORC1 (Neishabouri et al., 2015), which is discussed in detail later in this chapter. Alternatively, BCAAs can be broken down to generate intermediates for several other metabolic pathways as discussed below (Hutson et al., 2005; Neinast et al., 2019; Zhang et al., 2018).

The first step in the catabolism of a BCAA is reversible transamination, i.e., the transfer of the amine group from the BCAA to a-ketoglutarate (a-KG), to generate glutamate and a branched chain a-keto acid (BCKA) (Harper et al., 1984) (Figure1.1). A-KG is a rate-limiting intermediate in the TCA cycle (Cupp & McAlister-Henn, 1991), but also inhibits ATP synthase (Chin et al., 2014), and is thereby an important regulator of energy homeostasis. As an amino acid, glutamate can serve as a direct substrate for protein translation and can also be a substrate for the synthesis of non-essential amino acids, including glutamine and aspartate (Ballester et al., 2019; Hu et al., 2010; Mayers et al., 2016). Importantly, glutamate and its product, GABA, are major neurotransmitters in the brain (Curtis et al., 1960; Florey, 1954; Watkins, 2000). The transamination reaction requires pyridoxal phosphate as a cofactor and is catalyzed by branched chain aminotransferases (BCATs) (Hutson et al., 2005). BCATs are encoded by two genes, *BCAT1* and *BCAT2*, which produce proteins of similar size, ~43 kDa, that form homodimers (Hall et al., 1993; Hutson et al., 1988; Wallin et al., 1990). BCAT1 and BCAT2 are partitioned into the cytoplasm and mitochondria, respectively, and while robust expression of BCAT1 is normally limited to a small number of tissues, especially the brain, gonads and specific immune compartments, BCAT2 is ubiquitously expressed throughout the body (Suryawan et al., 1998; Sweatt et al., 2004).

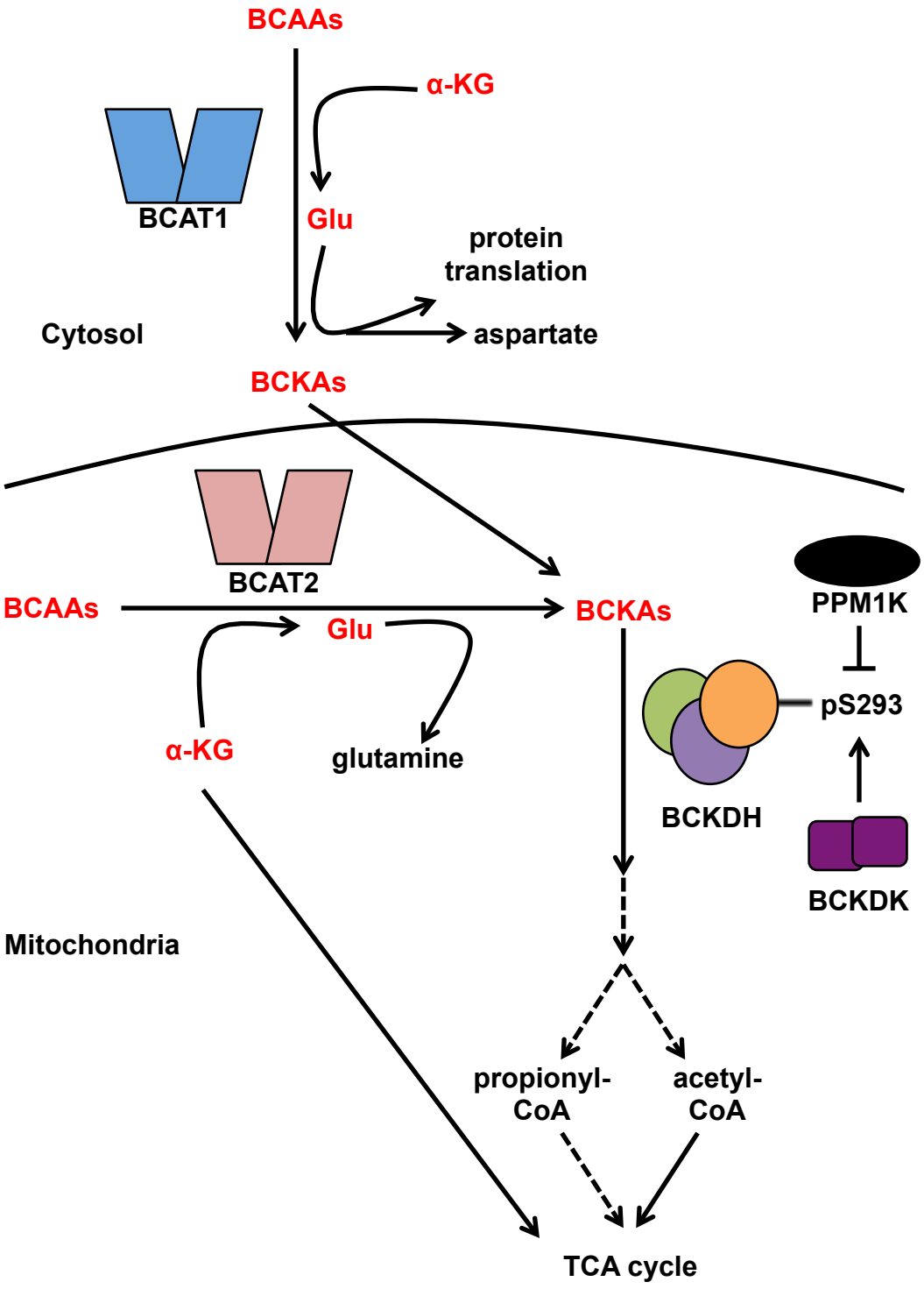

BCAAs
α-KG
BCAT1
Glu
protein
translation
Cytosol
aspartate
BCKAs
BCAT2
BCAAs
BCKAs
PPM1K
Glu
pS293
α-KG
glutamine
BCKDH
BCKDK
Mitochondria
propionyl-
CoA
acetyl-
CoA
TCA cycle

Figure 1.1. Branched chain amino acid catabolism.

Branched chain aminotransferase (BCAT) 1 in the cytosol and BCAT2 in the mitochondria generate branched chain keto-acids (BCKAs) and glutamate (Glu) from branched chain amino acids (BCAAs) and α-ketoglutarate (α-KG). Glutamate can serve as a direct substrate for protein translation and the biosynthesis of non-essential amino acids. The rate-limiting step in BCAA catabolism is catalyzed by the BCKA dehydrogenase (BCKDH) complex, which is inhibited by BCKDH kinase (BCKDK)-mediated phosphorylation of the E1 subunit at serine 293. This phosphorylation is removed by protein phosphatase, Mg2+/Mn2+ dependent 1K (PPM1K), resulting in activation of BCKDH. Through further enzymatic steps, the end products of BCAA catabolism are propionyl-CoA and acetyl-CoA for isoleucine, acetyl-CoA for leucine, and propionyl-CoA for valine. Acetyl-CoA and propionyl-CoA can be further processed through the TCA cycle. Red: direct metabolites of BCAT1; solid lines: direct relationships; dashed lines: indirect relationships.

Following the transamination of BCAAs, BCKAs (α-ketoisocaproate for leucine, α-ketoisovalerate for valine, and α-ketomethylvalerate for isoleucine) are usually exported from the cell into blood and then taken up by cells in other tissues, especially the liver (Harper et al., 1984; Hutson, 1989; Suryawan et al., 1998). The next step in BCAA catabolism is rate-limiting, irreversible and mediated by the BCKA dehydrogenase (BCKDH) complex, which resides in the mitochondria (Harris et al., 1990). This complex consists of 3 subunits: E1, E2 and E3. The E1 subunit is a tetramer of 2 α-subunits and 2 β-subunits (Ævarsson et al., 2000); BCKDH activity is dependent on the phosphorylation status of the E1a subunits at serine 293. This phosphorylation depends on the activity of BCKDH kinase (BCKDK), which phosphorylates E1a and inactivates the complex (Harris et al., 1990), and a mitochondrial protein phosphatase (PPM1K), which dephosphorylates E1a and activates the complex (Lu et al., 2009). The activities of these regulatory enzymes are dependent on substrate concentration such that high amounts of BCKAs activates BCKDH and low amounts of BCKAs deactivates it (Harris et al., 1990; Hutson, 1989). This balance is critical, as mutations in the *BCKDHA* or *BCKDHB* genes, which encode the E1a and E1B subunits, respectively, lead to buildup of BCAAs and BCKAs, which is toxic to the brain (Chuang et al., 1995; Harris et al., 1990; Nobukuni et al., 1991). This induces a condition called maple syrup urine disease, which leads to neurological dysfunction and can result in death if left untreated (Dancis et al., 1960; Dancis et al., 1959). Following further enzymatic steps, the end products of BCAA catabolism are propionyl-CoA and acetyl-CoA for isoleucine, acetyl-CoA for leucine, and propionyl-CoA for valine (Harper et al., 1984). Acetyl-CoA

and propionyl-CoA feed into the TCA cycle and therefore contribute to energy homeostasis.

1.7.2 Deregulation of the branched chain amino acid pathway in cancer

Over the past few years, there has been an accumulation of evidence that suggests BCAA metabolism is an important factor in tumor development (Ananieva & Wilkinson, 2018; Selwan & Edinger, 2017). Several cancers exhibit overexpression of LAT1 and its expression indicates a negative prognosis (Asano et al., 2007; Enomoto et al., 2019; Fuchs & Bode, 2005; Kaira et al., 2008, 2010). Furthermore, JPH203/KYT-0353, a small molecule inhibitor of LAT1, has advanced to a clinical trial for advanced solid tumors (UMIN000016546); it appears to be well tolerated and showed promising results for biliary tract cancer (Okano et al., 2020). While some cancers increase uptake of BCAAs from blood (Lee et al., 2019; Li et al., 2020; Mayers et al., 2016), others increase endogenous production (Gu et al., 2019; Hattori et al., 2017); the fate of these amino acids varies depending on metabolic demands. For example, knockdown of BCAT1 in epithelial ovarian cancer (EOC) cells was associated with decreased intracellular concentrations of several non-essential amino acids such as glutamate and aspartate (Wang et al., 2015). Furthermore, BCAA-derived glutamate and other non-essential amino acids have been reported in PDAC cells and NSCLC tumors (Mayers et al., 2016). Nucleotide supplementation rescued colony formation of PDAC cells in which BCAT2 was depleted, suggesting BCAAs can be used to fuel nucleotide synthesis; this finding was supported by a separate group studying NSCLC (Li et al., 2020; Mayers et

al., 2016). Additionally, while proliferating cells require an abundance of lipids to generate membranes for cell growth, knockdown of BCAT1 in EOC cells was associated with decreased levels of certain lipids (Wang et al., 2015). In some tumors, such as breast cancer, BCATs enhance mitochondrial function, but not in others such as PDAC and NSCLC (Lee et al., 2019; Mayers et al., 2016; Zhang & Han, 2017). By improving mitochondrial function, BCAT1 can improve energy homeostasis (ATP synthesis) and decrease levels of toxic reactive oxygen species (Zhang & Han, 2017). The aforementioned pathways are important mediators of the cell cycle and indeed, BCAT1 promotes cell cycle progression in some tumors (Wang et al., 2015; Zhou et al., 2013).

MYC-dependent expression of BCAT1 has been reported in multiple tumors (Zheng et al., 2016; Zhou et al., 2013), yet the contribution of BCAT1 to cancer growth was largely unrecognized until its overexpression was described in isocitrate dehydrogenase 1 (IDH1)-wildtype glioma (Tönjes et al., 2013). Knockdown of BCAT1 was associated with increased intracellular BCAAs and decreased glutamate release by cancer cells. More importantly, BCAT1 knockdown resulted in decreased growth of gliomas in mice. Similarly, subsequent reports indicate BCATs support the growth of NSCLC tumors (Mayers et al., 2016) and myeloid leukemias (García-Martínez et al., 2009; Hattori et al., 2017) and while knockdown of BCAT1 did not reduce tumor burden in a model of EOC, it significantly prolonged survival of the mice (Wang et al., 2015). There is also an emerging role for BCAA metabolism in HCC: BCAT1 expression increases tumor growth, whereas BCAAs are anti-fibrotic, reduce the incidence of

tumors and were shown to increase survival of a cohort of patients (Cha et al., 2013; Imanaka et al., 2016; Kawaguchi et al., 2014; Tada et al., 2019; Takegoshi et al., 2017; Zheng et al., 2016). *In vitro*, BCATs support the growth, proliferation, invasion and migration of a variety of cancer cells (Thewes et al., 2017; Tönjes et al., 2013; Wang et al., 2018; Wang et al., 2015; Zhou et al., 2013). Due to a growing appreciation for the role of BCAT1 in tumorigenesis, there have been recent suggestions of its utility as a prognostic biomarker (Chang et al., 2016; Cho et al., 2017; Xu et al., 2018). In chapter 2, we show that BCAT1 is over-expressed in mouse SCLC and is detectable in human SCLC cell lines. Whether and how BCAT1 promotes the growth of SCLC is the primary focus of this thesis; importantly, BCAAs enhance the activity of the mechanistic target of rapamycin kinase (MTOR), which is discussed in the following section.

1.8 Mechanistic Target of Rapamycin Complex 1

1.8.1 Structure of mTORC1

MTOR was identified as the target of a complex composed of a peptidyl-prolylisomerase, FKBP12, and rapamycin (also known as sirolimus) (Chiu et al., 1994; Heitman et al., 1991; Sabatini et al., 1994; Sabers et al., 1995), a drug with antifungal (Sehgal et al., 1975; Vezina et al., 1975), immunosuppressive (Bertagnolli et al., 1994; Bierer et al., 1990; Blazar et al., 1993; Blazar et al., 1998; Eghtesad et al., 2012; Ferraresso et al., 1994; Hackstein et al., 2003; Kahan et al., 1999; Passerini et al.,

2020), and antitumor properties (Dilling et al., 1994; Grewe et al., 1999; Hosoi et al., 1999; Ishizuka et al., 1997; Muthukkumar et al., 1995; Seufferlein & Rozengurt, 1996; Shi et al., 1995). MTOR is a member of the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family (Bosotti et al., 2000; Keith & Schreiber, 1995; Perry & Kleckner, 2003) and is a serine/threonine protein kinase (Brunn, Fadden, et al., 1997), which is evolutionarily conserved from yeast to mammals (Chiu et al., 1994) and is the catalytic subunit of two enzyme complexes known as mTORC1 and mTORC2 (Hresko & Mueckler, 2005; Kim et al., 2002). mTORC1 forms a 1-mDa ‘‘lozenge’’-shaped dimer and is composed of MTOR, regulatory associated protein of mTOR complex 1 (RPTOR also known as KOG1), mammalian lethal with SEC13 protein (MLST8 also known as GβL), DEP domain containing MTOR interacting protein (DEPTOR) and AKT1 substrate 1 (AKT1S1 also known as PRAS40) (Haar et al., 2007; Kim et al., 2003; Peterson et al., 2009; Sancak et al., 2007; Yip et al., 2010). mTORC2 is composed of MTOR, RPTOR independent companion of mTOR complex 2 (RICTOR also known as AVO3), MLST8, DEPTOR, mitogen-activated protein kinase associated protein 1 (MAPKAP1 also known as SIN1) and proline rich 5 (PRR5) or proline rich 5 like (PRR5L) (Frias et al., 2006; Jacinto et al., 2004; Peterson et al., 2009; Thedieck et al., 2007; Woo et al., 2007). While mTORC2 regulates cytoskeleton organization and cell survival (Jacinto et al., 2004; Khan et al., 2015; Tenkerian et al., 2015), the major cellular role of mTORC1 is to control cell growth (Kim et al., 2002).

1.8.2 Roles of mTORC1

mTORC1 is an important regulator of cell growth and can be pro- or anti-tumorigenic in different settings (Saxton & Sabatini, 2017). For example, multiple components of the PI3K/AKT/mTORC1 pathway are mutated or amplified in SCLC, such that over 35% of patient tumors harbor alterations (Umemura et al., 2014). Furthermore, human SCLC cell lines are sensitive to pharmacological inhibition of this pathway (Seufferlein & Rozengurt, 1996; Umemura et al., 2014). Additionally, inhibition of LAT1 attenuates mTORC1 activity in anaplastic thyroid cancer cells and tumors, which decreases growth by blocking cell cycle progression (Enomoto et al., 2019). In contrast, BCAA accumulation enhances mTORC1 activity and promotes the growth of clear cell renal carcinoma (ccRCC) (Qu et al., 2020). Similarly, BCAT1-driven activity of mTORC1 promotes the growth of breast cancer, endometrial cancer, and myeloid leukemia (Gu et al., 2019; Hattori et al., 2017; Wang et al., 2018; Zhang & Han, 2017). However, while BCAA supplementation has been shown to increase mTORC1 signaling in HCC, this resulted in a reduction in tumor incidence (Takegoshi et al., 2017).

The downstream pathways underlying these growth phenotypes vary; for example, mTORC1 can induce fatty acid synthesis (Qu et al., 2020), promote mitochondrial biogenesis (Zhang & Han, 2017), and inhibit autophagy (Hosokawa et al., 2009; Jung et al., 2009; Kim et al., 2011; Shang et al., 2011). Autophagy is a normal housekeeping process in which a cell's own autophagosomes engulf cytosolic organelles and macromolecules and then join with lysosomes to facilitate the

degradation of their contents (Gatica et al., 2018; Glick et al., 2010). This ultimately helps to prevent old and damaged components from disrupting cellular homeostasis and inducing death (Elmore et al., 2001; Lemasters et al., 1998; Wang et al., 2012). Importantly, autophagy can promote cancer cell resistance to chemotherapy; indeed, expression of BCAT1 enhances autophagy in HCC cells, which was associated with resistance to cisplatin (Zheng et al., 2016). By recycling macromolecules down to their precursors, such as amino acids or lipids, cells can also utilize autophagy to alleviate metabolic stress during times of nutrient starvation (Efeyan et al., 2013; Singh et al., 2009). However, this process must be regulated because excessive cellular degradation triggers death (Feng et al., 2005; Gozuacik & Kimchi, 2004; Schwarze & Seglen, 1985; Xue et al., 2001). Hence, mTORC1 helps control autophagy when sufficient nutrients are available. For example, mTORC1 binds to, phosphorylates, and inactivates the unc-51-like autophagy activating kinase 1 (ULK1) complex, which is an essential mediator of autophagy induced by nutrient starvation (Hosokawa et al., 2009; Kim et al., 2011; Shang et al., 2011).

Additionally, mTORC1 promotes protein translation (Showkat et al., 2014; Terada et al., 1994), which is a vulnerability of cancers such as SCLC (Kim et al., 2016), most notably by phosphorylating both ribosomal protein S6 kinase (RPS6K) and EIF4E binding protein 1 (EIF4EBP1) (Burnett et al., 1998). The majority of eukaryotic mRNAs contain a 5’ end cap in the form of a 7 methylguanosine triphosphate structure, to which the ribosome must dock in order to initiate translation (Ramanathan et al., 2016). Upon mTORC1-mediated phosphorylation at threonine 389, RPS6K is stimulated to

phosphorylate and activate, among other proteins, ribosomal protein S6 (RPS6), which is an important component of the small (40S) subunit of the ribosome that mediates cap-dependent protein translation initiation (Blenis et al., 1987; Bommer et al., 1980; Jenö et al., 1988; Price et al., 1989; Ramanathan et al., 2016; Terao & Ogata, 1979; Tolan & Traut, 1981). Conversely, mTORC1-mediated phosphorylation at threonines 37, 46, and 70 and serine 65 inactivates EIF4EBP1, which is a protein that directly binds to and inactivates EIF4E (Brunn et al., 1997; Brunn et al., 1997). EIF4E binds the 5' cap of mRNA as well as the scaffolding protein, EIF4G, which then binds the helicase, EIF4A, to form the EIF4F complex (Merrick & Pavitt, 2018). Association of EIF4E with the EIF4F complex facilitates the recruitment of the 40S subunit and is thought to be the rate-limiting step in cap-dependent translation initiation (Merrick & Pavitt, 2018). Thus, by indirectly activating RPS6 and EIF4E, mTORC1 can enhance cap-dependent protein translation initiation.

1.8.3 Regulation of mTORC1

The roles of growth factors, hormones, and amino acids in the regulation of mTORC1 have been studied extensively. Through multiple distinct signaling pathways, growth factors and hormones indirectly activate mTORC1 by eventually inhibiting the tuberous sclerosis complex (TSC), which is a heterotrimer consisting of TSC1, TSC2 and TBC1D7 (Dibble et al., 2012; Van Slegtenhorst et al., 1998). TSC functions as a a GTPase activating protein (GAP) and a negative regulator of the Ras homolog, mTORC1 binding protein, RHEB (Castro et al., 2003; Garami et al., 2003; Inoki et al.,

2003; Tee et al., 2003; Zhang et al., 2003). In the absence of growth factors, TSC stimulates the GTPase activity of RHEB to catalyze hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP), which is inactivating. Some growth factors and hormones, such as epidermal growth factor (EGF) and insulin, stimulate RTKs, which may eventually signal through PI3K and AKT or through mitogen-activated protein kinase 1 (MAPK1 or ERK), leading to phosphorylation and inactivation of TSC2 (Inoki et al., 2002; Ma et al., 2005; Manning et al., 2002). Additionally, the WNT signaling pathway inhibits glycogen synthase kinase 3 (GSK3), which also results in decreased activity of TSC2 (Avrahami et al., 2020; Cook et al., 1996; Inoki et al., 2006; Ruel et al., 1999). In contrast, the tumor necrosis factor (TNF) pathway that inactivates TSC through phosphorylation and inactivation of TSC1 (Lee et al., 2007, 2008). When bound to GTP (activated form), RHEB directly binds to mTORC1 at the lysosomal membrane to stimulate its activity (Inoki et al., 2003).

Amino acids, especially arginine and leucine, mainly stimulate mTORC1 by activating the Ras-related GTP-binding proteins (RRAGs) (Figure 1.2) (Durán et al., 2012; Han et al., 2012; Jewell et al., 2013; Sancak et al., 2008; Wolfson et al., 2015). RRAGs, which form heterodimers of RRAGA or RRAGB with RRAGC or RRAGD (Sekiguchi et al., 2001), are tethered to the lysosomal membrane by associating with the Ragulator complex, which is composed of MP1, p14, p18, HBXIP, and C7ORF59 (Bar-Peled et al., 2012; Sancak et al., 2010). The absence of amino acids stimulates the GTPase activity of RRAGs A and B, which is inactivating, but upon amino acid stimulation, the Ragulator complex acts as a guanine exchange factor (GEF) for RRAGs

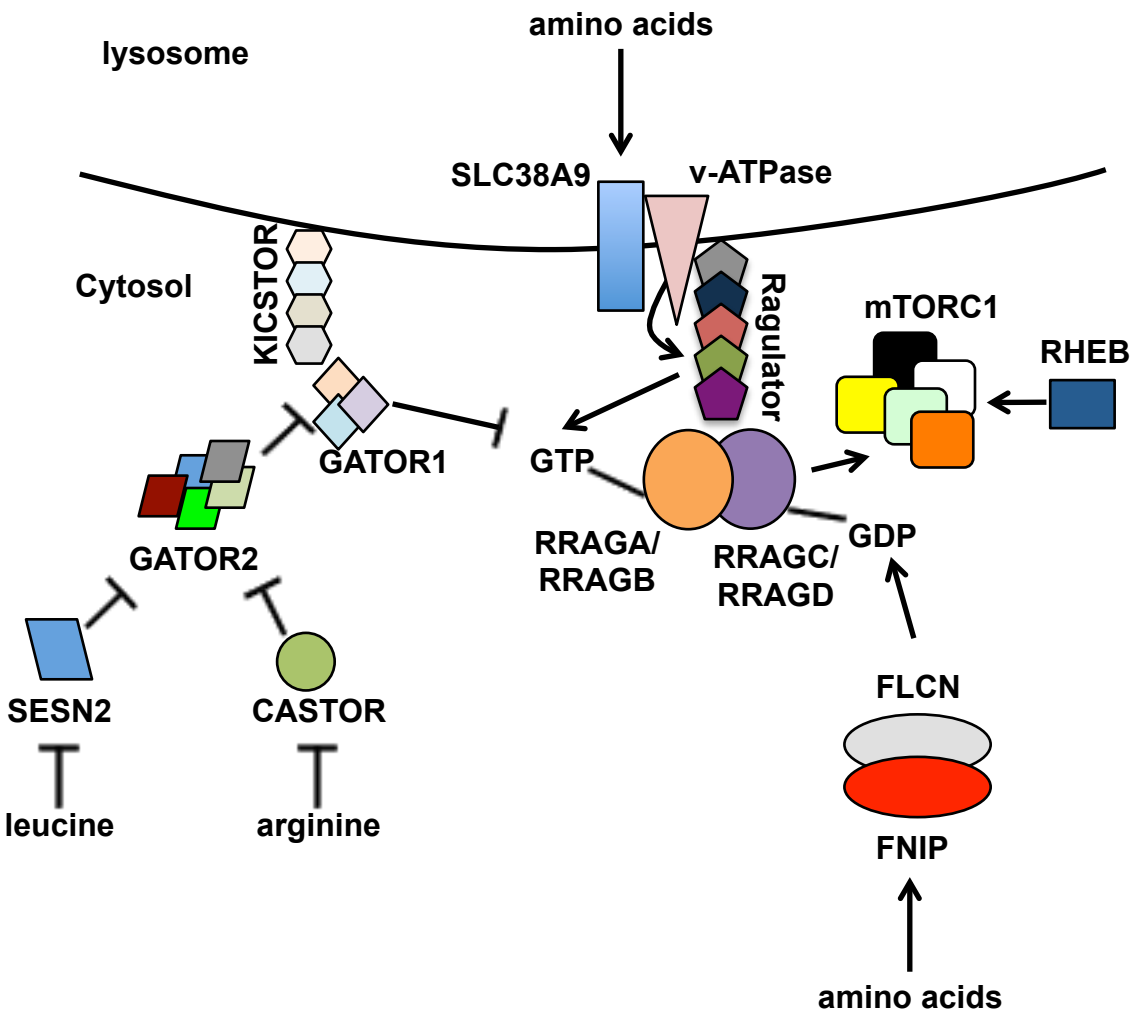

amino acids
lysosome
SLC38A9
v-ATPase
Cytosol
KICSTOR
Ragulator
mTORC1
RHEB
GATOR1
GTP
GATOR2
RRAGA/
RRAGB
RRAGC/
RRAGD
GDP
FLCN
SESN2
CASTOR
FNIP
leucine
arginine
amino acids

Figure 1.2. Mechanisms through which amino acids are sensed by mTORC1.

Intralysosomal amino acids signal through the transporter SLC38A9, which interacts with a complex of the Ras-related GTP-binding proteins (RRAGs), Ragulator, and v-ATPase. v-ATPase promotes the activity of Ragulator, which tethers the RRAGs to the lysosomal membrane. Ragulator is a guanine exchange factor for RRAGs A and B that promotes their association with GTP, which is activating. Cytosolic amino acids activate the folliculin-folliculin interacting protein (FLCN/FNIP) complex, which is a GTPase activating protein (GAP) and positive regulator of RRAGs C and D. Additionally, cytosolic leucine and arginine inactivate sestrin 2 (SESN2) and CASTOR, respectively, which both inhibit GATOR2. GATOR2 is a complex, which, when activated, inhibits the GATOR1 complex that is a GAP for and inhibitor of RRAGs A and B. Activated RRAGs associate with RPTOR and recruit mTORC1 to the lysosomal surface to be activated by RHEB.

A and B, which facilitates their association with GTP (Bar-Peled et al., 2012). Furthermore, amino acids activate the folliculin-folliculin interacting protein (FLCN/FNIP) complex, which is a GAP and positive regulator of RRAGs C and D (Tsun et al., 2013). This permits the RRAGS to associate with RPTOR and recruit mTORC1 to the lysosomal surface to be activated by RHEB (Sancak et al., 2008). Thus, mTORC1 is fully stimulated only when both RHEB and RRAGs are activated under conditions when both growth factors and amino acids are in sufficient availability.

The mechanisms through which intra-lysosomal amino acids are sensed are different from the mechanisms through which cytosolic amino acids are sensed. Intra-lysosomal amino acids signal through the transporter SLC38A9, which interacts with a complex composed of RRAGs, Ragulator and the lysosomal v-ATPase (Rebsamen et al., 2015; Zoncu et al., 2011). v-ATPase promotes the activity of Ragulator and thus, promotes the activity of RRAGs and mTORC1. In contrast, cytosolic amino acids typically signal through two complexes known as GATOR1 and GATOR2. GATOR1, which consists of DEPDC5, NPRL2, and NPRL3, is tethered to the lysosomal membrane by the recently identified KICSTOR complex, which is composed of KPTN, ITFG2, C12orf66, and SZT2, and inhibits mTORC1 by acting as a GAP of RRAGs A and B (Bar-Peled et al., 2013; Shen et al., 2018; Wolfson et al., 2017). In contrast, GATOR2, which is composed of MIOS, WDR24, WDR59, SEH1L, and SEC13, interacts with GATOR1 at the lysosomal membrane and is a positive regulator of mTORC1 (Bar-Peled et al., 2013). In the absence of amino acids, a direct sensor of leucine, called sestrin 2, associates with and inhibits GATOR2 and thus, inhibits the activity of

mTORC1; additionally, prolonged amino acid deprivation induces transcription of *sestrin 2* (Wolfson et al., 2015). Separately, CASTOR (cytosolic arginine sensor for mTORC1) was recently identified as a direct sensor of arginine and also inhibits GATOR2 upon amino acid deprivation (Chantranupong et al., 2016). Thus, both leucine and arginine stimulate mTORC1 at least in part by dissociating inhibitory proteins from GATOR2. Independently of the RRAGs, glutamine activates mTORC1 through the ADP-ribosylation factor (ARF) family of GTPases (Jewell et al., 2015).

Beyond growth factors and amino acids, mTORC1 activity is modulated by energy availability (ATP level) and DNA damage. Energy insufficiency may be caused by environmental factors such as low oxygen (hypoxia) or low glucose. In response to low levels of ATP, the adenosine monophosphate-activated protein kinase (AMPK) inactivates mTORC1 directly through inhibitory phosphorylation of RPTOR and indirectly through activating phosphorylation of TSC2 (Gwinn et al., 2008; Inoki et al., 2003). Additionally, glucose deprivation can inhibit the RRAG GTPases (Efeyan et al., 2013; Kalender et al., 2010) and promote the expression of p53 target genes, including the AMPK regulatory subunit (*AMPKß*) and *TSC2* (Feng et al., 2007; Jones et al., 2005). Both hypoxia and DNA damage can inhibit mTORC1 through induction of REDD1 (Regulated in DNA damage and development 1), which activates TSC (Brugarolas et al., 2004; Dennis et al., 2013; Deyoung et al., 2008; Feng et al., 2005; Horton et al., 2002; Schwarzer et al., 2005; Sofer et al., 2005; Yoshida et al., 2010). The realization that mTORC1 is an important regulator of cancer growth has prompted the development of several classes of inhibitors for clinical application.

1.8.4 Clinical application of mTORC1 inhibitors

MTOR inhibitors are generally divided into two categories: allosteric inhibitors of mTORC1 and those that directly bind to and inhibit the kinase domain. While mTORC1 was first identified as the target of rapamycin (Heitman et al., 1991), an allosteric inhibitor, and despite the ability of this drug to slow oncogenic transformation and cancer growth in preclinical models (Aoki et al., 2001; Cai et al., 2013; Dilling et al., 1994; Douros & Suffness, 1981; Houchens et al., 1983), rapamycin has not been clinically successful. Subsequently, several analogs, referred to as rapalogs, were developed, which exhibit improved solubility and pharmacokinetic properties (Schuler et al., 1997). Like rapamycin, these inhibitors complex with FKBP12 to inhibit mTORC1. In phase II and III clinical trials, two rapalogs, everolimus and temsirolimus, showed efficacy in treating certain cancers (Atkins et al., 2004; Smith et al., 2010; Strosberg, 2012; Witzig et al., 2011; Yao et al., 2016); however, these drugs showed limited success in treating SCLC as monotherapy or in combination with chemotherapeutics (Pandya et al., 2007; Tarhini et al., 2010). The efficacy of rapalogs is limited, partly due to the subsequent loss of a negative-feedback loop involving RPS6K-mediated phosphorylation and inhibition of insulin receptor substrate (IRS), which leads to activation of upstream oncogenic signaling pathways, such as MAPK and PI3K/AKT (Carracedo et al., 2008; O'Reilly et al., 2006; Sun et al., 2005; Tremblay et al., 2001). Additionally, although prolonged treament with rapamycin or rapalogs inhibits the pro-

survival complex mTORC2 (Sarbassov et al., 2006), acute treatment does not (Jacinto et al., 2004).

The development of MTOR kinase inhibitors (KIs), which directly target the MTOR catalytic site to block ATP, helped to circumvent these limitations. These inhibitors (e.g., Ku-006379, Torin, PP242) block the mTORC1-mediated phosphorylation of RPS6K and EIF4EBP1 as well as the mTORC2-mediated phosphorylation AKT (Feldman et al., 2009; García-Martínez et al., 2009; Thoreen et al., 2009; Xing et al., 2014). As such, compared to rapalogs, KIs exhibit improved antitumorigenic effects in preclinical models (Chresta et al., 2010; García-Martínez et al., 2009; Mecca et al., 2018; Thoreen et al., 2009). However, one caveat shared between MTOR KIs and rapalogs is that both classes of inhibitors can induce autophagy, which is cytoprotective at moderate levels (Thoreen et al., 2009; Zeng et al., 2018). Additionally, sensitivity to MTOR KIs can be limited by downregulation of EIF4EBPs, which results in a greater EIF4E/EIF4EBP ratio (Alain et al., 2012). These concerns prompted the development of dual PI3K/MTOR inhibitors, which is facilitated by the high degree of similarity in their kinase domains. These drugs, such as XL-765, effectively inhibit the phosphorylation of AKT, RPS6K, and EIF4EBP1, and have shown efficacy in phase I/II clinical trials (Awan et al., 2016; Brana et al., 2010; Brown et al., 2013, 2018; Papadopoulos et al., 2015).

1.9 Significance and Overview

Metabolism utlizes a diverse network of pathways that can be exploited to reduce the growth of cancer cells (Huang et al., 2018; Kim et al., 2016; Morita et al., 2018). This could be particularly useful to treat recalcitrant cancers, such as SCLC, for which the standard of care has not changed significantly in the past few decades (Farago & Keane, 2018). SCLC is highly metastatic and most patients present with late-stage disease, which limits standard treatment to chemotherapy. However, after first-line treatment, SCLC often reemerges resistant to further chemotherapy. Thus, there is an urgent need for robust biomarkers and novel therapeutic targets in order to detect SCLC earlier and improve patient outcomes. While there have been significant advancements in mapping the SCLC genome and developing robust model systems, the functional significance of the majority of genomic alterations is not well understood (Drapkin et al., 2018; Gazdar et al., 1980; George et al., 2015; Kim et al., 2016; Meuwissen et al., 2003; Schaffer et al., 2010). However, there is growing evidence of distinct subtypes of SCLC (Rudin et al., 2019), which are characterized by differential expression of neuroendocrine transcription factors and *MYC* family members (the SCLC-A, *MYCL*-driven subtype being the majority) and which display distinct metabolic dependencies, including amino acid metabolism (Chalishazar et al., 2019; Huang et al., 2018). Given the fact that the overwhelming majority of human SCLC exhibits inactivation of p53 and RB (over 90%), and a smaller percentage exhibits mutation in *RBL2* (5-7%) (George et al., 2015), the Park lab previously utilized GEMMs to determine the role of *Mycl* in SCLC and observed that expression of this transcription factor is sufficient to induce

malignant transformation of precancerous cells deficient of *Rb1*, *Trp53*, and *Rbl2* (Kim et al., 2016). Additionally, the Park lab found that while expression of *Mycl* increased the synthesis of pre-rRNAs and increased protein translation, targeting RNA Pol-I in the *Rb1*/*Trp53*-mutant GEMM reduced pre-rRNA synthesis and suppressed tumor growth. These results indicated that *Mycl* is a potent oncogene for SCLC and that ribosome biogenesis and protein translation may be targeted to inhibit the growth of SCLC.

However, because *MYC* family members are currently not feasible drug targets (Carabet et al., 2019; Clausen et al., 2010; Yu et al., 2016), we sought to identify genes expressed downstream of *Mycl* that could be targeted instead. Therefore, gene expression was compared between non-transformed preSC and *Mycl*-transformed preSC; *BCAT1*, which executes the reversible transamination of BCAAs and has been implicated in the growth of some cancers, was identified as one of the most upregulated genes following transformation (Kim et al., 2016). This dissertation first examined whether the levels of BCAT1 protein are increased in SCLC compared to precancerous cells. Most importantly, its overexpression prompted us to ask whether BCAT1 is important for the growth of SCLC. Next, the reversible nature of BCAA transamination raised the question of whether the expression of BCAT1 promotes the genesis of BCAAs or induces their catabolism. Furthermore, leucine potently increases the activity of mTORC1 (Gran & Cameron-Smith, 2011) and, in certain cancers, this pathway is enhanced by the expression of BCAT1 (Gu et al., 2019; Hattori et al., 2017; Wang et al., 2018; Zhang & Han, 2017); however, in other cancers, BCAT1 enhances autophagy (Zheng et al., 2016), which is inhibited by mTORC1 (Kim et al., 2011). The reversible

nature of the transamination, and this discrepancy between cancers, led us to ask whether the expression of BCAT1 in SCLC promotes the activity of mTORC1 or inhibits it, and whether this affects autophagy. Also, because a-ketoglutarate and BCKAs can feed into the TCA cycle (Harper et al., 1984), and because mTORC1 can promote mitochondrial biogenesis (Zhang & Han, 2017), we asked whether BCAT1 expression affects mitochondrial respiration. Finally, because the BCAAs are direct substrates for protein translation and because mTORC1 usually enhances protein translation (Duan et al., 2015; Showkat et al., 2014), we asked whether expression of BCAT1 affects protein translation in SCLC. We hypothesized that expression of BCAT1 supports the growth of SCLC; therefore, the primary aim of this thesis was to elucidate the relationship between BCAT1 expression, the growth of cells and tumors, BCAA catabolism, and the activity of downstream pathways, such as mTORC1, protein translation, autophagy, and mitochondrial respiration, to reveal how BCAT1 affects the tumorigenesis of SCLC (Figure 1.3).

In Chapter 2, we demonstrate BCAT1 is important for the growth of SCLC. Specifically, we show BCAT1 protein is overexpressed in mouse SCLC cells relative to preSC and that BCAT1 is expressed in a subset of human SCLC cell lines. We also show that genetic or pharmacological targeting of BCAT1 inhibits the growth of SCLC colonies in soft agar. Furthermore, while inhibition of BCAT1 does not significantly decrease the growth of SCLC tumors in subcutaneous allograft models, deletion of BCAT1 inhibits the growth of tumors in an autochthonous GEMM. In addition, we show that loss of BCAT1 inhibits leucine catabolism and decreases the intracellular

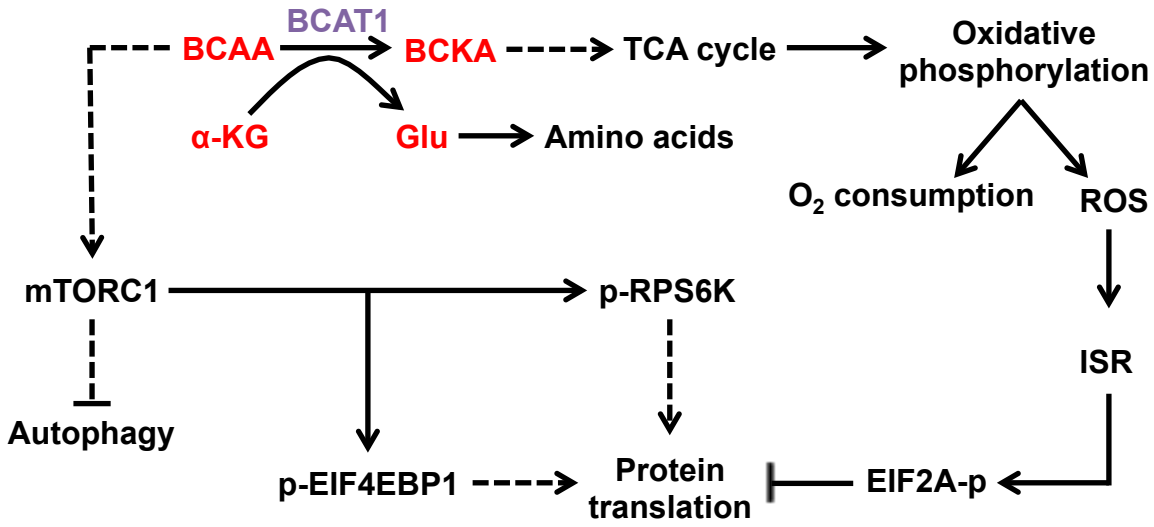

BCAT1
BCAA
BCKA
TCA cycle
Oxidative phosphorylation
α-KG
Glu
Amino acids
O2 consumption
ROS
mTORC1
p-RPS6K
ISR
Autophagy
p-EIF4EBP1
Protein translation
EIF2A-p

Figure 1.3 Diagram of pathways potentially altered by expression of branched chain aminotransferase 1 (BCAT1) in SCLC.

BCAT1 generates a branched chain keto-acid (BCKA) and glutamate (Glu) by transferring the amine group from an essential branched chain amino acid (BCAA) to α-ketoglutarate (α-KG). Through glutamate, BCAAs can fuel the synthesis of non-essential amino acids, such as glutamine and aspartate. The BCKA can be further oxidized to feed into the tricarboxylic acid (TCA) cycle, which facilitates oxidative phosphorylation. Oxidative phosphorylation consumes atmospheric oxygen (O_2) and can induce the integrated stress response (ISR) by generating reactive oxygen species (ROS). The ISR results in phosphorylation of EIF2A, which inhibits the initiation of protein translation. BCAAs, especially leucine, activate mTORC1, which can indirectly promote protein translation by activating RPS6K and inhibiting EIF4EBP1. Protein translation must be tightly controlled to avoid activation of the ISR. Activation of mTORC1 can inhibit autophagy, which relieves stress at normal levels, but can induce cell death if prolonged. Red: direct metabolites of BCAT1; solid lines: direct relationships; dashed lines: indirect relationships.

concentrations of certain non-essential amino acids. Unexpectedly, we find that loss of BCAT1 promotes mTORC1 signaling, but is associated with reduced protein translation. Finally, we show loss of BCAT1 does not significantly affect autophagy, mitochondrial respiration, or the levels of reactive oxygen species. These observations raise important questions about the mechanisms underlying the observed effects on protein translation and tumor growth as well as the therapeutic potential of targeting BCAT1 in SCLC, which we explore further in Chapter 3.

Chapter 2: Characterization of branched chain aminotransferase 1 in small cell lung cancer

2.1 Introduction

Altered metabolism of cancer cells was observed over ninety years ago when Warburg and Cori observed that cancer cells exhibited increased rates of glycolysis, which is the metabolic pathway by which cells generate energy from glucose (Warburg O, Posener K, Negelein E, 1924; Carl Cori & Cori, 1925; Warburg et al., 1927; Warburg, 1956). This was a surprising finding, given that mitochondrial respiration is much more efficient at producing ATP. Glycolysis only produces two ATPs, whereas the tricarboxylic acid (TCA) cycle and oxidative phosphorylation produces up to thirty-six. We now realize that in addition to ATP, glycolysis generates metabolic intermediates, such as acetyl-CoA, that facilitate rapid cell division (Boros et al., 1997; Chesney et al., 1999; Lee et al., 1995). Thus, increased uptake of glucose is critical to cancer cell proliferation (Chan et al., 2011). However, there is a growing appreciation of a wide variety of metabolic processes that contribute to the biosynthesis of macromolecules.

Cancer cells require increased levels of amino acids to sustain nucleotide biosynthesis for DNA and RNA synthesis (Cano-Crespo et al., 2019; Kondo et al., 2000; Sigoillot et al., 2003). For example, de novo synthesis of purine and pyrimidine nucleotides is dependent on non-essential amino acids, such as glutamine and

aspartate (Huang et al., 2018; Jones, 1980; Labuschagne et al., 2014; Pedley & Benkovic, 2017). Amino acids also help to fuel the synthesis of proteins, which are important components of the cell membrane, which relay signals from the outside environment to the nucleus, and which catalyze various reactions within the cell . Oxidation of amino acids can also support the biogenesis of lipids, which are also major components of the cell membrane and participate in signaling (Green et al., 2016; Halama et al., 2015; Mondal et al., 2009; Singer & Nicolson, 1972). For these reasons, cancer cell metabolism is an attractive target to treat cancers that do not respond well to standard therapy, or for which standard therapy does not produce durable responses, such as small cell lung cancer (SCLC).

Lung cancer is the second most common type of tumor in men and women, behind prostate and breast cancers, respectively (Siegel et al., 2019). Lung cancer is divided into subtypes such as non-small cell lung cancer (NSCLC), lung carcinoid cancer, and SCLC (Travis et al., 2015). SCLC is a high-grade neuroendocrine tumor that develops from mutant neuroendocrine cells in the lung epithelium, and is one of three categories of pulmonary neuroendocrine tumors (Park et al., 2011; Travis et al., 2015). In the United States, SCLC represents 15% of lung cancer and is strikingly more aggressive than other lung cancers; it is highly proliferative and the majority of patients (70%) present with metastatic disease (Farago & Keane, 2018). This contributes significantly to the dismal outcome for patients, as evidenced by the 5-year survival rates for localized and distant disease of 27% and 3%, respectively (Howlader et al., 2020).

Despite the limited treatment options and poor clinical outcomes, the treatment strategy for SCLC has remained stagnant for decades, contributing to the National Cancer Institute designation of recalcitrant cancer (Farago & Keane, 2018). Clearly, treatment options need to be expanded. Importantly, treatment options have begun to expand with the recent advent of immunotherapy. However, first-line immunotherapy only increases the median survival by 2-3 months (Horn et al., 2018; Paz-Ares et al., 2019). Thus, a deeper understanding of the underlying genetic factors that contribute to SCLC initiation and progression will facilitate the development of more effective targeted therapies for this disease.

While few studies have explored the potential for targeting tumor metabolism in SCLC, SCLC subtypes show selective dependencies on different metabolic pathways associated with amino acid metabolism and nucleotide biosynthesis (Chalishazar et al., 2019; Huang et al., 2018). Additionally, our group found that SCLC driven by MYCL is dependent on ribosome biogenesis and protein translation (Kim et al., 2016). However, since MYC family members are not currently feasible drug targets (Carabet et al., 2019; Clausen et al., 2010; Yu et al., 2016), and drugs targeting MYCs could potentially induce undesirable side effects due to modulating the expression of many genes simultaneously, we sought to identify and test the role of individual genes downstream of MYCL that might be targeted to attenuate its oncogenic effects. In essence, we sought to identify transcriptional targets of MYCL that contribute to the growth of SCLC. We isolated pre-cancerous cells of SCLC (preSC) and ectopically expressed *Mycl* to

induce malignant transformation (Kim et al., 2016). Then, we compared gene expression between the parental cells and the cells ectopically expressing *Mycl* and found that the expression of branched chain aminotransferase 1 (*Bcat1*) was one of the most highly upregulated in the transformed cells. BCAT1 catalyzes the reversible transamination of branched chain amino acids (BCAAs), transferring the amine group from BCAAs to α-ketoglutarate to generate glutamate and a branched chain α-keto acid (BCKA) (Hall et al., 1993). Recently, the BCAA pathway has gained attention as an important mediator of tumorigenesis (Ananieva & Wilkinson, 2018; Selwan & Edinger, 2017); indeed BCAT1 was shown to enhance the growth of some tumors, such as IDH1-wildtype glioma, ovarian cancer, endometrial cancer and NSCLC (Mayers et al., 2016; Tönjes et al., 2013; Wang et al., 2018; Wang et al., 2015). BCAT1 has been shown to impact many cellular processes in a variety of cancers, such as the synthesis of non-essential amino acids, the synthesis of nucleotides, the synthesis of proteins, and mitochondrial biogenesis (Mayers et al., 2016; Zhang & Han, 2017). By enhancing these processes, BCAT1 promotes progression through the cell cycle, and increases the proliferation, migration, and invasion of cancer cells (Thewes et al., 2017; Wang et al., 2018; Wang et al., 2015; Zhou et al., 2013). BCAT1 may also be utilized as a prognostic biomarker for cancers, such as glioma, hepatocellular carcinoma and gastric cancer (Chang et al., 2016; Cho et al., 2017; Xu et al., 2018).

The mechanistic target of rapamycin complex 1 (mTORC1) is a serine/threonine kinase that promotes cell growth, especially through phosphorylation of RPS6K and EIF4EBP1, resulting in increased protein translation initiation and elongation (Brunn et

al., 1997; Showkat et al., 2014). The activity of mTORC1 is stimulated by growth factors and amino acids like leucine (Bar-Peled & Sabatini, 2014; Dibble & Cantley, 2015). Although some reports suggest that BCAT1 catabolizes BCAAs to BCKAs in many cancer models (Mayers et al., 2016; Nishitani et al., 2013; Suh et al., 2019; Tönjes et al., 2013; Zheng et al., 2016), which can inhibit mTORC1, recent reports suggest that BCAT1 generates BCAAs from BCKAs in few cancers like endometrial cancer and leukemia, effectively enhancing the activity of mTORC1 (Gu et al., 2019; Wang et al., 2018; Zhang & Han, 2017). Thus, the ability of BCAT1 to either catabolize or generate BCAAs in different settings means BCAT1 can exert different effects on mTORC1 in different cancers, even though BCAT1 promotes the growth of cancer cells in general. Multiple components of the PI3K/AKT/mTORC1 pathway are mutated or amplified in SCLC, such that over 35% of patients harbor alterations, which suggests that the mTORC1 pathway is important for the growth of SCLC (Umemura et al., 2014). The high expression of BCAT1 following *Mycl*-induced malignant transformation of preSC, and the frequent alteration of the mTORC1 pathway in SCLC raised important questions about the role of BCAT1 in SCLC, which has not been investigated thus far.

Here we set out to 1) determine the expression of BCAT1 protein in mouse and human SCLC cell lines, 2) determine whether BCAT1 promotes the malignant transformation of preSC and/or enhances cancer cell growth, 3) determine the direction that the BCAT1-mediated transamination favors, and 4) determine how the expression of BCAT1 influences downstream pathways such as mTORC1, protein translation, autophagy, and mitochondrial respiration. We found that BCAT1 protein was highly

expressed in mouse SCLC cells relative to preSC and that BCAT1 was also expressed in human SCLC cell lines. Additionally, targeting BCAT1 genetically or pharmacologically resulted in the reduced growth of SCLC colonies in soft agar. While inhibition of BCAT1 did not consistently reduce the growth of subcutaneous allografts, it reduced the growth of SCLC tumors in an autochthonous GEMM. We also found that inhibition of BCAT1 attenuated the transamination of leucine, decreased the intracellular levels of certain non-essential amino acids, and enhanced the activity of mTORC1. Interestingly, we also found that knockdown of BCAT1 was associated with decreased protein translation. These findings suggest that BCAT1 alters the metabolism of SCLC toward the catabolism of BCAAs, which might increase the intracellular pools of glutamate, glutamine, and aspartate, and which might also enhance protein translation. These findings support the concept that targeting BCAT1 may be an effective approach for reducing the growth of SCLC.

2.2 Results

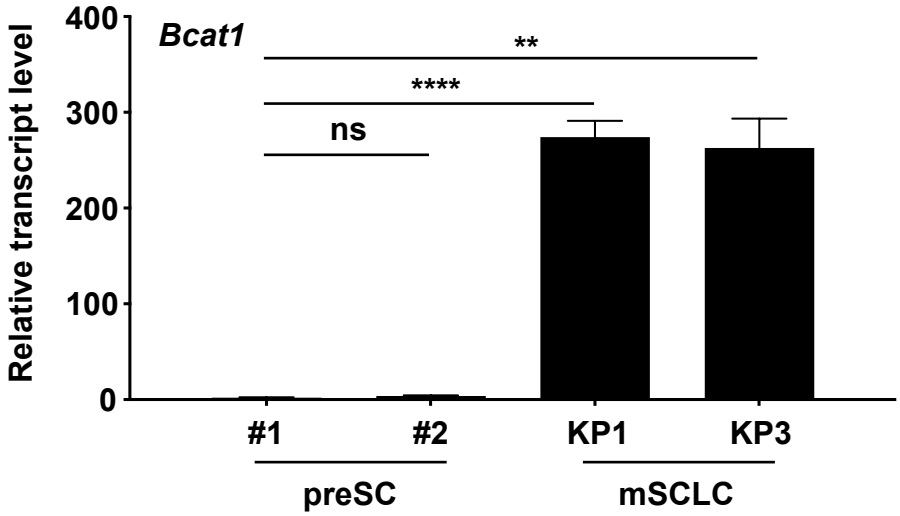
2.2.1 BCAT1 is highly expressed in murine SCLC cells and is also detectable in human SCLC cell lines

High expression of BCAT1 has been observed in multiple cancer types, such as glioma, breast cancer, ovarian cancer, endometrial cancer, NSCLC, hepatocellular carcinoma, and others (Mayers et al., 2016; Tönjes et al., 2013; Wang et al., 2018;

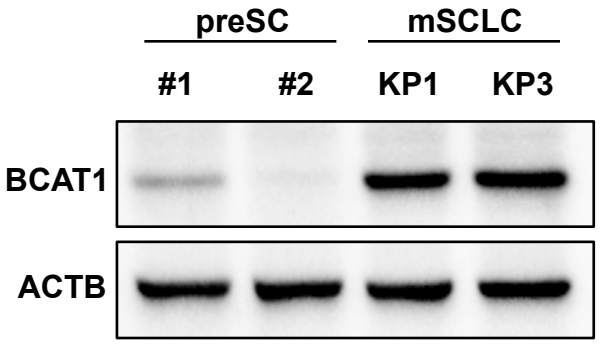
Zheng et al., 2016). Expression of *BCAT1* has also been proposed as a prognostic biomarker for some cancers, such as glioma, hepatocellular carcinoma and gastric cancer (Chang et al., 2016; Cho et al., 2017; Xu et al., 2018). Our preliminary data from microarray analysis indicated that the levels of *Bcat1* mRNA were increased in precancerous cells of SCLC (preSC) when they were transformed with the powerful oncogenic transcription factor MYCL (Kim et al., 2016), which suggested that *Bcat1* mRNA would be elevated in SCLC cells compared to preSC cells. To test this, we isolated mRNA from preSC and mouse SCLC cells, which were separately isolated from lungs of *Rb1*/*Trp53*-mutant mice (Park et al., 2011), and performed RT-qPCR for *Bcat1*. We found higher *Bcat1* transcript levels in the SCLC cells relative to the preSC cells (Figure 2.1A). Therefore, we hypothesized that the protein level of BCAT1 would also be elevated in SCLC cells compared to preSC cells. To investigate this, preSC and mouse SCLC cells that were separately isolated from lungs of *Rb1*/*Trp53*-mutant mice (Park et al., 2011) were cultured in complete medium (RPMI with 10% serum and 1X Pen/Strep/Glutamine) prior to lysis. Immunoblots on whole cell lysates showed that BCAT1 was elevated in extracts of SCLC cells compared to extracts of preSC cells (Figure 2.1B), suggesting that the protein level of BCAT1 increased during the progression of SCLC in the *Rb1*/*Trp53*-mutant GEMM.

As the levels of BCAT1 were increased in mouse SCLC, we hypothesized that BCAT1 would also be present in human SCLC. To this end, following culture in full medium, we analyzed whole-cell extracts of several human SCLC cell lines by immunoblot and detected BCAT1 in 100% of the extracts. Thus, human SCLC

A


Bcat1
Relative transcript level
400
300
200
100
0
**
****
ns
#1
#2
KP1
KP3
preSC
mSCLC

B


preSC
mSCLC
#1
#2
KP1
KP3
BCAT1
ACTB

C

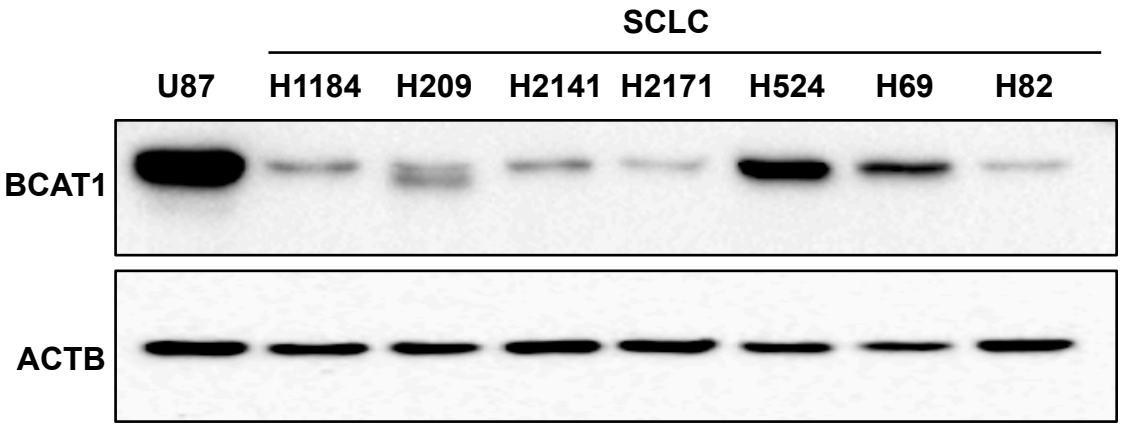

SCLC
U87
H1184
H209
H2141
H2171
H524
H69
H82
BCAT1
ACTB

Figure 2.1. Expression of BCAT1 was increased in SCLC. (Figure 2.1A was contributed by Kim DW)

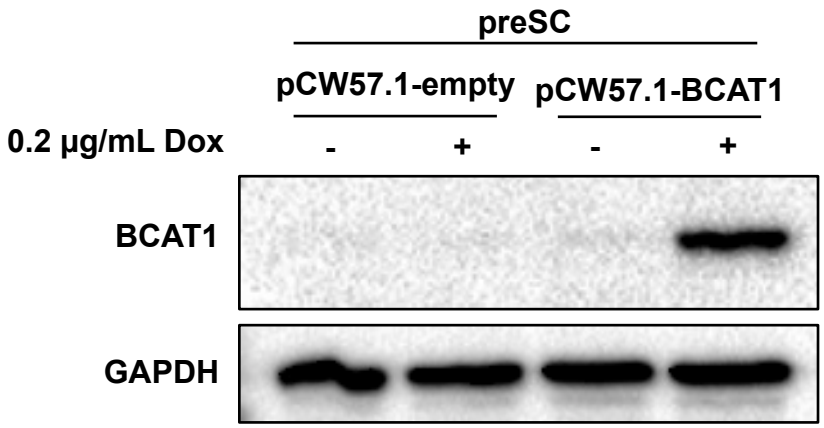
(A) RT-qPCR data showing relative expression of *Bcat1* transcript in preSC cells (left two columns) and mouse SCLC (mSCLC) cells (right two columns). n=3 replicates per cell type. Representative result from one of three independent experiments. Data are mean expression ± standard error of mean and expressed relative to preSC #1. ns: not significant, $p>0.05$; **: $p<0.01$; ****: $p<0.0001$, two-tailed Student's t-test. (B) Immunoblot for BCAT1 protein in preSC cells (lanes one and two) and mSCLC cells (lanes three and four). ACTB (actin beta) blot verified equal loading of total protein. Immunoblot representative of one experiment. (C) Immunoblot for BCAT1 protein in human glioma (lane one) and SCLC cells (lanes two through eight). U87: glioma positive control for BCAT1 protein expression. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

expressed BCAT1, although the degree of expression varied considerably (Figure 2.1C).

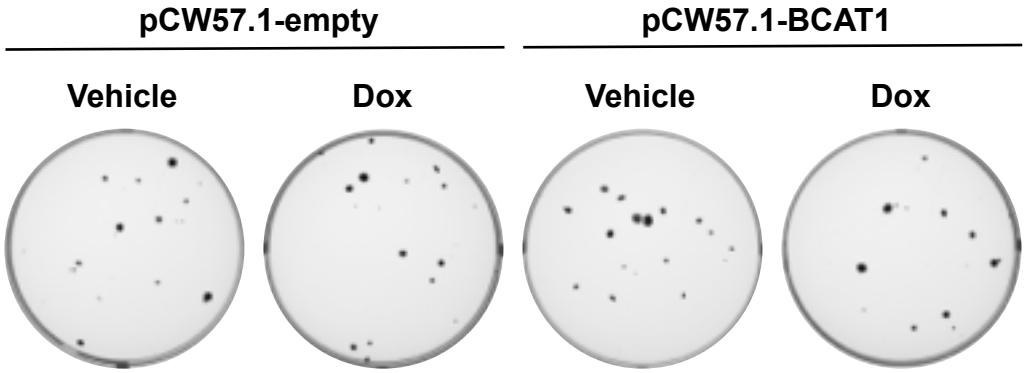
2.2.2 Ectopic expression of BCAT1 is not sufficient to induce the transformation of precancerous cells *in vitro*

Given the fact that the level of BCAT1 was increased in mouse SCLC compared to preSC, and that BCAT1 is a transcriptional target of the oncogenic MYC-family members (Zheng et al., 2016; Zhou et al., 2013), we hypothesized that BCAT1 promoted the cancerous transformation of precancerous cells. PreSC cells are adherent and usually die if separated from the substratum, but murine SCLC cells are non-adherent and are cultured in suspension (Kim et al., 2016). A test for cancerous transformation of adherent cells is anchorage-independent growth in soft agar (Yang et al., 1998), which is a semi-solid that immobilizes single cells above the bottom of the plate. To determine whether BCAT1 could induce anchorage-independent growth in preSC cells, control cells (empty vector) and cells in which BCAT1 was conditionally expressed by treatment with doxycycline (dox) (Figure 2.2A) were cultured in soft agar overlayed with complete medium for two months (Figure 2.2B and C). While we hypothesized that the ectopic expression of BCAT1 would enhance the colony formation of preSC cells, the number of colonies that formed when BCAT1 was expressed was not significantly different from either the vector ($p=0.94$) or the vehicle ($p=0.93$) control (Figure 2.2C). This indicated that ectopic expression of BCAT1 was not sufficient to induce the transformation of precancerous cells of SCLC.

A


preSC
pCW57.1-empty
pCW57.1-BCAT1
0.2 µg/mL Dox
-
+
-
+
BCAT1
GAPDH

B


pCW57.1-empty
pCW57.1-BCAT1
Vehicle
Dox
Vehicle
Dox

C

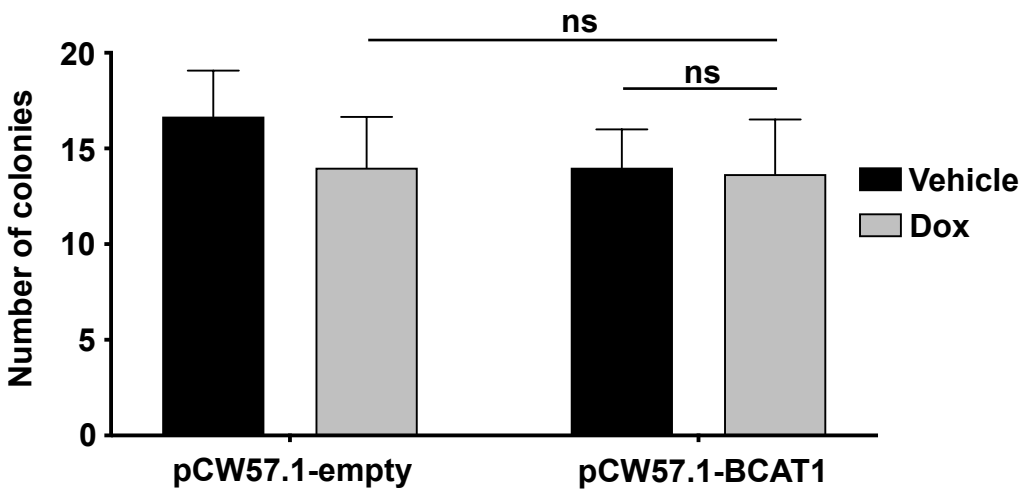

ns
ns
Number of colonies
20
15
10
5
0
Vehicle
Dox
pCW57.1-empty
pCW57.1-BCAT1

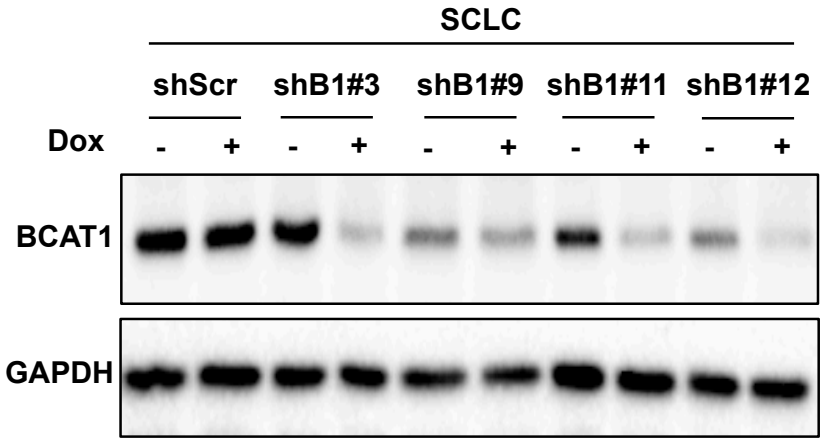
Figure 2.2. Expression of BCAT1 was not sufficient for transformation of precancerous cells.

(A) Immunoblot for BCAT1 protein in preSC cells transduced with pCW57.1-empty vector (lanes one and two) or pCW57.1-BCAT1 (lanes three and four) and treated with either the vehicle (ddH_2O) (lanes one and three) or doxycycline (dox) (lanes two and four). GAPDH blot verified equal loading of total protein. Immunoblot representative of three independent experiments. (B and C) Results of soft agar experiment with preSC cells ectopically expressing BCAT1 or not. (B) Visualization of soft agar assay after wells were stained and imaged. Representative images from one of three independent experiments. (C) Quantification of soft agar assay. After imaging, colonies ≥50 μm were counted. n=3 replicates per cell type per treatment. Representative result from one of three independent experiments. Data are mean number of colonies ± standard error of mean. ns: not significant, $p>0.05$, two-tailed Student's t-test.

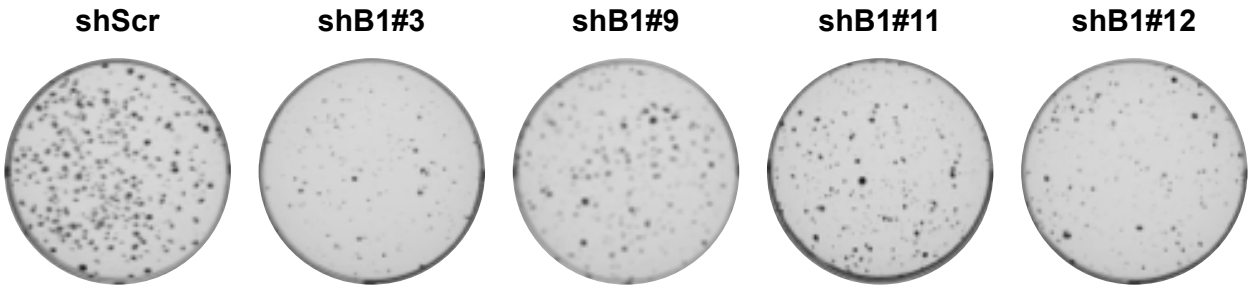
2.2.3 Knockdown of BCAT1 decreases the growth of SCLC cells *in vitro*

Elevated expression of BCAT1 had been shown to enhance the growth of several types of cancer cells *in vitro*, such as endometrial cancer, glioma, nasopharyngeal carcinoma, hepatocellular carcinoma, and others (Tönjes et al., 2013; Wang et al., 2018; Zheng et al., 2016; Zhou et al., 2013). However, the role of BCAT1 in the growth of SCLC cells had not been described. Although ectopic expression of BCAT1 did not induce the cancerous transformation of preSC, we asked the reciprocal question of whether inhibition of BCAT1 would reduce the growth of SCLC cells. To test this, we constructed twelve lentiviral vectors to express shRNAs targeting BCAT1 (shB1) and one vector to express scrambled shRNA (shScr) in mouse SCLC cells. Only four of the constructs resulted in decreased expression of BCAT1 (Figure 2.3A) and were used to assay growth over three weeks in soft agar (Figure 2.3B and C). As described before, the cells were cultured in full medium. Three of the four cells transduced with BCAT1-targeting shRNAs formed fewer colonies than the scramble control (shB1#3, shB1#11, and shB1#12), (shB1#3, $p=2.9x10^{-4}$; shB1#9, $p=0.17$; shB1#11, $p=1.4x10^{-3}$; shB1#12, $p=5.5x10^{-4}$), which was consistent with the hypothesis that BCAT1 required for the growth of SCLC cells. In contrast, cells transduced with shRNA #9 produced similar numbers of colonies to those generated by the scramble control, raising the possibility of off-target effects. It is well known that shRNAs have the potential to induce unintended effects by binding to sequences of mRNA that are similar, but not identical to, the intended target. Therefore, we pursued CRISPR-mediated gene editing as an additional genetic tool to compliment the shRNA

A


SCLC
shScr
shB1#3
shB1#9
shB1#11
shB1#12
Dox
- + - + - + - + - +
BCAT1
GAPDH

B


shScr
shB1#3
shB1#9
shB1#11
shB1#12

C

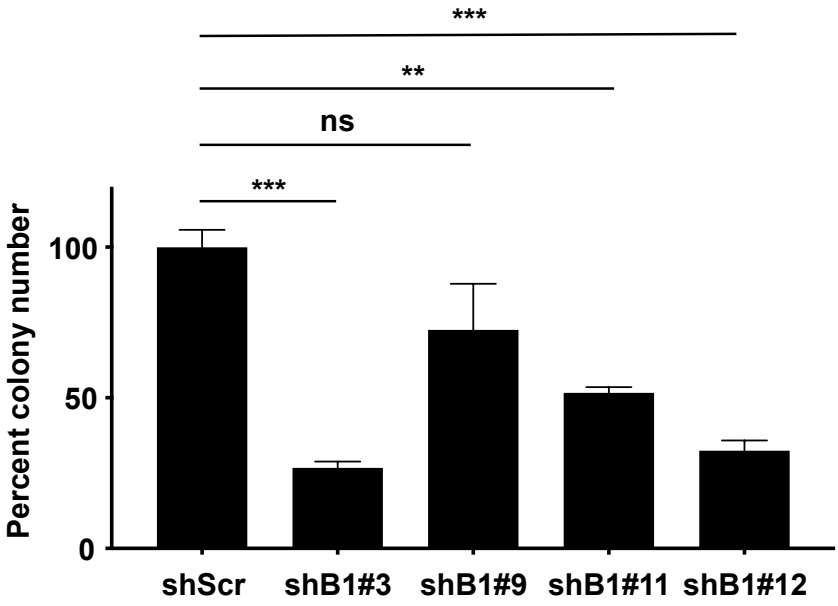

***
**
ns
***
Percent colony number
100
50
0
shScr
shB1#3
shB1#9
shB1#11
shB1#12

Figure 2.3. shRNA-mediated knockdown of BCAT1 reduced the growth of SCLC cells *in vitro*.

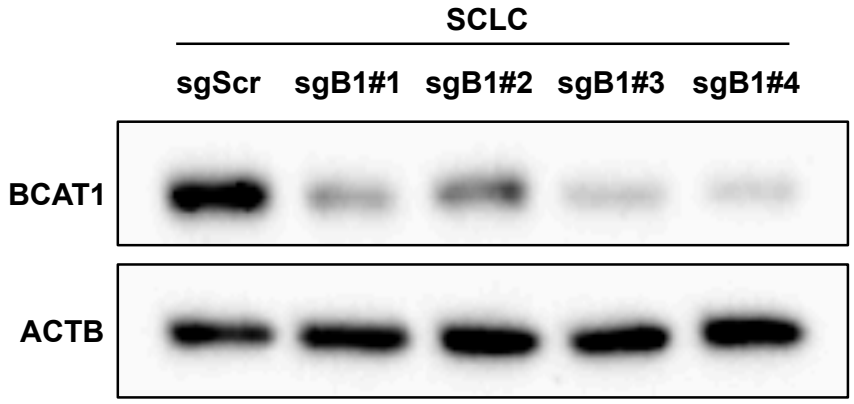
(A) Immunoblot for BCAT1 protein in KP1 cells transduced with Tet-pLKO-puro-shScr, -shB1#3, -shB1#9, -shB1#11, or -shB1#12 and treated with either the vehicle (ddH_2O) or doxycycline (dox). GAPDH blot verified equal loading of total protein. shScr: scramble shRNA control; shB1: shRNA targeting Bcat1. Immunoblot representative of two independent experiments. (B and C) Results of soft agar assay with BCAT1 knockdown cells. (B) Visualization of soft agar after wells were stained and imaged. Representative images from one of two independent experiments. (C) Quantification of soft agar. After imaging, colonies ≥100 μm were counted. n=3 replicates per cell type. Representative result from one of two independent experiments. Data are mean number of colonies ± standard error of mean relative to shScr. ns: not significant, $p>0.05$; **: $p<0.01$; ***: $p<0.001$, two-tailed Student's t-test.

experiment. We generated four lentiviral constructs encoding Cas9 and single guide RNA (sgRNA) against BCAT1 (sgB1), as well as a lentiviral vector encoding a scramble sequence (sgScr) and Cas9. We then transduced mouse SCLC cells and tested colony formation in soft agar. As expected, BCAT1 protein expression was reduced relative to the control cells (Figure 2.4A). We observed dramatically decreased colony growth in three of the four targeting cells compared to control (sgB1#1, $p=3.3x10^{-4}$; sgB1#2, $p=0.097$; sgB1#3, $p=1.4x10^{-4}$; sgB1#4, $p=6.5x10^{-4}$) (Figure 2.4B and C). Notably, sgB1#2 cells had the weakest knockdown of BCAT1 and also the least inhibition of colony formation (Figure 2.4A and B). The reduced growth of SCLC colonies following shRNA- and CRISPR-mediated inhibition of expression of BCAT1 indicated that BCAT1 is required for the growth of mouse SCLC cells *in vitro*.

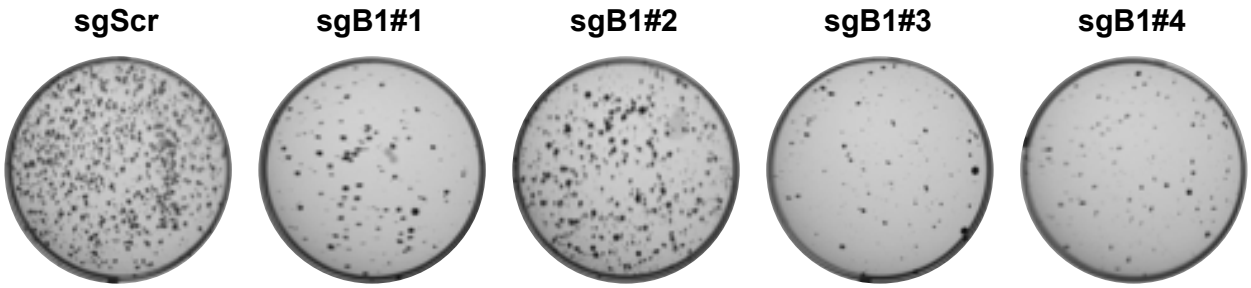
2.2.4 Cells with high expression of BCAT1 undergo strong selection to grow in subcutaneous allografts

Abnormally high expression of BCAT1 had been shown to increase the growth of multiple types of tumors *in vivo*, such as glioma, hepatocellular carcinoma, breast cancer, and others (Tönjes et al., 2013; Zhang & Han, 2017; Zheng et al., 2016). To further explore the role of BCAT1 in SCLC tumor growth, we asked whether the decreased growth observed in soft agar under conditions of BCAT1 knockdown is recapitulated *in vivo*. Due to the reduced growth of SCLC cells observed following inhibition of BCAT1 *in vitro*, we hypothesized that inhibition of BCAT1 would decrease the growth of SCLC tumors *in vivo* as well. We initially tested our hypothesis by

A


SCLC
sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4
BCAT1
ACTB

B


sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4

C

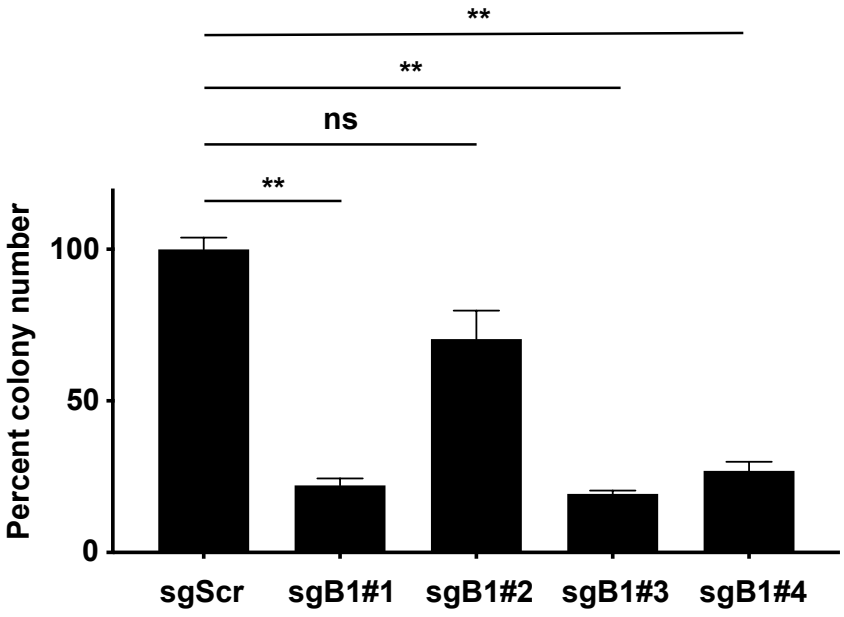

**
**
ns
**
Percent colony number
100
50
0
sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4

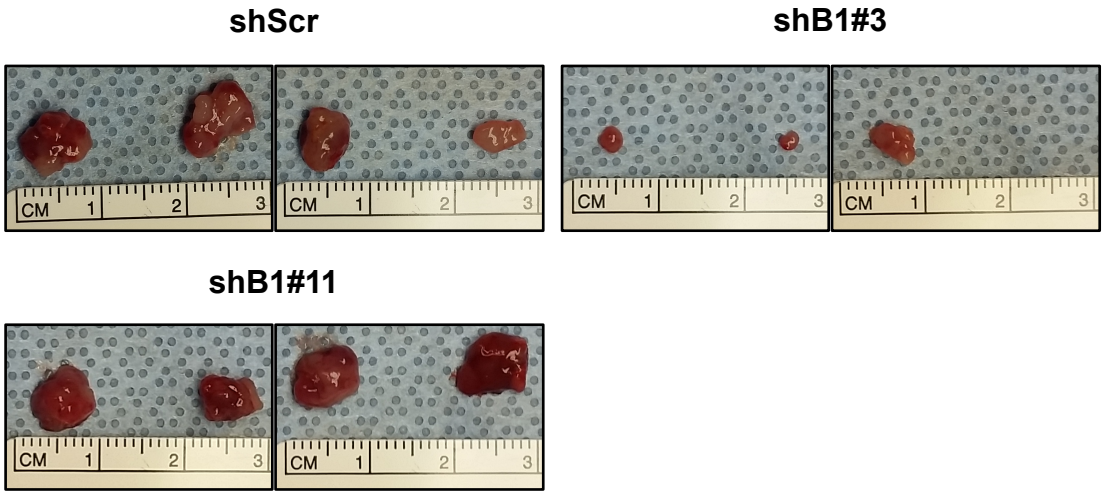
Figure 2.4. CRISPR-mediated knockdown of BCAT1 reduced the growth of SCLC cells *in vitro*.

(A) Immunoblot for BCAT1 protein in KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, sgB1#2, sgB1#3, or sgB1#4. ACTB blot verified equal loading of total protein. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting Bcat1. Immunoblot representative of three independent experiments. (B and C) Results of soft agar with BCAT1 knockdown cells. (B) Visualization of soft agar after wells were stained and imaged. Representative images from one of three independent experiments. (C) Quantification of soft agar. After imaging, colonies ≥50 μm were counted. n=2-3 replicates per cell type. Representative result from one of three independent experiments. Data are mean number of colonies ± standard error of mean relative to sgScr. ns: not significant $p>0.05$; **: $p<0.01$, two-tailed Student's t-test.

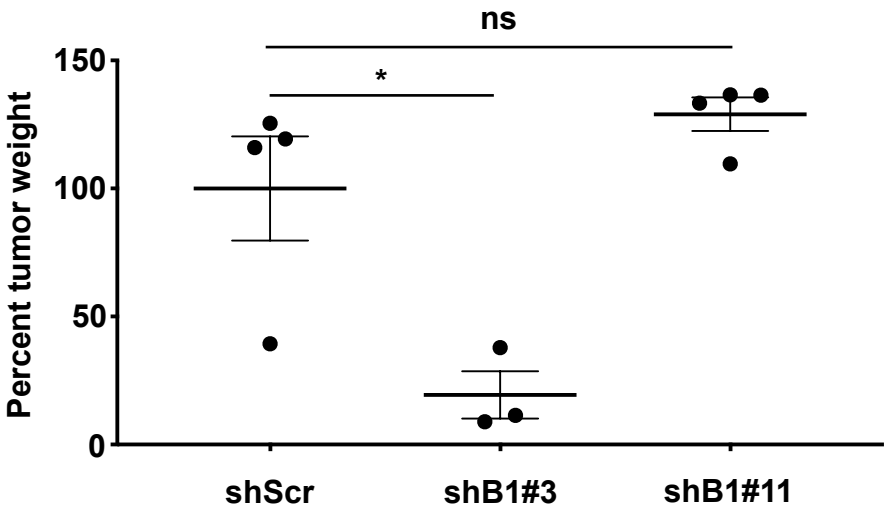
subcutaneous injection of shScr, shB1#3, and shB1#11 cells into athymic nude mice in order to bypass the significant amount of time required to develop a genetically modified knockout mouse. Mice were fed doxycycline beginning two days post-injection and euthanized one month following injection of the cells. Tumors from shB1#3 cells were smaller and weighed significantly less ($p=0.024$) than tumors from shScr cells, but tumors from sh#11 cells were similar in size to the controls (Figures 2.5A and B) ($p=0.22$). To help explain the discrepancy between the growths of the BCAT1-targeting cells, we measured the residual expression of BCAT1 in the tumors. We postulated that tumors arising from shB1#11 cells might contain higher levels of BCAT1 than those arising from shB1#3 cells, which could explain the discrepancy in growth phenotypes. To determine the levels of BCAT1 in the tumors, we froze the tumors, lysed them, and analyzed the protein extracts through immunoblot (Figure 2.5C). Both shB1#3 and shB1#11 tumors contained lower levels of BCAT1 than the control tumors, and BCAT1 expression was in fact lowest in shB1#11, indicating BCAT1 expression was not responsible for the difference in phenotypes. While it is possible that the robust growth of shB1#11 *in vivo* might be due to off-target effects, we were unable to draw conclusions from the shRNA allograft experiment. However, the decreased growth of the tumors from shB1#3 cells, and the lack of growth from one of the injections, prompted us to further investigate whether BCAT1 might be important for the growth of SCLC *in vivo*.

To further investigate whether inhibition of BCAT1 reduced SCLC tumor growth, we tested the tumor-forming capacity of sgScr, sgB1#2, sgB1#3, and sgB1#4 cells,

A


shScr
shB1#3
shB1#11
CM 1 2 3

B


ns
*
150
100
50
0
Percent tumor weight
shScr
shB1#3
shB1#11

C

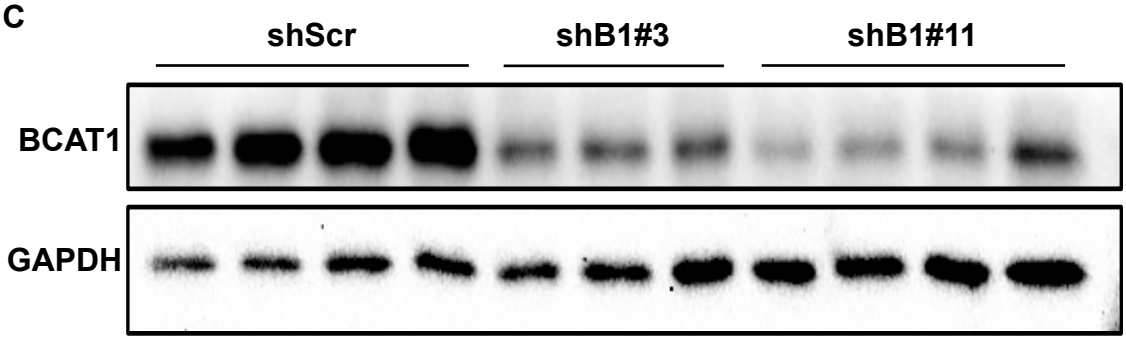

shScr
shB1#3
shB1#11
BCAT1
GAPDH

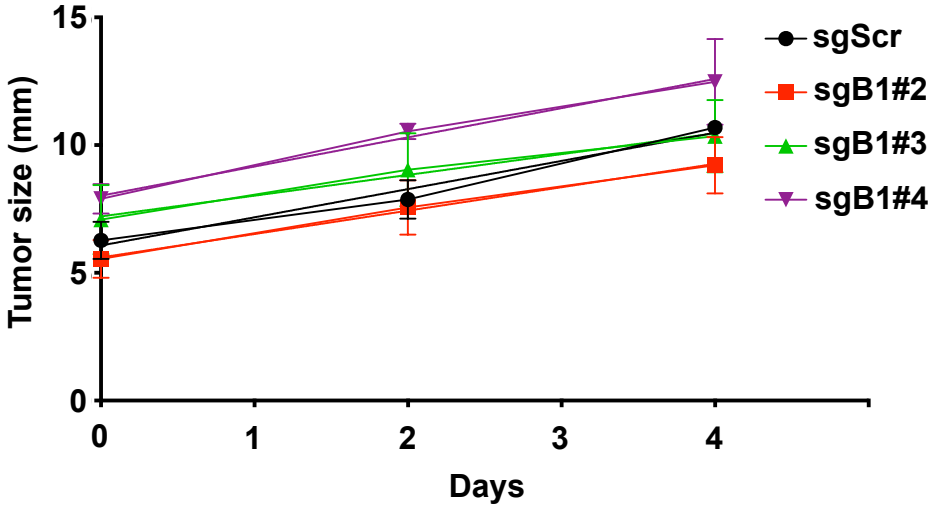
Figure 2.5. shRNA-mediated knockdown of BCAT1 did not consistently reduce the growth of allografts.

(A) Visualization of allografts following knockdown of BCAT1. $5x10^5$ KP1 cells transduced with Tet-pLKO-puro-shScr, -shB1#3, or -shB1#11 were injected into flanks of athymic nude mice, with each cell type injected into two flanks of two mice. After two days, expression of shRNA was induced with doxycycline-containing rodent chow. After one month, all mice were individually euthanized and both tumors of each mouse were excised and imaged. shScr: scrambled shRNA control; shB1: shRNA targeting BCAT1. Results are representative of two independent experiments. (B) Quantification of allograft weights. After each tumor was imaged, it was weighed and frozen on dry ice, until all were frozen. n=3-4 tumors per cell type. Data are mean mg of tumor ± standard error of mean relative to shScr. ns: not significant, $p>0.05$; *: $p<0.05$, two-tailed Student's t-test. (C) Visualization of residual BCAT1 protein in allografts. When all tumors were frozen, they were processed for protein extract and knockdown of BCAT1 protein was verified by immunoblot analysis. GAPDH blot verified equal loading of total protein. Immunoblot representative of two independent experiments.

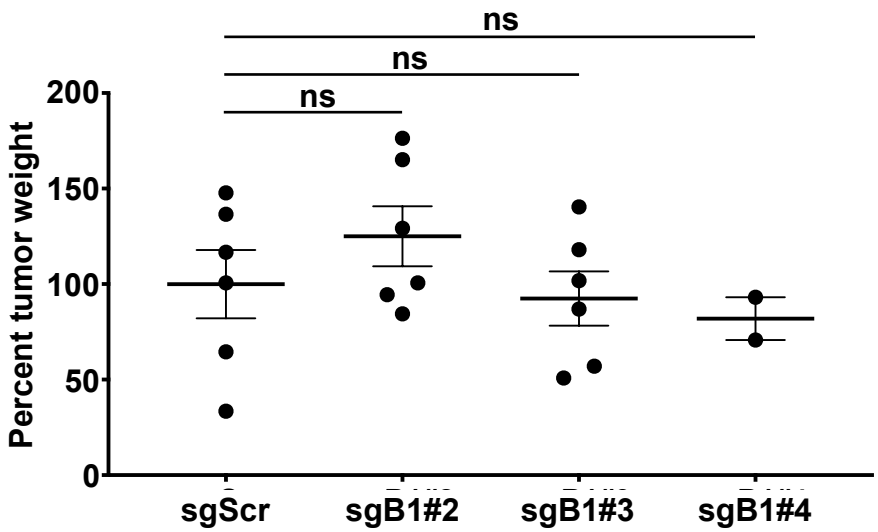
which were injected into the flanks of athymic nude mice. To determine how BCAT1 affects the growth rate of the tumors, we measured tumor sizes over time. In this experiment, the tumors were allowed to grow until any one of them reached 15 mm, at which time we euthanized all the mice and determined the final weights of the tumors. Due to the higher expression of BCAT1 in sgB1#2 cells *in vitro* (see Figure 2.4A), we anticipated that any impaired growth of tumors caused by BCAT1 knockdown would be less robust in sgB1#2 tumors compared to tumors arising from sgB1#3 and sgB1#4 cells. However, as shown in Figure 2.6A, all of the BCAT1-targeting cells produced tumors that grew at similar rates to controls. Similarly, the average weight of BCAT1-targeting tumors was not significantly different from the control (sgB1#2: $p=0.32$; sgB1#3: $p=0.75$; sgB1#4: $p=0.61$) ($n=2$ for sgB1#4 and $n=6$ for the rest) (Figure 2.6B). Interestingly, however, four of the six mice injected with sgB1#4 cells, which exhibited the best knockdown *in vitro* (see Figure 2.4C), did not form tumors at all, which suggested knockdown of BCAT1 could have inhibited the initiation of those tumors. We postulated that over time in mice, there was selective pressure for cells expressing high levels of BCAT1 to grow, which resulted in tumors with expression of BCAT1 similar to that of control tumors. To determine the levels of BCAT1 in the tumors, we froze the tumors, lysed them, and analyzed the protein extracts through immunoblot. While the expression of BCAT1 in individual tumors of each group was variable, the expression in the targeting tumors was similar to that of the control tumors (Figure 2.6C), which was consistent with the concept of strong selection for cells with more BCAT1 to grow.

The immunoblot analysis of tumor lysates helped to explain the differences

A


15
10
5
0
Tumor size (mm)
0
1
2
3
4
Days
sgScr
sgB1#2
sgB1#3
sgB1#4

B


ns
ns
ns
200
150
100
50
0
Percent tumor weight
sgScr
sgB1#2
sgB1#3
sgB1#4

C

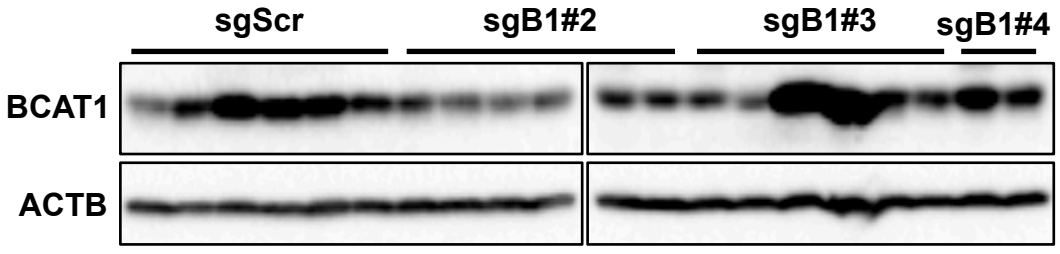
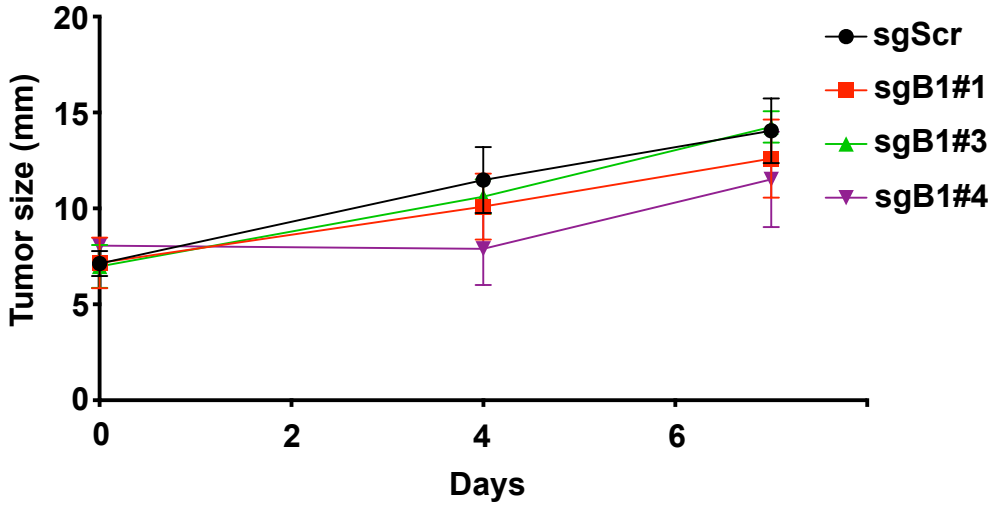

sgScr
sgB1#2
sgB1#3
sgB1#4
BCAT1
ACTB

Figure 2.6. Constitutive CRISPR-mediated knockdown of BCAT1 did not reduce the growth of allografts in immunocompromised athymic nude mice.

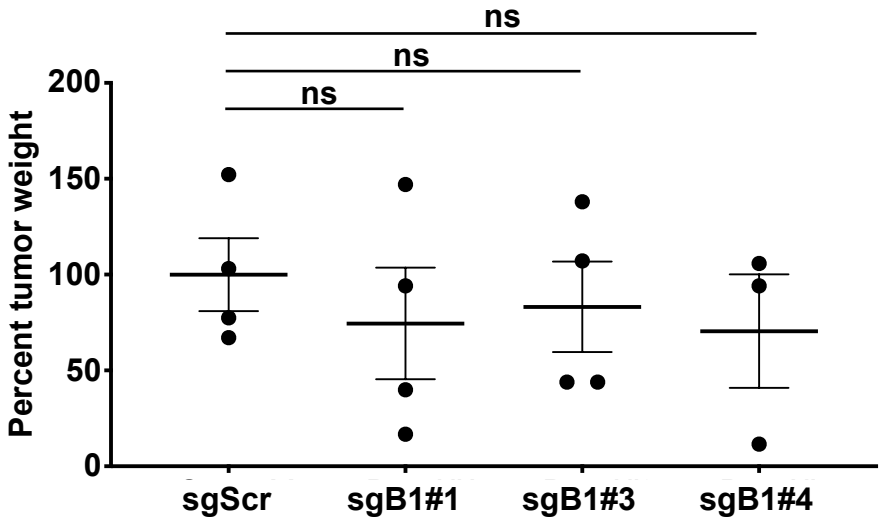
(A) Quantification of allograft lengths. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#2, -sgB1#3, or -sgB1#4 were injected into flanks of athymic nude mice. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. n=2-6 tumors per cell type. Data are mean mm of tumor ± standard error of mean. Results are representative of one independent experiment. (B) Quantification of allograft weights. When any tumor reached 15 mm in length, all mice were individually euthanized and each tumor was excised, weighed, and frozen on dry ice. n=2-6 tumors per cell type. Data are mean mg of tumor ± standard error of mean relative to sgScr. ns: not significant, $p>0.05$, two-tailed Student's t-test. (C) Visualization of residual BCAT1 protein in allografts. When all tumors were frozen, they were processed for protein extract and knockdown of BCAT1 protein was evaluated by immunoblot analysis. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

between the phenotypes observed *in vitro* and *in vivo*, and prompted us to thaw previously frozen cells in order to initiate the allograft experiment with cells exhibiting more robust knockdown of BCAT1. Additionally, we postulated that the immune system, which is compromised in athymic nude mice, could play a role in regulating the growth of SCLC. The concept that BCAA metabolism in cancer cells could modulate the immune system to promote tumor growth was evidenced by the finding that BCKAs secreted by glioma cells were taken up by macrophages, which resulted in reduced phagocytic activity (Silva et al., 2017). Therefore, we tested the growth of the freshly thawed CRISPR cells injected into immunocompetent mice. These first filial generation (F1) B6129SF1/J mice were the products of crossing C57BL/6J females (B6) and 129S1/SvImJ males (129S). Since our mouse SCLC cells were derived from mice with a 129S/B6 mixed background, the F1 hybrid mice were less likely to reject the cells, reducing the need for a compromised immune system. In this experiment, tumors were allowed to grow until any one of them reached about 20 mm. The results obtained with the freshly thawed cells were similar to the earlier experiment in nude mice: the average size of BCAT1-targeting tumors was similar to control (Figure 2.7A) and their final weights were similar to control (sgB1#1: p=0.49; sgB1#3: p=0.60; sgB1#4: p=0.42) (Figure 2.7B). However, one of the four sgB1#4 injections failed to form a tumor, which again suggested that knockdown of BCAT1 inhibited the tumor initiation. Additionally, while the residual levels of BCAT1 in protein extracts were variable, the levels in BCAT1-targeting tumors were similar to control (Figure 2.7C), which again suggested selective pressure to retain expression of BCAT1.

A


Tumor size (mm)
20
15
10
5
0
0
2
4
6
Days
sgScr
sgB1#1
sgB1#3
sgB1#4

B


ns
ns
ns
Percent tumor weight
200
150
100
50
0
sgScr
sgB1#1
sgB1#3
sgB1#4

C

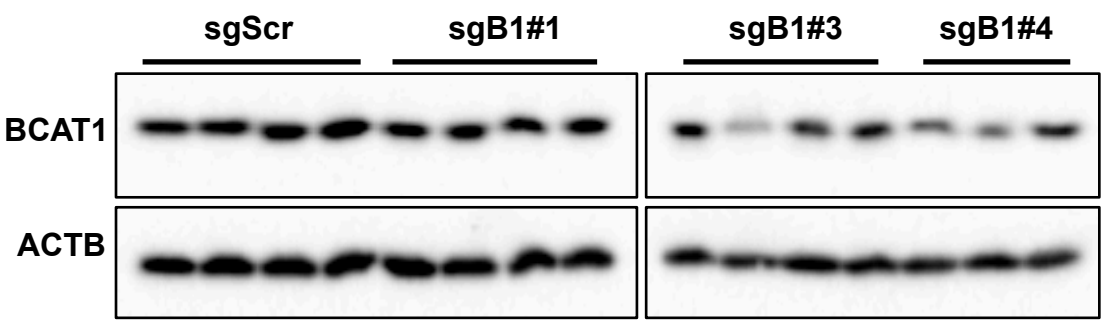

sgScr
sgB1#1
sgB1#3
sgB1#4
BCAT1
ACTB

Figure 2.7. Constitutive CRISPR-mediated knockdown of BCAT1 did not reduce the growth of allografts in immunocompetent B6129SF1/J mice.

(A) Quantification of allograft lengths. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, -sgB1#3, or -sgB1#4 were injected into flanks of immunocompetent B6129SF1/J mice. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. n=3-4 tumors per cell type. Data are mean mm of tumor ± standard error of mean. Results are representative of one experiment. (B) Quantification of allograft weights. When any tumor exceeded 18 mm in length, all mice were individually euthanized and each tumor was excised, weighed, and frozen on dry ice. n=3-4 tumors per cell type. Data are mean mg of tumor ± standard error of mean relative to sgScr. ns: not significant, $p>0.05$, two-tailed Student's t-test. (C) Visualization of residual BCAT1 protein in allografts. When all tumors were frozen, they were processed for protein extract and knockdown of BCAT1 was evaluated by immunoblot analysis. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

In an effort to avoid selection against loss of BCAT1 while growing cells in culture prior to injection into mice, we generated conditional sgScr, sgB1#3, or sgB1#4 cells, which expressed Cas9 and knocked down BCAT1 only when induced with doxycycline. After injection into the F1 mice, we induced Cas9 expression with doxycycline-treated rodent chow. In order to determine the effect of BCAT1 on tumor initiation, we measured the number of days to visually detect tumors by observation of raised masses under the skin. We measured the sizes of tumors over time to determine the effect of BCAT1 on the growth rate of established tumors, and we also measured the final weights of the tumors. Each individual tumor was allowed to grow until it reached 15 mm. While mice injected with sgB1#3 cells did not exhibit significantly increased durations of tumor-free survival ($p=0.13$, Gehan-Breslow-Wilcoxon test) compared to those injected with sgScr cells, the appearance of tumors in mice injected with sgB1#4 cells was significantly delayed ($p=0.022$, Gehan-Breslow-Wilcoxon test) (Figure 2.8A). However, the average sizes of the tumors were similar to control (Figure 2.8B), as were the average weights (sgB1#3: $p=0.40$; sgB1#4: $p=0.61$) (Figure 2.8C). This suggested that inhibition of BCAT1 might have disrupted the initiation of SCLC tumors, at least for the sgB1#4 cells, but not their ongoing growth. However, an increase in tumor latency without a decrease in final mass led us to postulate that there were some cells that retained more expression of BCAT1 and eventually grew rapidly to form the tumors. To determine whether there was selective pressure for SCLC cells with high expression of BCAT1 to grow, we used immunoblot (Figure 2.8D) to assess the levels of BCAT1 in parental cells, which were induced with doxycycline *in vitro*, and compared them to the levels of BCAT1 in cancer cells cultured from the final tumors. In

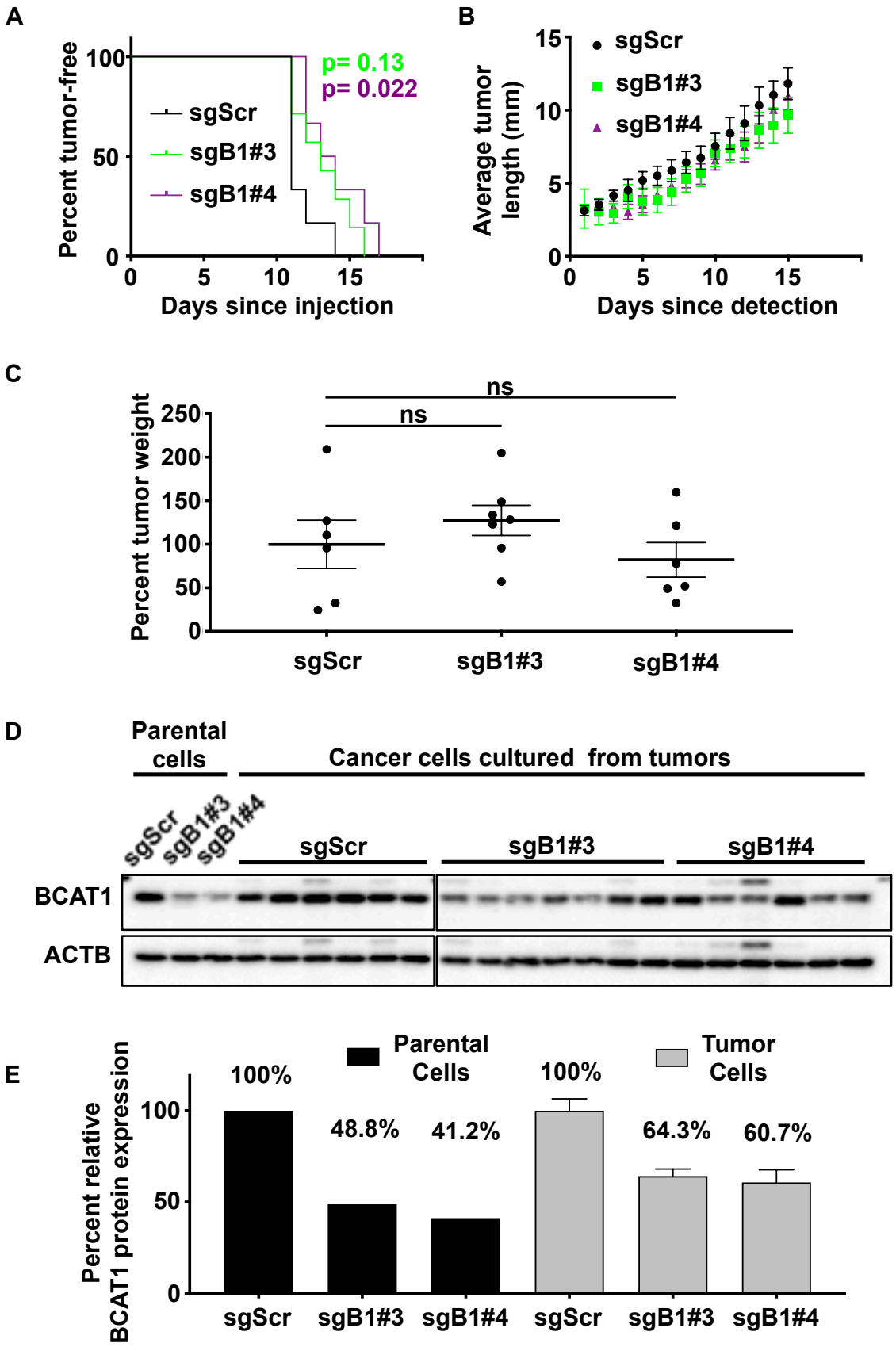

A
Percent tumor-free
100
50
0
0
5
10
15
Days since injection
sgScr
sgB1#3
sgB1#4
p= 0.13
p= 0.022
B
Average tumor length (mm)
15
10
5
0
0
5
10
15
Days since detection
sgScr
sgB1#3
sgB1#4
C
Percent tumor weight
250
200
150
100
50
0
ns
ns
sgScr
sgB1#3
sgB1#4
D
Parental cells
Cancer cells cultured from tumors
sgScr
sgB1#3
sgB1#4
sgScr
sgB1#3
sgB1#4
BCAT1
ACTB
E
Percent relative BCAT1 protein expression
100
50
0
Parental Cells
Tumor Cells
100%
48.8%
41.2%
100%
64.3%
60.7%
sgScr
sgB1#3
sgB1#4
sgScr
sgB1#3
sgB1#4

Figure 2.8. Doxycycline-inducible knockdown of BCAT1 did not reduce the growth of allografts in immunocompetent B6129SF1/J mice.

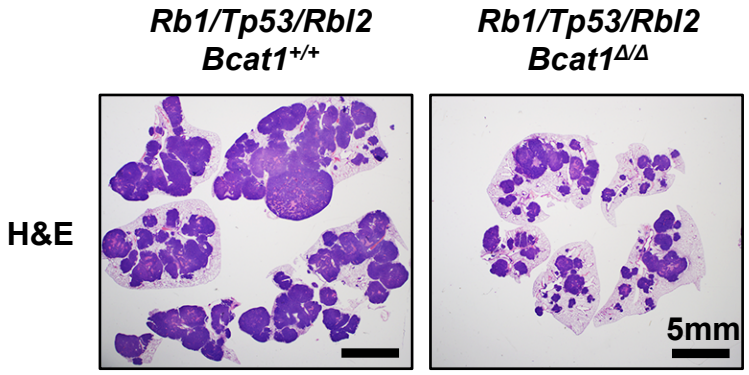
(A) Quantification of tumor-free survival. KP1-Cas9 cells transduced with LV-gRNA-zeocin-sgScr, -sgB1#3, or -sgB1#4 were injected into flanks of B6129SF1/J mice. Expression of Cas9 was induced with doxycycline (dox)-containing rodent chow. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. n=6-7 mice per cell type. Data are percentage of mice, per cell type, remaining without visible tumors since date of injection. sgB1#3: p=0.13; sgB1#4: p=0.022, Gehan-Breslow-Wilcoxon test. Results are representative of one experiment. (B) Quantification of allograft lengths. Upon visual detection, tumor lengths were measured over time. n=6-7 tumors per cell type. Data are mean mm of tumor ± standard error of mean. (C) Quantification of allograft weights. When each tumor reached 15 mm, the mice were euthanized and the tumors were excised, weighed and frozen on dry ice. n=6-7 tumors per cell type. Data are mean mg of tumor ± standard error of mean relative to sgScr. ns: not significant, $p>0.05$, two-tailed Student's t-test. (D) Visualization of knockdown of BCAT1 before and after allograft. A subset of parental cells was treated with dox for forty-eight hours and processed for protein extract. After weighing allografts, half of each tumor was used to culture cancer cells, which were also processed for protein extract and knockdown of BCAT1 protein was compared through immunoblot analysis. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment. (E) Quantification of knockdown of BCAT1 before and after allograft. ImageJ was used for densitometry analysis of (E). n=1 for parental cells and n=6-7 for tumor cancer cells. Data are the mean level of BCAT1 protein normalized to ACTB ± standard error of mean relative to sgScr.

comparison to their parental cells, we observed reduced knockdown efficiency in the majority of cells cultured from tumors: the knockdown in sgB1#3 and sgB1#4 parental cells were 51.2% and 58.8%, respectively, while the average knockdown in sgB1#3 and sgB1#4 tumor cells dropped to 35.7% and 39.3%, respectively (Figure 2.8E). However, while this suggested there was strong pressure for cells with high levels of BCAT1 to grow, which was consistent with the concept that BCAT1 is required for the growth of SCLC *in vivo*, it did not provide direct evidence.

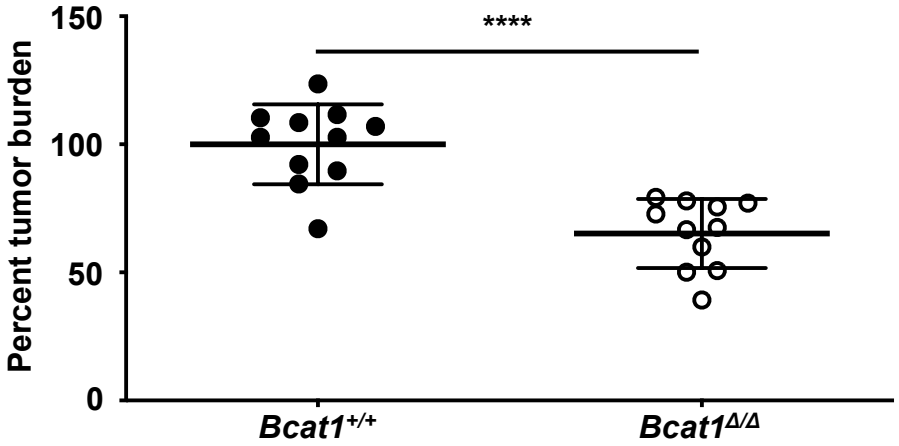
2.2.5 Deletion of BCAT1 decreases the growth of SCLC tumors in an autochthonous mouse model

The reduced knockdown efficiency in cells cultured from subcutaneous tumors suggested that there was selective pressure against cells upon loss of BCAT1, which was consistent with the concept that BCAT1 is required for the continued growth of SCLC in vivo. Additionally, the allograft experiment excludes any potential roles for the lung microenvironment interacting with SCLC tumor to regulate the growth of SCLC. Therefore, we sought a more robust autochthonous GEMM. To this end, we generated a new GEMM by crossing the *Rb1*/*Trp53*/*Rbl2*-floxed model (Schaffer et al., 2010) with a strain harboring floxed *Bcat1* alleles (Ananieva et al., 2014); importantly, this model facilitated the assessment of the function of BCAT1 without the confounding factor of time cultured *in vitro*. We induced lung tumor through intratracheal instillation of Ad-CMV-Cre virus (Dupage et al., 2009). Six months after the virus infection, we euthanized the mice, stained the lungs with hematoxylin and eosin (Figure 2.9A),

A


Rb1/Tp53/Rbl2
Bcat1+/+
Rb1/Tp53/Rbl2
Bcat1Δ/Δ
H&E
5mm

B


****
Percent tumor burden
150
100
50
0
Bcat1+/+
Bcat1Δ/Δ

C

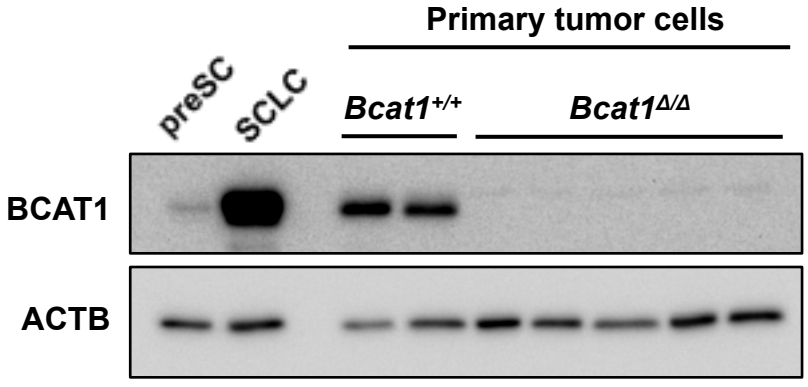

Primary tumor cells
preSC
SCLC
Bcat1+/+
Bcat1Δ/Δ
BCAT1
ACTB

Figure 2.9. Deletion of *Bcat1* reduced tumor burden in an autochthonous GEMM of SCLC. (Figure contributed by Kim, KB)

Through intratracheal instillation of Ad-CMV-Cre virus, lung tumors were induced in ten-week-old *Rb1/Tp53/Rbl2*$^{lox/lox}$ mice containing wild-type or floxed alleles of *Bcat1*. The infected mice (*Bcat1*$^{+/+}$ or *Bcat1*$^{\Delta/\Delta}$, respectively) were aged for 6 months and then euthanized. Results representative of one experiment. (A) Representative images of hematoxylin and eosin (H&E) stained lungs from *Rb1/Tp53/Rbl2*-mutant mice with or without *Bcat1* (*Bcat1*$^{+/+}$ or *Bcat1*$^{\Delta/\Delta}$, respectively). n=11 mice per genotype. (B) Quantification of tumor burden. H&E slides were analyzed and the area of tumors divided by the total area of the lung was calculated as tumor burden. n=11 mice per genotype. Data are mean percent tumor burden ± standard error of mean relative to *Bcat1*$^{+/+}$. ****: $p<0.0001$, two-tailed Student's t-test. (C) Primary cells were cultured from tumors and analyzed for expression of BCAT1 protein by immunoblot. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

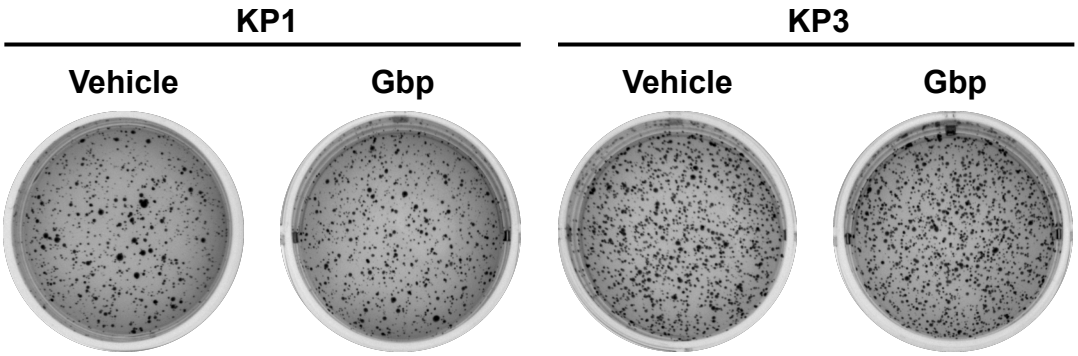
determined tumor burden by dividing the area of tumors by total lung area (Figure 2.9B), and verified knockout of BCAT1 protein in primary tumor cells by immunoblot (Figure 2.9C). We found that knockout of BCAT1 protein in *Rb1*$^{\Delta/\Delta}$*/Tp53*$^{\Delta/\Delta}$*/Rbl2*$^{\Delta/\Delta}$*/Bcat1*$^{\Delta/\Delta}$ mice resulted in significantly reduced tumor burden compared to control *Rb1*$^{\Delta/\Delta}$*/Tp53*$^{\Delta/\Delta}$*/Rbl2*$^{\Delta/\Delta}$*/Bcat1*$^{+/+}$ mice ($p=1.7x10^{-5}$). Taken together, the apparent selection against loss of BCAT1 in CRISPR allografts and the decreased tumor burden following deletion of *Bcat1* in the autochthonous GEMM indicated that inhibition of BCAT1 decreases the growth of SCLC *in vivo*.

2.2.6 Pharmacological inhibition of BCAT1 reduces the growth of SCLC cells *in vitro*

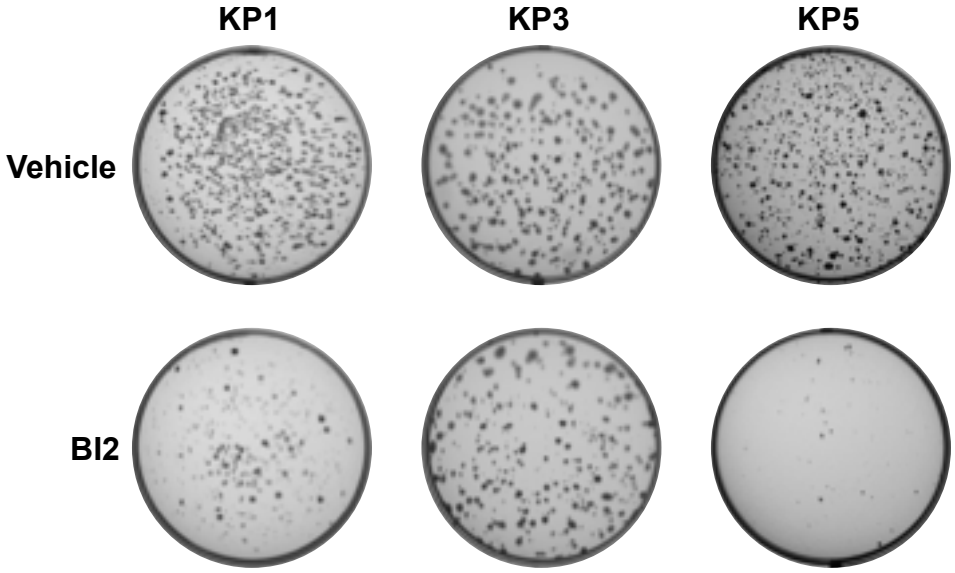
Despite nearly universal recurrence of SCLC following standard therapies, including etoposide with cisplatin or carboplatin, the treatment strategy for SCLC has remained largely unchanged for the past few decades (Farago & Keane, 2018). These chemotherapeutics also damage normal, rapidly dividing cells, and induce a multitude of undesirable side effects, such as hair loss, loss of appetite, nausea and digestive issues. Moreover, because they damage DNA, these chemotherapeutics can lead to other cancers in the future (Lawley, 1980). The long-term failure of the standard of care, along with its lack of cell specificity, underscores the necessity of more effective and less toxic therapeutic strategies. Robust expression of BCAT1 is normally limited to a small number of tissues, including the brain, gonads, and specific immune compartments (Suryawan et al., 1998; Sweatt et al., 2004), which we postulated could

mean less severe side effects from targeted therapy. Small molecules that inhibit BCAT1, including gabapentin (Gbp) and BCATc inhibitor 2 (BI2), reduced the growth of cancer cells, such as glioma (Tönjes et al., 2013) and leukemia (García-Martínez et al., 2009). As our data indicated that BCAT1 promotes the growth of SCLC both *in vitro* and *in vivo*, we sought to determine whether BCAT1 could be targeted pharmacologically to reduce the growth of SCLC cells. To test the hypothesis that pharmacological inhibition of BCAT1 reduced the growth of SCLC cells, we first assessed the growth of mouse SCLC cells in soft agar during three weeks of treatment with Gbp, which is a leucine analog that has about 50-fold specificity for BCAT1 over BCAT2 and blocks the binding of leucine to BCAT1 (Goto et al., 2005). Treatment with 20 mM gabapentin, which was the concentration used to inhibit glioma growth (Tönjes et al., 2013), did not reduce the growth of KP1 or KP3 cells in full medium, which suggested that targeting BCAT1 pharmacologically did not inhibit SCLC growth (Figure 2.10A). However, Gbp was originally designed to relieve neurological disorders by targeting voltage-sensitive Ca^{2+} channels (Taylor, 1997), and a recent report indicated that the reduced proliferation of HCT116 colon cancer cells by Gbp was independent of BCAT activity (Grankvist et al., 2018). We postulated that Gbp could have effects independent of BCAT1 in SCLC; therefore, we next chose to test the growth of SCLC cells following three weeks of treatment with BI2 in full medium (Figure 2.10B and C). BI2 is a sulfonyl hydrazide (N'-[(5-chloro-1-benzofuran-2-yl) carbonyl]-2-(trifluoromethyl) benzene-sulfonohydrazide), which docks in the BCAT active site, presumably blocking ligand binding, and has fifteen-fold specificity for BCAT1 over BCAT2 (Caballero et al., 2009; Hu et al., 2006). In two of the three cells tested, KP1 and KP5, treatment with 10 μM BI2 dramatically

A


KP1
KP3
Vehicle
Gbp
Vehicle
Gbp

B


KP1
KP3
KP5
Vehicle
BI2

C

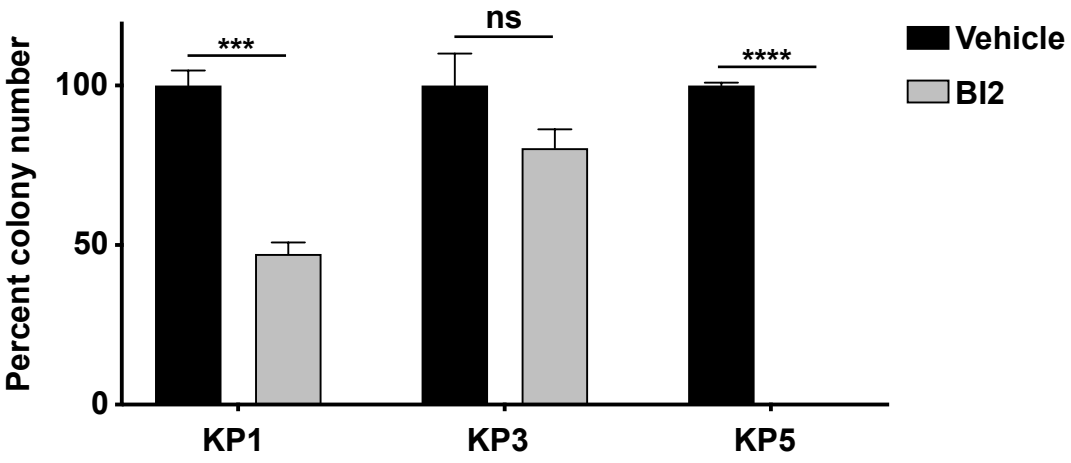

Percent colony number
100
50
0
***
ns
****
KP1
KP3
KP5
Vehicle
BI2

Figure 2.10. Pharmacological inhibition of BCAT1 reduced the growth of SCLC cells *in vitro*.

(A) Results of SCLC growth in soft agar with or without 20 mM gabapentin (Gbp). After three weeks, wells were stained and imaged. Representative result from one of three independent experiments. (B) Results of SCLC growth in soft agar with or without 10 μM BCATc Inhibitor 2 (BI2). After three weeks, wells were stained and imaged. Representative result from one of at least three independent experiments. (C) After imaging, colonies ≥50 μm were quantified. n=3 replicates per cell and treatment. Representative result from one of at least three independent experiments. Data are mean number of colonies ± standard error of mean relative to vehicle control for each cell type. ns: not significant, $p>0.05$; ***: $p<0.001$, ****: $p<0.0001$, two-tailed Student's t-test.

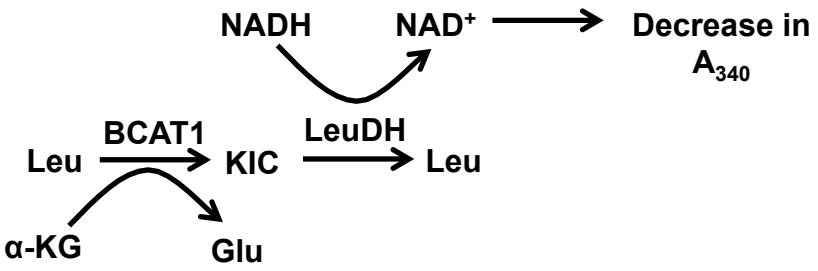
decreased the number of colonies formed ($p= 9.24x10^{-4}$ and $p=4.64x10^{-8}$, respectively), indicating that BCAT1 can be targeted pharmacologically to inhibit the growth of a subset of SCLC cells. However, while the growth of the third cell line tested, KP3, was somewhat reduced in the presence of the inhibitor, the reduction in growth was not significant ($p=0.17$).

2.2.7 Inhibition of BCAT1 reduces the transamination of leucine in SCLC cells

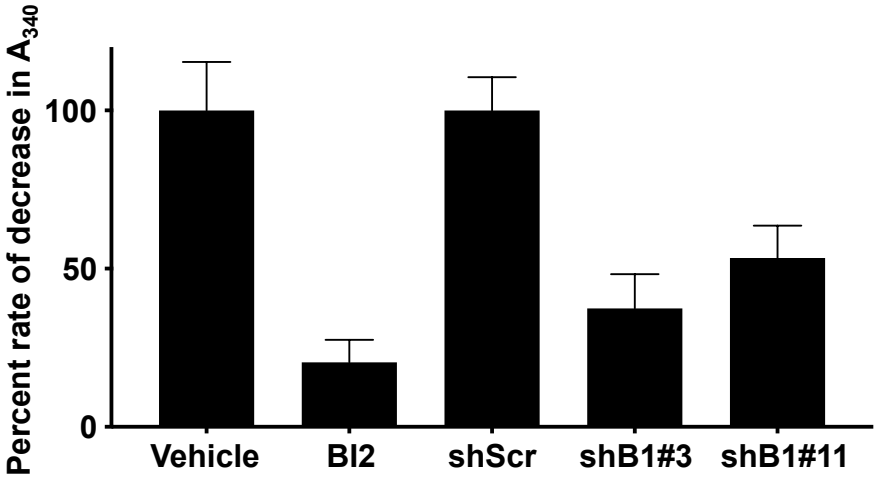
The reduced growth of SCLC cells in soft agar following genetic or pharmacological inhibition of BCAT1 indicated that BCAT1 promoted the growth of the SCLC cells. We next sought to determine the mechanism of action. Rapidly dividing cells require increased rates of biosynthesis of macromolecules, such as non-essential amino acids, nucleotides, and proteins, which are all supported by BCAA metabolism (Holeček, 2018; Mayers et al., 2016). To investigate the mechanism of action underlying the reduced growth of SCLC following inhibition of BCAT1, we first focused on the most well known function of the enzyme, which is the reversible transfer of the BCAA amine group to α-ketoglutarate (α-KG) (Hall et al., 1993; Harper et al., 1984). We studied this reaction *in vitro* because cell cultures are easier to propagate, handle, and process than whole tumors. Furthermore, the growth phenotypes observed *in vitro* were more apparent than *in vivo*, suggesting we might observe a more robust phenotype in cell culture. We reasoned that changes in the direct metabolites (e.g., BCAAs, α-KG, BCKAs, and glutamate) would inform us to which downstream pathways are potentially

altered, which could explain the phenotype of decreased growth of SCLC. For example, in leukemia cells, BCAT1 synthesizes BCAAs to enhance the activity of mTORC1 and promote cell growth (García-Martínez et al., 2009). However, the majority of published literature indicates that BCAT1 usually drives the reaction toward the transamination of BCAAs (Neinast et al., 2019; Suryawan et al., 1998), which has been shown to enhance downstream processes like the synthesis of non-essential amino acids and nucleotides (Mayers et al., 2016). Therefore, we hypothesized that BCAT1 promotes the transamination of BCAAs in SCLC. Since SCLC expresses higher levels of BCAT1, we suspected that SCLC would exhibit increased transamination of BCAAs compared to preSC. As a proof of concept, we first compared the rate of α-ketoisocaproate (KIC) production between lysates of SCLC cells treated with either vehicle or the inhibitor BI2, as well as between lysates of shRNA cells. In this assay, BCAT in cell lysates transaminated leucine to KIC, which was then transaminated back to leucine by a bacterial enzyme called leucine dehydrogenase. Leucine dehydrogenase utilized NADH and decreased the absorbance of light at 340 nm (A_{340}) (Figure 2.11A). Hence, a faster rate of decrease in A_{340} indicated a faster rate of production of KIC, which meant a faster rate of leucine transamination. Compared to DMSO-treated SCLC lysates, lysates treated with BI2 showed a slower rate of decrease in A_{340} (Figure 2.11B). Additionally, lysates of BCAT1-targeting shRNA cells exhibited slower rates of decrease in A_{340}, suggesting that BCAT1 directly promotes the transamination of leucine in cell lysates. To further test the role of BCAT1 in the transamination of leucine, we evaluated CRISPR cells in the colorimetric assay. As shown in Figure 2.11C, both sgB1#1 and sgB1#2 cell lysates exhibited slower rates of decrease in A_{340}, supporting the concept

A


NADH
NAD+
Decrease in
A340
BCAT1
LeuDH
Leu
KIC
Leu
α-KG
Glu

B


Percent rate of decrease in A340
100
50
0
Vehicle
BI2
shScr
shB1#3
shB1#11

C

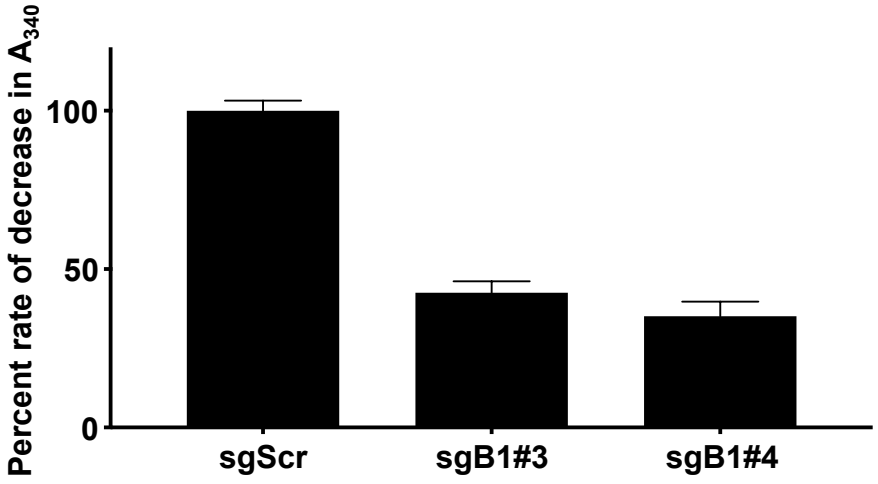

Percent rate of decrease in A340
100
50
0
sgScr
sgB1#3
sgB1#4

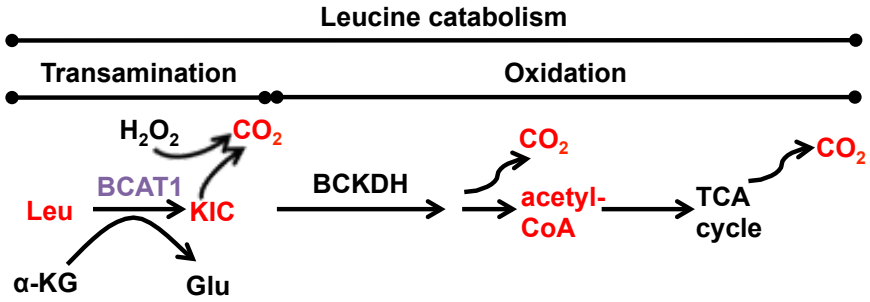
Figure 2.11. Targeting Bcat1 reduced the transamination of leucine in SCLC cell lysates.

(A) Schematic of the colorimetric coupled-enzyme reaction. Leucine was transaminated to KIC by BCAT. KIC was transaminated back to leucine by leuDH. The leuDH-mediated reaction utilized NADH, which resulted in decreased A_{340}. Leu: leucine; α-KG: α-ketoglutarate; KIC: α-ketoisocaproate; Glu: glutamate; LeuDH: leucine dehydrogenase; NADH: nicotinamide adenine dinucleotide (NAD) + hydrogen (H); A_{340}: absorbance of light at 340 nm. (B) Quantification of the rate of decrease in A_{340} following inhibition of BCAT1. KP1 lysates were treated either with vehicle (DMSO), or 5 μM BCATc inhibitor 2 (BI2). shScr, shB1#3, or shB1#11 cells were treated with dox for forty-eight hours and lysed. shScr: scramble shRNA control; shB1: shRNA targeting Bcat1. n=2 replicates per cell type and treatment. Data are mean rate of decrease in A_{340} ± standard error of mean relative to vehicle or shScr. Representative result from one of three independent experiments. (C) Quantification of the rate of decrease in A_{340} using sgScr, sgB1#3, or sgB1#4 lysates. sgScr: scramble sgRNA control; sgB1: sgRNA targeting Bcat1. n=2 replicates per cell type. Data are mean rate of decrease in A_{340} ± standard error of mean relative to sgScr. Representative result from one of two independent experiments.

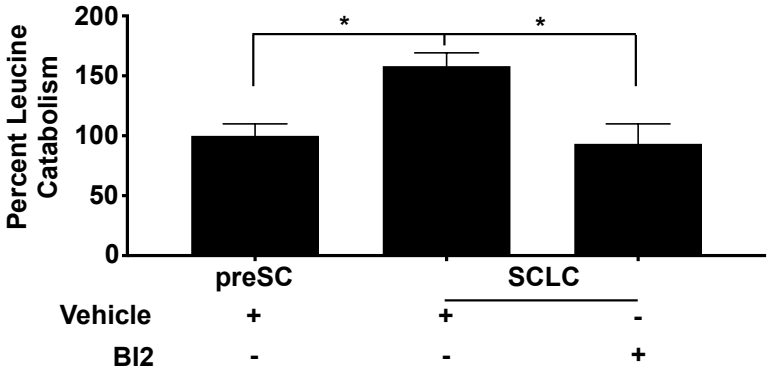
that BCAT1 promotes the transamination of leucine in SCLC cell lysates.

The cell-free colorimetric assay lacked certain factors that can influence the BCAT reaction, such as a cell membrane with functional amino acid transporters. To investigate whether BCAT1 enhances the catabolism of BCAAs in living cells, we first incubated preSC and SCLC cells in Krebs buffer containing L- [^{14}C(U)]-leucine and measured the amount of labeled CO_2 released over time. In this assay, the release of CO_2 occurs in two parts (Figure 2.12A). First, the cells produce labeled KIC, which can be secreted from the cell and oxidized following addition of hydrogen peroxide to the medium. Alternatively, the cells can oxidize the KIC themselves, which occurs before the addition of hydrogen peroxide. Thus, the measurement of total labeled CO_2 is an indirect measurement of both the production of KIC, i.e., transamination, as well as its further degradation, i.e., oxidation. Compared to preSC, we detected a higher rate of radiolabeled CO_2 produced from SCLC ($p=0.017$) (Figure 2.12B). To investigate whether BCAT1 was responsible for the increased rate, we also included a group of SCLC cells that were treated with 10 μM BI2 for 24 hours: the rate of radiolabeled CO_2 production from BI2-treated SCLC was attenuated to a level comparable to preSC ($p=0.031$) (Figure 2.12B), suggesting BCAT1 enhances the catabolism of leucine. Again, although BI2 has more affinity for BCAT1 than for BCAT2, and the tested concentration only attenuated leucine catabolism in SCLC to that near preSC, there was still the possibility that this phenotype was due to the combined inhibition of BCAT1 and BCAT2. To determine whether BCAT1, specifically, enhanced leucine catabolism in SCLC, we assessed the rate of radiolabeled CO_2 production in CRISPR cells when

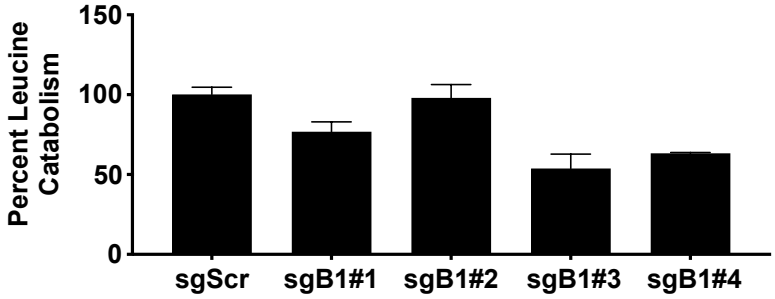
A


Leucine catabolism
Transamination
Oxidation
H2O2
CO2
BCAT1
Leu
KIC
α-KG
Glu
BCKDH
CO2
acetyl-
CoA
TCA
cycle
CO2

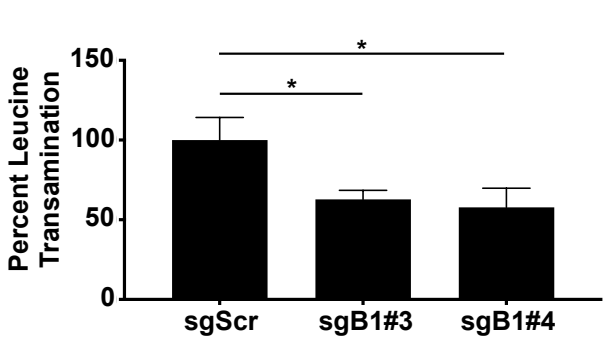
B


Percent Leucine Catabolism
200
150
100
50
0
*
*
preSC
SCLC
Vehicle + + -
BI2 - - +

C


Percent Leucine Catabolism
150
100
50
0
sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4

D


Percent Leucine Transamination
150
100
50
0
*
*
sgScr
sgB1#3
sgB1#4

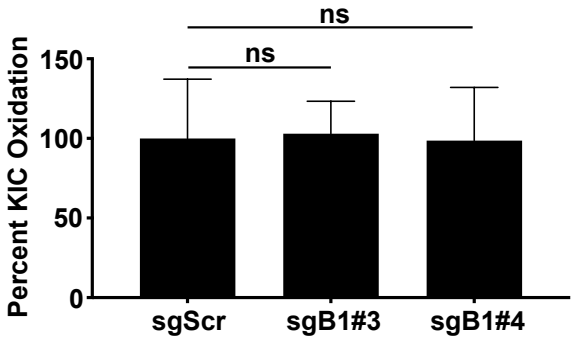

Percent KIC Oxidation
150
100
50
0
ns
ns
sgScr
sgB1#3
sgB1#4

Figure 2.12. Targeting Bcat1 reduced the transamination of leucine in live SCLC cells. (Figure 2.12D was contributed by Ananieva, AS)

(A) Schematic of the radiometric leucine catabolism assay. Cells were rotated in sealed scintillation vials containing Krebs buffer with [^{14}C]-leucine. [^{14}C]-leucine was taken up by cells and was transaminated to [^{14}C]-KIC by BCAT. BCKDH and other enzymes oxidized [^{14}C]-KIC onward to the TCA cycle and released [^{14}C]-CO_2, which was trapped in NaOH following addition of $HClO_4$ to the cells. H_2O_2 was used to oxidize [^{14}C]-KIC that was transaminated and secreted into solution, and the resulting [^{14}C]-CO_2 was also trapped by NaOH. In (B) and (C), all [^{14}C]-CO_2 was collected in the same NaOH trap, which reflected total catabolism. In (D), the CO_2 from transamination and oxidation were collected in separate NaOH traps. NaOH samples were analyzed by liquid scintillation counting. Leu: leucine; α-KG: α-ketoglutarate; KIC: α-ketoisocaproate; BCKDH: branched chain ketoacid dehydrogenase; Glu: glutamate; TCA: tricarboxylic acid. (B) Quantification of the rate of leucine catabolism in preSC cells or SCLC cells treated with vehicle (DMSO) or BCAT1c inhibitor 2 (BI2). n=2 replicates per cell type and treatment. Data are mean pmol [^{14}C]-CO_2 per hour ± standard error of mean relative to preSC and are the average of three independent experiments. (C) Quantification of the rate of leucine catabolism in CRISPR-edited SCLC cells. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. n=2 replicates per cell type. Representative result of one of three independent experiments. Data are mean pmol [^{14}C]-CO_2 per hour ± standard error of mean relative to relative to sgScr. (D) Quantification of [^{14}C]-leu transamination (left graph) and [^{14}C]-KIC oxidation (right graph) in CRISPR-edited SCLC cells. n=5-6 replicates per cell type. Representative result from one of three independent experiments. Data are mean pmol [^{14}C]-CO_2 per hour ± standard error of mean relative to sgScr. ns: not significant, $p>0.05$ (D); *: $p<0.05$ (B) & (D), two-tailed Student's t-test.

given L- [^{14}C(U)]-leucine. BCAT1-targeting cells tended to have decreased rates of leucine catabolism compared to sgScr cells (Figure 2.12C). BCAA catabolism involves reversible transamination and irreversible oxidation of the amino acids, and the majority of tissues within the body do not perform both (Neinast et al., 2019; Sweatt et al., 2004). Thus, we sought to determine whether the cells exhibited decreased transamination of leucine, decreased KIC oxidation, or both. Therefore, we separated the two parts of the catabolism by measuring labeled CO_2 before and after addition of hydrogen peroxide and found that the BCAT1-targeting cells exhibited reduced rates of KIC production (sgB1#3: p=0.035; sgB1#4: p=0.046), but not further oxidation (sgB1#3: p=0.94; shB1#4: p=0.98) (Figure 2.12D). Therefore, we conclude that BCAT1 directly promoted the transamination of leucine in SCLC without affecting the downstream degradation.

2.2.8 Knockdown of BCAT1 reduces the concentrations of glutamate, glutamine, and aspartate in SCLC cells

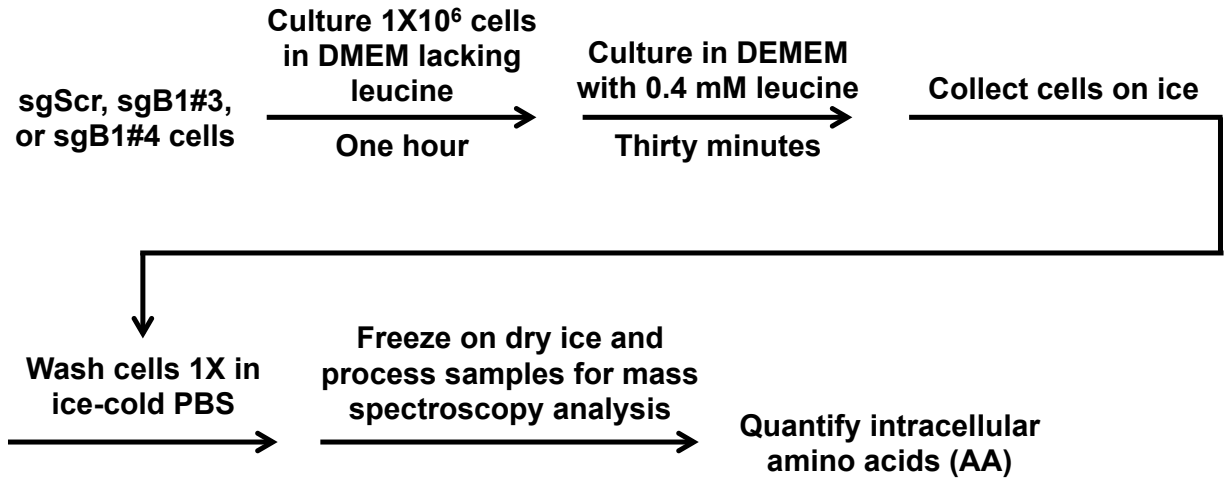
The catabolism of BCAAs results in the production of glutamate, which can be further utilized toward the endogenous production of other non-essential amino acids, such as glutamine and aspartate (Mayers et al., 2016). Hence, the decreased catabolism of leucine following inhibition of BCAT1 led us to hypothesize that BCAT1 increased the intracellular levels of glutamate, glutamine, and aspartate. Importantly, the pyrimidine synthesis pathway utilizes glutamine and aspartate to generate uridine monophosphate, which is the foundation for the pyrimidine nucleotides necessary for DNA and RNA synthesis (Jones, 1980). The purine synthesis pathway also utilizes

glutamine (Pedley & Benkovic, 2017), which further suggests that BCAA catabolism can support the synthesis of DNA and RNA. Additionally, protein translation is heavily dependent on amino acid and energy availability (Chee et al., 2019; Proud, 2004), and glutamine can also be utilized to maintain energy homeostasis (Yang et al., 2014). To investigate the relationship between BCAT1 and glutamate, glutamine, and aspartate, we measured their concentrations inside CRISPR cells following withdrawal of leucine and subsequent culture in leucine-containing medium. As shown in Figure 2.13 inhibiting BCAT1 through CRISPR-mediated editing resulted in significantly reduced intracellular concentrations of glutamate (sgB1#3: $p=3.4x10^{-4}$; shB1#4: $p=2.5x10^{-3}$), glutamine (sgB1#3: $p=3.3x10^{-4}$; shB1#4: $p=5.3x10^{-3}$), and aspartate (sgB1#3: $p=2.9x10^{-4}$; sgB1#4: $p=9.2x10^{-4}$). The decreased intracellular concentrations of these amino acids following inhibition of BCAT1 were also consistent with the concept that there was a shift toward the transamination of leucine in SCLC, which was enhanced by BCAT1.

2.2.9 Ectopic expression of BCAT1 promotes mTORC1 activity and protein translation in preSC cells

To explore how BCAT1 influences metabolism in preSC cells, we sought to determine how BCAT1 affected downstream signaling like the mTORC1 pathway and protein translation. Since inhibition of BCAT1 reduced the transamination of leucine in SCLC cells (see Figure 2.12D), and leucine is known to stimulate the activity of mTORC1 (Jewell et al., 2015), we hypothesized that the ectopic expression of BCAT1 in preSC would result in decreased activity of mTORC1. However, because mTORC1

A


sgScr, sgB1#3, or sgB1#4 cells
Culture 1X10^6 cells in DMEM lacking leucine
One hour
Culture in DEMEM with 0.4 mM leucine
Thirty minutes
Collect cells on ice
Wash cells 1X in ice-cold PBS
Freeze on dry ice and process samples for mass spectroscopy analysis
Quantify intracellular amino acids (AA)

B

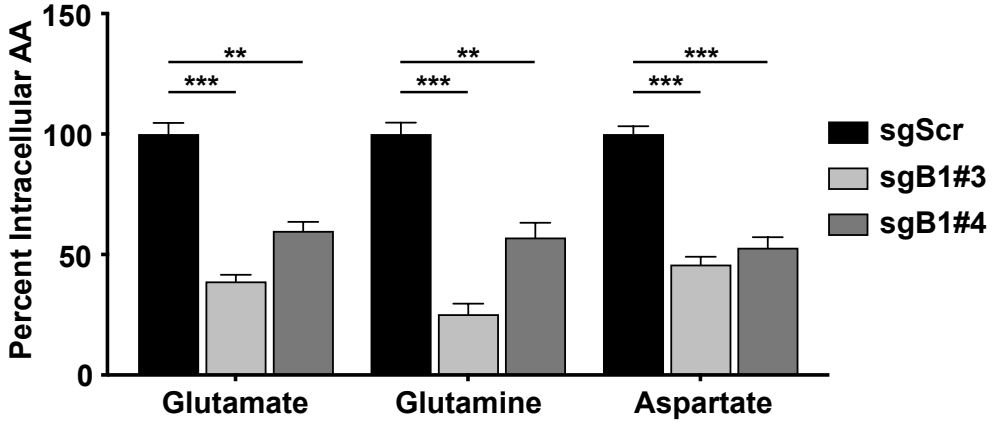

Percent Intracellular AA
150
100
50
0
**
***
**
***
***
***
Glutamate
Glutamine
Aspartate
sgScr
sgB1#3
sgB1#4

Figure 2.13. CRISPR-mediated knockdown of BCAT1 reduced the intracellular levels of glutamate, glutamine, and aspartate. (Figure 2.12B was partially contributed by Park, JJ)

(A) Schematic of the mass spectroscopy experiment to analyze intracellular amino acids. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#3, or -sgB1#4 were starved of leucine and then incubated in leucine-containing medium for the indicated times. After freezing the cells, intracellular amino acids were determined by mass spectroscopy. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Quantification of intracellular glutamate, glutamine, and aspartate in CRISPR-edited cells. n=3 replicates per cell. Representative result from one of three independent experiments. Data are mean pmol of amino acid ± standard error of mean relative to sgScr. **: $p<0.01$ and ***: $p<0.001$, two-tailed Student's t-test.

usually promotes protein translation, and our group previously found that ectopic expression of *Mycl* increased the expression of *Bcat1* and increased protein translation in preSC cells (Kim et al., 2016), there remained the possibility that BCAT1 could promote the activity of mTORC1 and increase protein translation. Additionally, EIF2A signaling was among the most significant molecular pathways related to the *Mycl*-driven transformation of preSC cells (Kim et al., 2016). As EIF2A is critical to the initiation of protein translation (Hinnebusch, 2014), we also asked whether BCAT1 could affect the phosphorylation EIF2A signaling. To test these possibilities, we induced expression of BCAT1 in preSC cells with doxycycline for forty-eight hours, followed by culture in low-serum medium for eighteen hours (Figure 2.14A). Then, we cultured the cells in full medium for eight hours and pulsed them with puromycin, a tRNA analog, for ten minutes. After processing the cells for protein extract, we analyzed the extracts for phosphorylation of RPS6K, which is indicative of mTORC1 activity, by immunoblot. To determine the effect on protein translation, we also blotted for puromycin. To investigate whether ectopic expression of BCAT1 affected EIF2A signaling, we blotted for phosphorylation of EIF2A. preSC cells overexpressing BCAT1 exhibited increased phosphorylation of RPS6K compared to control, which indicated an increase in the activity of mTORC1 (Figure 2.14B). The overexpressing cells also had an increased rate of puromycin incorporation into protein, which indicated an increased rate of protein translation compared to control, and decreased phosphorylation of EIF2A, which indicated increased activity of EIF2A.

A

preSC cells transduced with empty lentivirus or virus containing *Bcat1*

Culture 5X10^6 cells in 2 mL RPMI-1640 with 0.1% serum in one well of a 6-well plate per cell type — 18 hours

Culture in 1.5 mL RPMI-1640 with 10% serum — 8 hours

10 minutes before collection, treat cells with 10 µg/mL puromycin — 10 minutes

Collect cells on ice, wash once with ice-cold PBS and lyse on ice

Run 20 µg total protein on SDS-PAGE

Immunoblot for phosphorylated and total EIF2A and RPS6K, puromycin, BCAT1, and ACTB

B

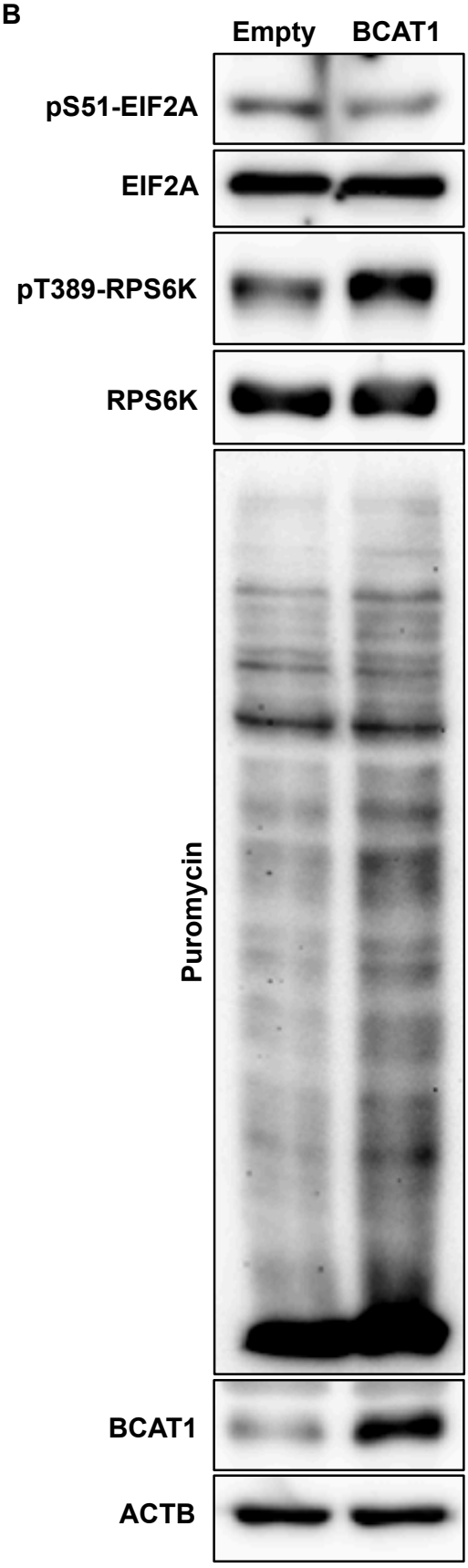


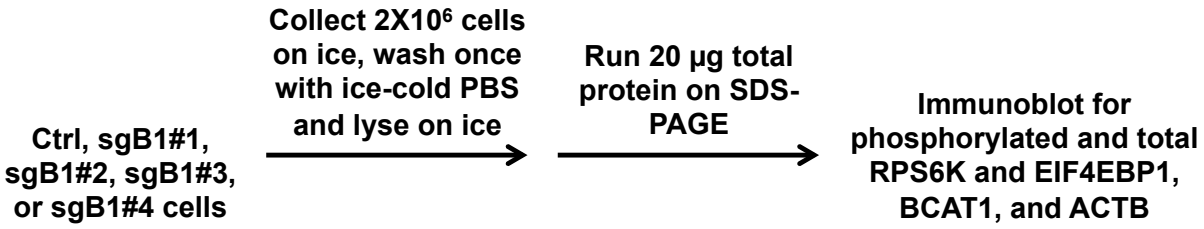
Figure 2.14. Ectopic expression of BCAT1 in precancerous cells resulted in increased mTORC1 activity and increased protein translation.

(A) Schematic of experiment to assess mTORC1 activity, protein translation, and EIF2A inhibition in preSC cells. preSC cells transduced with pCW57.1-empty vector (lane one) or pCW57.1-BCAT1 (lane two) were treated with doxycycline (dox). After incubation in low-serum medium, followed by incubation in complete medium, cells were pulsed puromycin. After cell collection and lysis, 20 µg of total protein from each sample was run on SDS-PAGE gels. (B) Immunoblot for puromycin, BCAT1, total and phosphorylated RPS6K, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. The effect on EIF2A was observed in one blot, the effect on RPS6K was observed in two blots, and the effect on puromycin incorporation was observed in three blots.

2.2.10 Knockdown of BCAT1 activates mTORC1, but not protein translation, in SCLC cells

The mTORC1 pathway, which normally promotes protein translation, is widely accepted to be pro-tumorigenic (Guertin & Sabatini, 2007) and is frequently activated in SCLC (Umemura et al., 2014), suggesting that it may promote the growth of SCLC. In a previously published report, our group determined that ectopic expression of *Mycl* increased the expression of *Bcat1* and drove the development of SCLC by supporting protein translation (Kim et al., 2016). Since *Bcat1* mRNA was increased in SCLC compared to preSC (see Figure 2.1A), and products of BCAT1, e.g., glutamate, are incorporated into protein during translation, we hypothesized that BCAT1 enhanced protein translation in SCLC cells. However, over the course of this dissertation, we found that knockdown of BCAT1 reduced the transamination of leucine, an amino acid known to increase the activity of mTORC1 (see Figure 2.12D). This presented a potential contradiction whereby knockdown of BCAT1 could increase the activity of mTORC1 while decreasing protein translation. Thus, we sought to investigate how the expression of BCAT1 affects these pathways in SCLC. We assessed the activity of mTORC1 in BCAT1 CRISPR cells through immunoblot detection of phosphorylated RPS6K at threonine 398 and phosphorylated EIF4EBP1 at threonine 37 and 46 following culture in full medium (Figure 2.15A). These post-translational modifications are directly mediated by mTORC1 and are commonly used to evaluate its activity (Burnett et al., 1998). As shown in Figure 2.15B, the phosphorylation of RPS6K was increased in BCAT1-targeted cells compared to control, which suggested an increase in

A


Ctrl, sgB1#1, sgB1#2, sgB1#3, or sgB1#4 cells
Collect 2X10^6 cells on ice, wash once with ice-cold PBS and lyse on ice
Run 20 µg total protein on SDS-PAGE
Immunoblot for phosphorylated and total RPS6K and EIF4EBP1, BCAT1, and ACTB

B

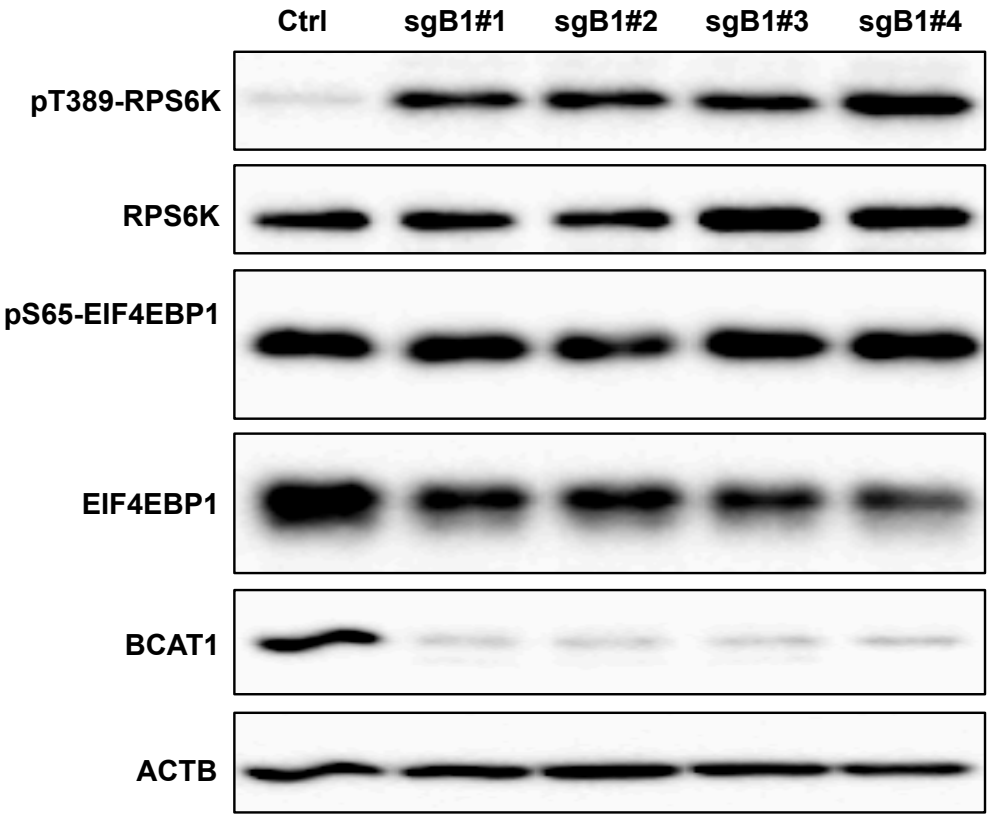

Ctrl
sgB1#1
sgB1#2
sgB1#3
sgB1#4
pT389-RPS6K
RPS6K
pS65-EIF4EBP1
EIF4EBP1
BCAT1
ACTB

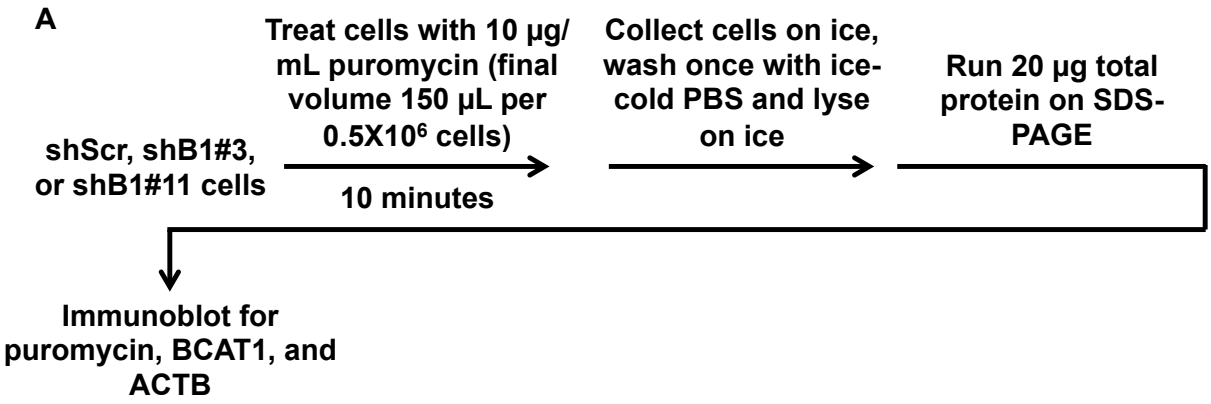
Figure 2.15. CRISPR-mediated knockdown of Bcat1 increased mTORC1 activity.

(A) Schematic of experiment to assess mTORC1 activity in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-empty, -sgB1#1, -sgB1#2, -sgB1#3, or -sgB1#4 were continuously cultured in complete medium and processed for protein extract. 20 μg of total protein from each sample was run on SDS-PAGE gels. Control: empty vector without sgRNA; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for BCAT1, RPS6K, and EIF4EBP1 proteins, as well as pT389-RPS6K and pS65-EIF4EBP1. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

the activity of mTORC1.

To determine the role of BCAT1 in protein translation, we pulsed cells with puromycin for ten minutes and then measured puromycin incorporation through immunoblot (Figure 2.16A). We first investigated the effect on protein translation in shRNA cells in full medium. As shown in Figure 2.16B, the amount of puromycin incorporated into protein was decreased in shB1#3 and shB1#11 cells compared to shScr cells, which indicated a reduced rate of protein translation. To expand on this, we assessed sgScr, sgB1#3, and sgB1#4 cells using the same assay (Figure 2.17A), but did not observe similar reductions in the incorporation of puromycin into protein (Figure 2.17B). However, we found that primary BCAT1 knockout cells, which were recently cultured from tumors formed in the autochthonous GEMM, exhibited decreased puromycin incorporation (Figure 2.18A and B). Thus, the similar rates of puromycin incorporated into the CRISPR cells indicated that inhibition of BCAT1 did not increase protein translation, and interestingly, the increased puromycin incorporation in the shRNA cells and the primary knockout cells, which were cultured for less time than the CRISPR cells, suggested that inhibition of BCAT1 in fact decreased protein translation in SCLC under these conditions. The increased activity of mTORC1 in the BCAT1-targeting CRISPR cells without a corresponding increase in the incorporation of puromycin into protein raised the question of how inhibiting BCAT1 upregulates the activity of mTORC1 without increasing protein translation. Thus, the experiments described in the remaining sections were conducted in order to determine whether the increased activity of mTORC1 induced various forms of metabolic stress, which could

A


shScr, shB1#3, or shB1#11 cells
Treat cells with 10 µg/mL puromycin (final volume 150 µL per 0.5X10^6 cells)
10 minutes
Collect cells on ice, wash once with ice-cold PBS and lyse on ice
Run 20 µg total protein on SDS-PAGE
Immunoblot for puromycin, BCAT1, and ACTB

B

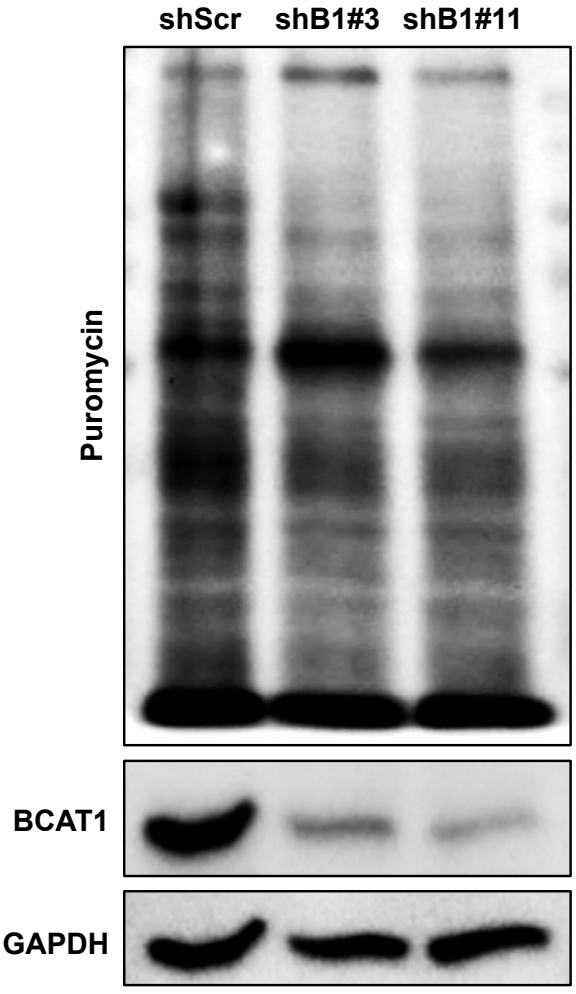
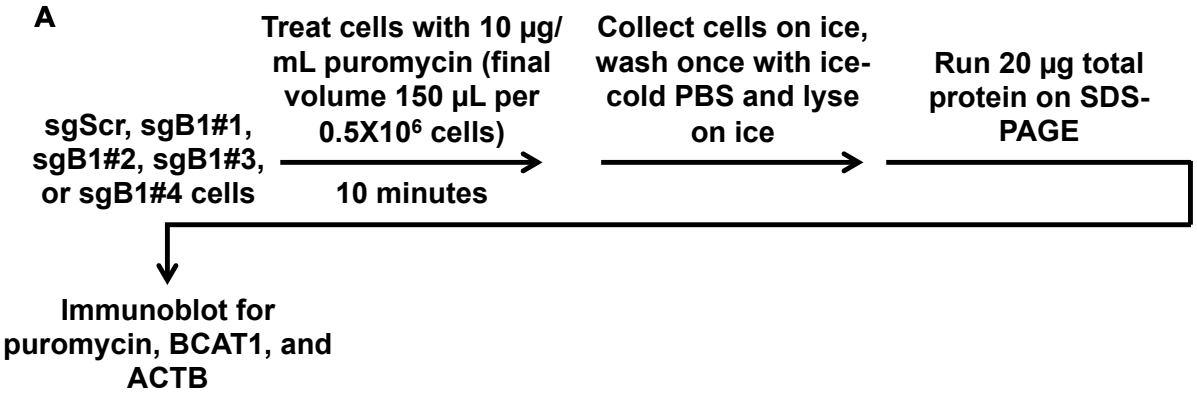

shScr
shB1#3
shB1#11
Puromycin
BCAT1
GAPDH

Figure 2.16. shRNA-mediated knockdown of BCAT1 decreased protein translation.

(A) Schematic of experiment to assess protein translation in SCLC cells. KP1 cells transduced with Tet-pLKO-puro-shScr, -shB1#3, or -shB1#11 were treated with either the vehicle (ddH_2O) or doxycycline. They were then pulsed with puromycin and processed for protein extract. 20 µg of total protein from each sample was run on SDS-PAGE gels. shScr: scrambled shRNA control; shB1: shRNA targeting BCAT1. (B) Immunoblot for puromycin and BCAT1. ACTB blot verified equal loading of total protein. Immunoblot representative of two independent experiments.

A


sgScr, sgB1#1, sgB1#2, sgB1#3, or sgB1#4 cells
Treat cells with 10 µg/mL puromycin (final volume 150 µL per 0.5X10^6 cells)
10 minutes
Collect cells on ice, wash once with ice-cold PBS and lyse on ice
Run 20 µg total protein on SDS-PAGE
Immunoblot for puromycin, BCAT1, and ACTB

B

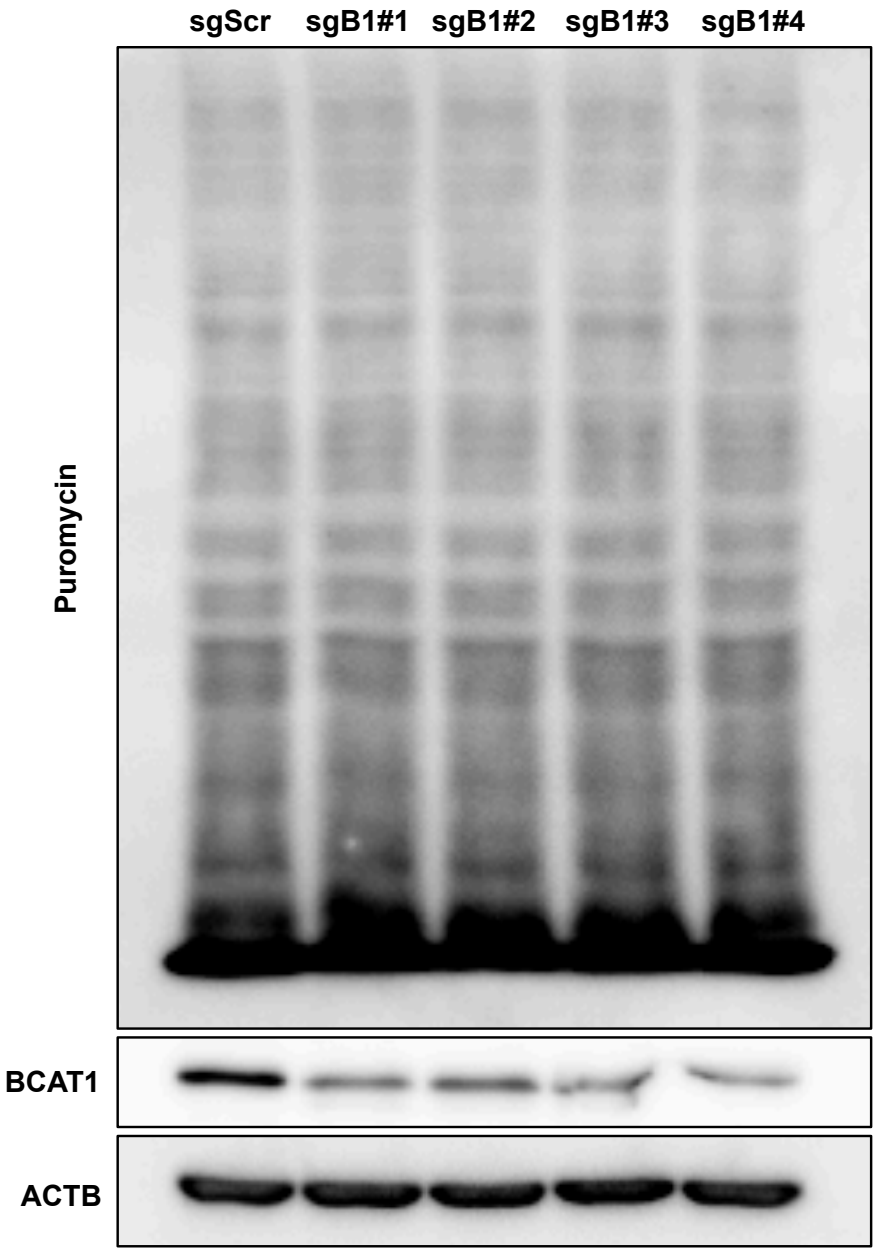
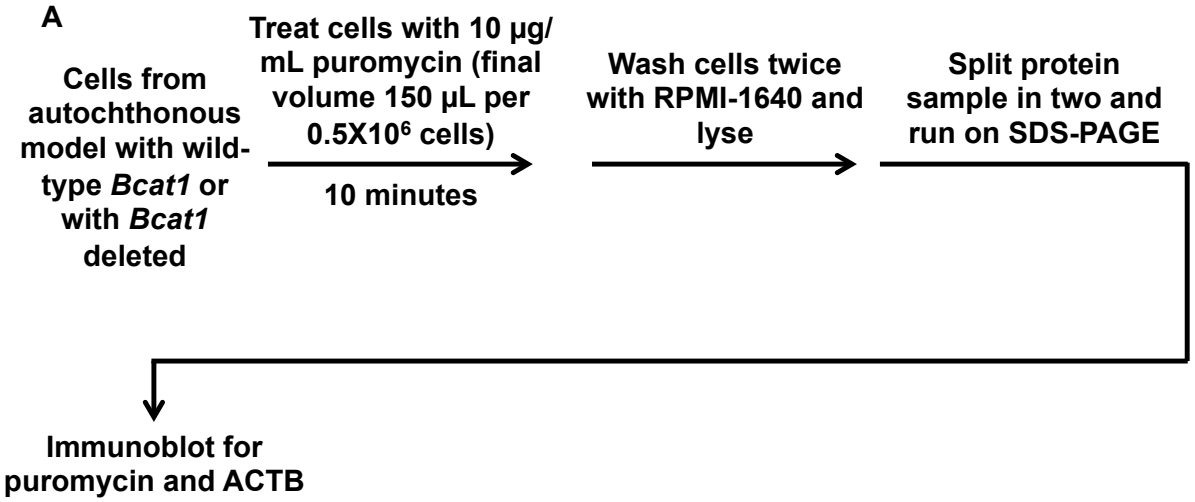

sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4
Puromycin
BCAT1
ACTB

Figure 2.17. CRISPR-mediated knockdown of BCAT1 did not decrease protein translation.

(A) Schematic of experiment to assess protein translation in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, -sgB1#2, -sgB1#3, or -sgB1#4 were continuously cultured in complete medium, pulsed with puromycin, and processed for protein extract. 20 µg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for puromycin and BCAT1. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

A


Cells from autochthonous model with wild-type *Bcat1* or with *Bcat1* deleted
Treat cells with 10 µg/mL puromycin (final volume 150 µL per 0.5X10^6 cells)
10 minutes
Wash cells twice with RPMI-1640 and lyse
Split protein sample in two and run on SDS-PAGE
Immunoblot for puromycin and ACTB

B

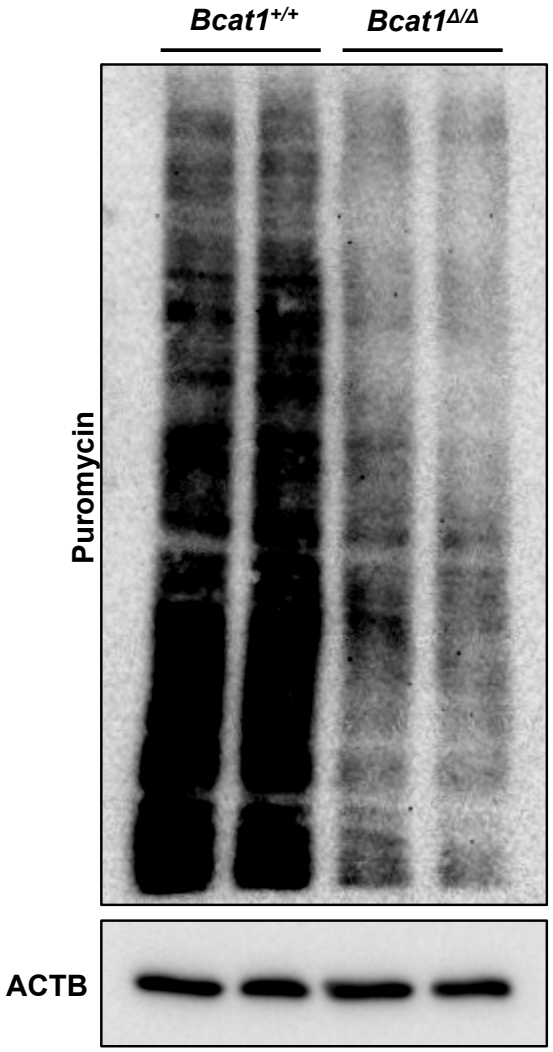

Bcat1$^{+/+}$
Bcat1$^{\Delta/\Delta}$
Puromycin
ACTB

Figure 2.18. Deletion of BCAT1 in primary cancer cells cultured from tumors resulted in decreased protein translation. (Figure contributed by Kim, KB)

(A) Schematic of experiment to assess protein translation in SCLC cells. Primary cancer cells harvested from *Bcat1*$^{+/+}$ (left two lanes) and *Bcat1*$^{\Delta/\Delta}$ (right two lanes) lung tumors (figure 2.9) were continuously cultured in complete medium, pulsed with puromycin, and processed for protein extract. 20 µg of total protein from each sample was run on SDS-PAGE gels. (B) Immunoblot for puromycin. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

have explained the uncoupling of mTORC1 to protein translation. However, we did not obtain significant findings from those experiments.

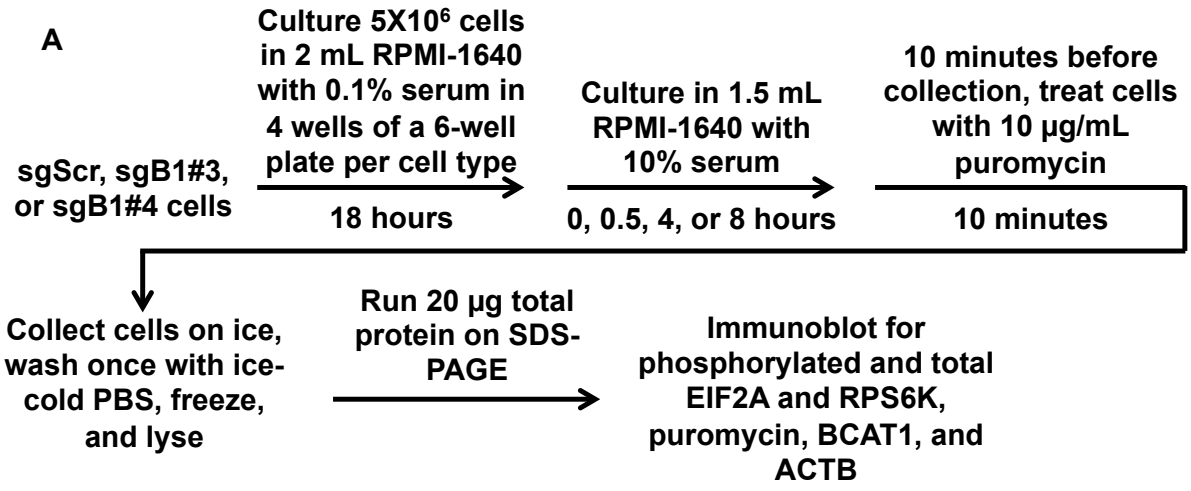
2.2.11 BCAT1-mediated activity of mTORC1 does not influence the integrated stress response in SCLC cells

We next asked how the inhibition of BCAT1 promoted the activity of mTORC1, but did not increase protein translation. Downstream targets of mTORC1, e.g., RPS6K and EIF4EBP1, are not the only regulators of protein translation; there are many components of the translation initiation machinery besides RPS6 and EIF4E (Hinnebusch, 2014). Thus, we sought to investigate whether the reduced expression of BCAT1 was associated with the inhibition of critical components of the translational machinery, which we postulated might counteract pro-translational signals from mTORC1. For example, over the past decade, there have been multiple reports linking aberrant activity of mTORC1 to ER stress and the UPR, which inhibits translation (Appenzeller-Herzog & Hall, 2012; Inoki et al., 2011; Ito et al., 2011; Kato et al., 2012; Panda et al., 2018). Furthermore, EIF2A signaling was among the most significant molecular pathways related to the *Mycl*-driven transformation of preSC cells (Kim et al., 2016). Thus, we hypothesized that the increased activity of mTORC1 following inhibition of BCAT1 could promote the phosphorylation and inactivation of EIF2A, which is a marker for the integrated stress response (ISR), including the UPR (Walter & Ron, 2011). We postulated that the activity of mTORC1 and the phosphorylation of EIF2A could be regulated by the cell cycle. Therefore, we sought to synchronize the CRISPR

cells through overnight culture in a low-serum medium containing only 0.1% bovine growth serum (BGS) and then track changes in biomarkers, e.g., phosphorylation of RPS6K at threonine 398, phosphorylation of EIF2A at serine 51, and puromycin, over time following subsequent culture in full medium (Figure 2.19A). As shown in Figure 2.19B, while both sgB1#3 and sgB1#4 cells exhibited increased phosphorylation of RPS6K compared to sgScr at thirty minutes of culture in full medium, the effect was not as robust for sgB1#3. Correspondingly, while the phosphorylation of EIF2α was increased in sgB1#4 cells at four hours, this was not observed in sgB1#3 cells. Furthermore, while both targeting sgRNAs were associated with decreased puromycin incorporation into protein, particularly at eight hours of full medium, this effect was minor. The lack of increased puromycin incorporation supported our earlier findings, which suggested that the activation of mTORC1, which resulted from the inhibition of BCAT1, did not promote protein translation in SCLC cells. While the pattern of RPS6K and EIF2A phosphorylation was inconclusive in determining the relationship between mTORC1 and EIF2A, we postulated that the activity of mTORC1 in the sgB1#3 cells, which was higher than in sgScr, but lower than in sgB1#4 was not sufficient to increase the phosphorylation of EIF2A relative to control.

Next, we sought to achieve more consistent activation of mTORC1 between the two targeting cells. As serum is made of many components, in addition to amino acids and growth factors, we explored other experimental conditions to avoid potential complications arising from manipulating such a complex component of the medium. We next assessed the cells without pre-incubation in the low-serum medium because this

A


sgScr, sgB1#3, or sgB1#4 cells
Culture 5X10^6 cells in 2 mL RPMI-1640 with 0.1% serum in 4 wells of a 6-well plate per cell type
18 hours
Culture in 1.5 mL RPMI-1640 with 10% serum
0, 0.5, 4, or 8 hours
10 minutes before collection, treat cells with 10 µg/mL puromycin
10 minutes
Collect cells on ice, wash once with ice-cold PBS, freeze, and lyse
Run 20 µg total protein on SDS-PAGE
Immunoblot for phosphorylated and total EIF2A and RPS6K, puromycin, BCAT1, and ACTB

B

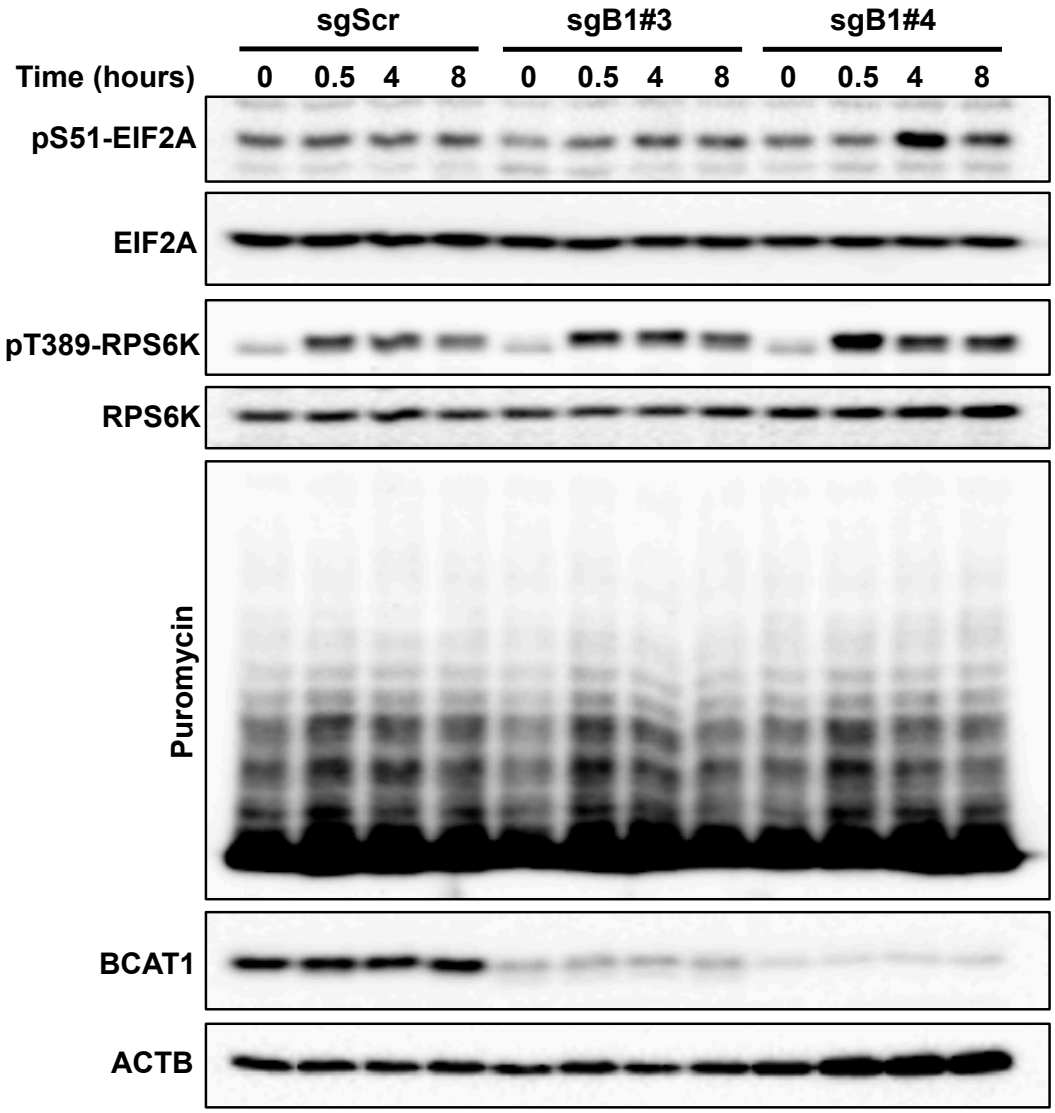

sgScr
sgB1#3
sgB1#4
Time (hours)
0 0.5 4 8 0 0.5 4 8 0 0.5 4 8
pS51-EIF2A
EIF2A
pT389-RPS6K
RPS6K
Puromycin
BCAT1
ACTB

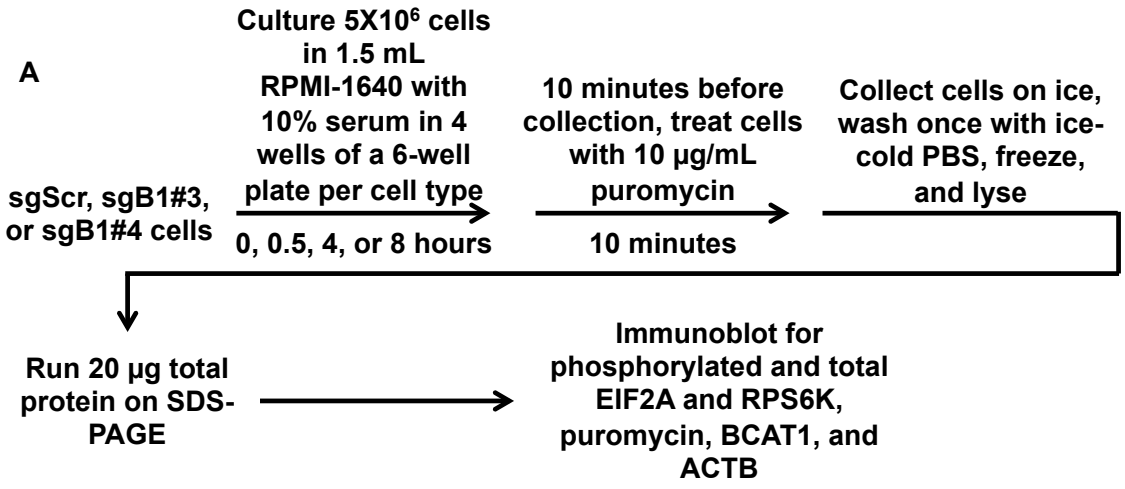
Figure 2.19. CRISPR-mediated knockdown of BCAT1 did not consistently inhibit EIF2A following serum synchronization.

(A) Schematic of experiment to assess mTORC1 activity, protein translation, and EIF2A inhibition in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#3, or -sgB1#4 were incubated in low serum medium and then incubated in complete medium for the indicated times. They were then pulsed with puromycin and processed for protein extract. 20 μg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for puromycin, BCAT1, total and phosphorylated RPS6K, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

step was not necessary to observe the increased activity of mTORC1 previously (Figure 2.20A) (see Figure 2.15B). As shown in Figure 2.20B, while the BCAT1-targeting cells exhibited similar increases in the phosphorylation of RPS6K compared to sgScr at thirty minutes after seeding, the phosphorylation of EIF2A was similar to the control at all time points. Although sgB1#3 cells exhibited decreased incorporation of puromycin compared to sgScr cells, sgB1#4 cells surprisingly displayed increased incorporation of puromycin. These results suggested that the increased activity of mTORC1 did not promote the phosphorylation of EIF2A, and were consistent with the concept that mTORC1 did not regulate protein translation in these cells; additionally, the phosphorylation of EIF2A was not related to the incorporation of puromycin into protein.

The lack of increased phosphorylation of EIF2A in sgB1#4 cells, which was observed in the previous two experiments, and the discrepancy in the incorporation of puromycin, led us to postulate that synchronizing the cells was a necessary step. Next, we assessed the cells after treatment with lovastatin for twenty-four hours and then mevalonate for six hours (Figure 2.21A). This method is commonly used to arrest cells in the G1 phase of the cell cycle and then release them from arrest once synchronized (Keyomarsi, 1996). Under this condition, both BCAT1-targeting cells exhibited increased phosphorylation of RPS6K compared to sgScr, especially at four and eight hours after release (Figure 2.21B). However, the phosphorylation EIF2A was similar to the control at all time points and neither BCAT1-targeting sgRNA affected the incorporation of puromycin into protein. This result suggested that mTORC1 did not promote the phosphorylation of EIF2A, and was consistent with the concept that mTORC1 did not

A


Culture 5X10^6 cells in 1.5 mL RPMI-1640 with 10% serum in 4 wells of a 6-well plate per cell type
10 minutes before collection, treat cells with 10 µg/mL puromycin
Collect cells on ice, wash once with ice-cold PBS, freeze, and lyse
sgScr, sgB1#3, or sgB1#4 cells
0, 0.5, 4, or 8 hours
10 minutes
Run 20 µg total protein on SDS-PAGE
Immunoblot for phosphorylated and total EIF2A and RPS6K, puromycin, BCAT1, and ACTB

B

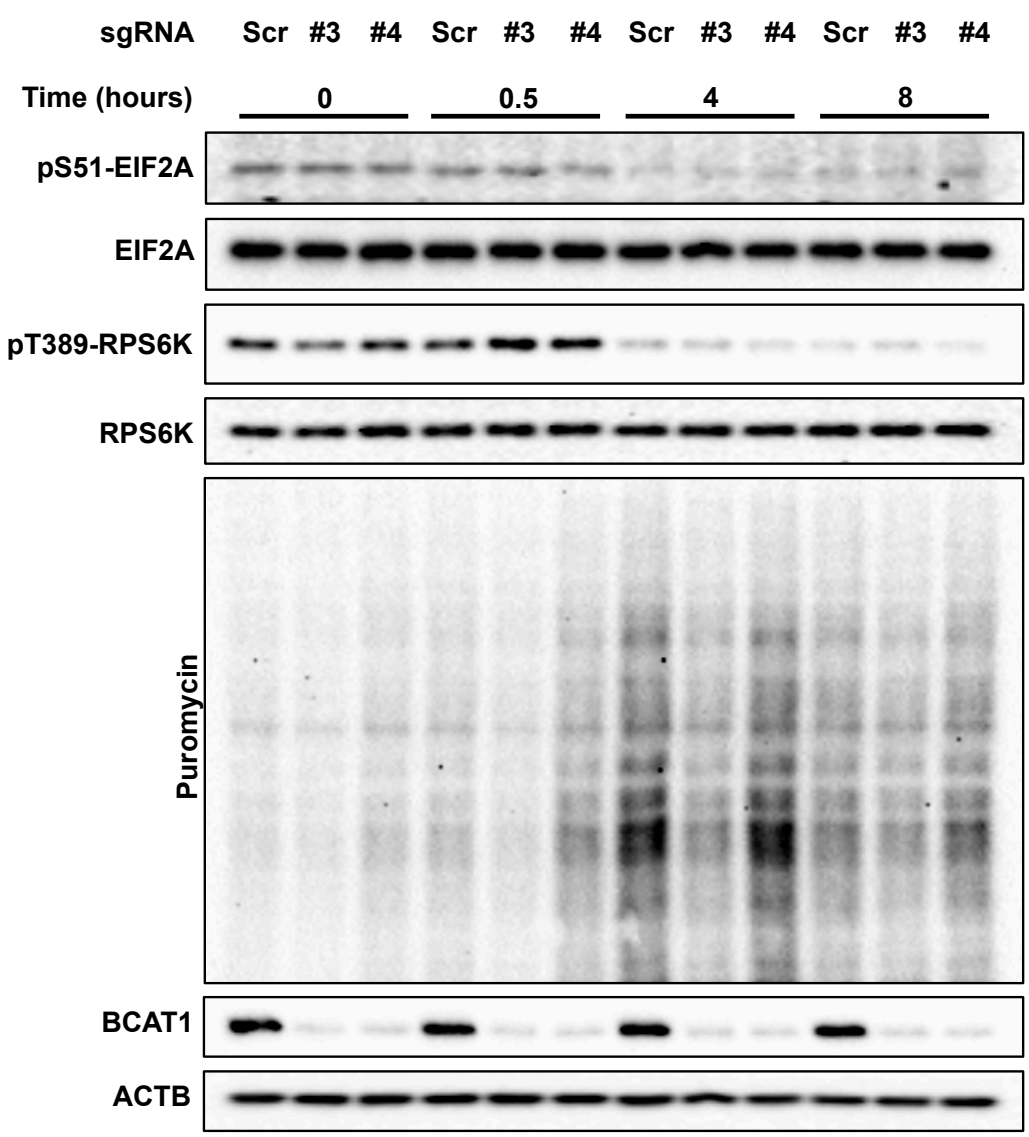
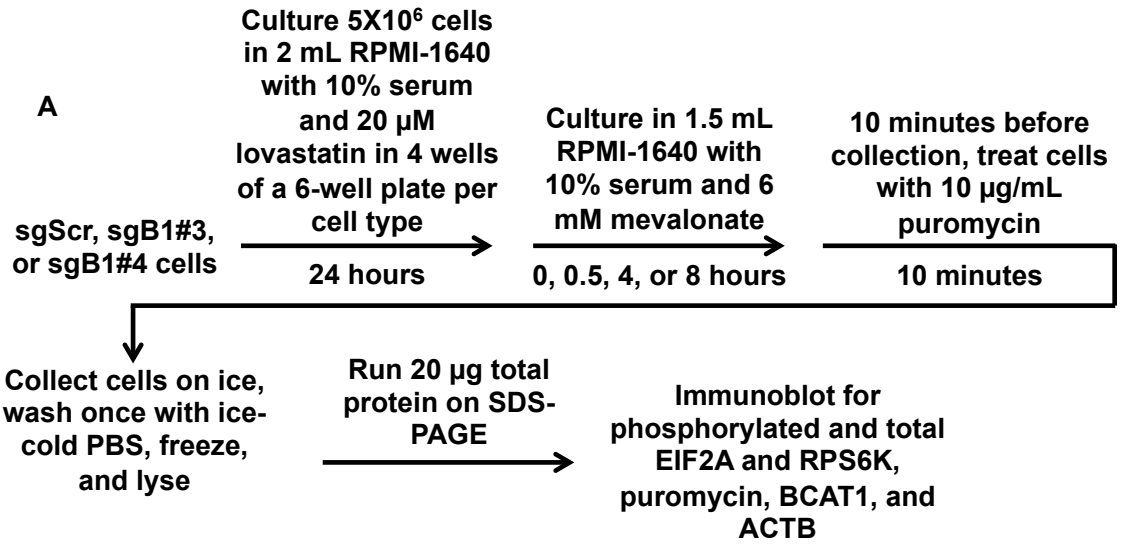

sgRNA
Scr #3 #4 Scr #3 #4 Scr #3 #4 Scr #3 #4
Time (hours)
0
0.5
4
8
pS51-EIF2A
EIF2A
pT389-RPS6K
RPS6K
Puromycin
BCAT1
ACTB

Figure 2.20. CRISPR-mediated knockdown of BCAT1 did not inhibit EIF2A without synchronization of cells.

(A) Schematic of experiment to assess mTORC1 activity, protein translation, and EIF2A inhibition in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#3, or -sgB1#4 were continuously cultured in complete medium, pulsed with puromycin, and processed for protein extract. 20 µg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for puromycin, BCAT1, total and phosphorylated RPS6K, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

A


sgScr, sgB1#3, or sgB1#4 cells
Culture 5X10^6 cells in 2 mL RPMI-1640 with 10% serum and 20 μM lovastatin in 4 wells of a 6-well plate per cell type
24 hours
Culture in 1.5 mL RPMI-1640 with 10% serum and 6 mM mevalonate
0, 0.5, 4, or 8 hours
10 minutes before collection, treat cells with 10 μg/mL puromycin
10 minutes
Collect cells on ice, wash once with ice-cold PBS, freeze, and lyse
Run 20 μg total protein on SDS-PAGE
Immunoblot for phosphorylated and total EIF2A and RPS6K, puromycin, BCAT1, and ACTB

B

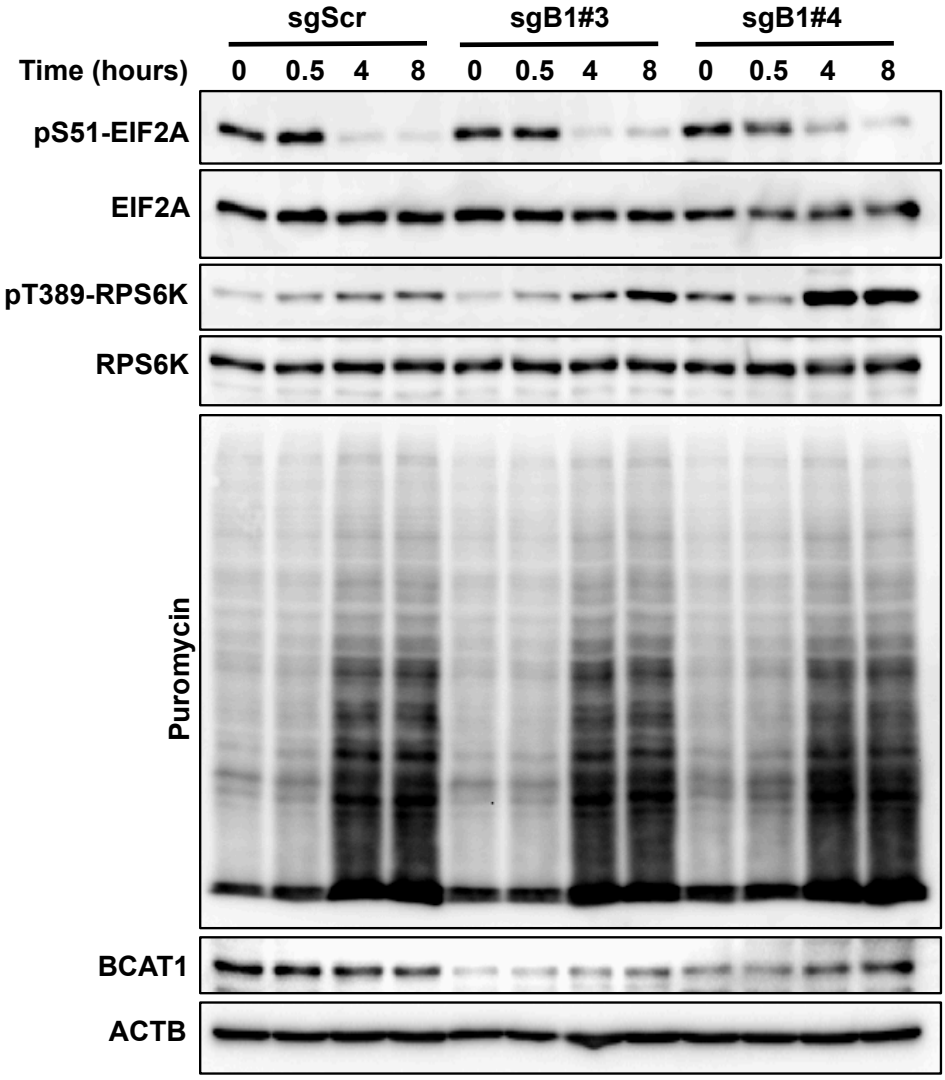

sgScr
sgB1#3
sgB1#4
Time (hours)
0 0.5 4 8 0 0.5 4 8 0 0.5 4 8
pS51-EIF2A
EIF2A
pT389-RPS6K
RPS6K
Puromycin
BCAT1
ACTB

Figure 2.21. CRISPR-mediated knockdown of BCAT1 did not inhibit EIF2A following synchronization of cells with lovastatin.

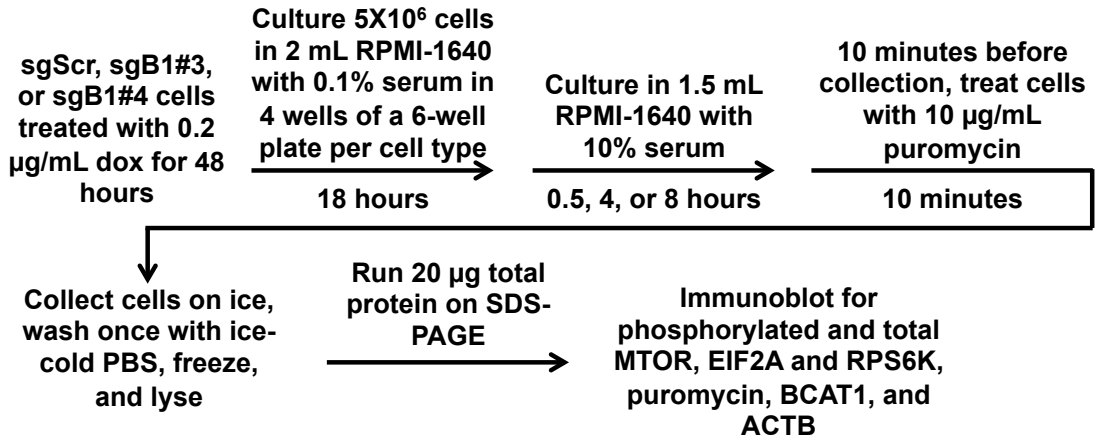
(A) Schematic of experiment to assess mTORC1 activity, protein translation, and EIF2A inhibition in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#3, or -sgB1#4 were incubated in lovastatin and then mevalonate for the indicated times. They were then pulsed with puromycin and processed for protein extract. 20 μg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for puromycin, BCAT1, total and phosphorylated RPS6K, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. Immunoblot representative of two independent experiments.

regulate protein translation.

We postulated that over time, the cells might have experienced selective pressure against loss of BCAT1, so that they had somehow compensated to uncouple the activity of mTORC1 from the phosphorylation of EIF2A. Therefore, we assessed the inducible CRISPR cells following preincubation in the low-serum medium and subsequent culture in full medium (Figure 2.22A). We chose this condition because it initially resulted in increased phosphorylation of RPS6K and EIF2A in the non-inducible sgB1#4 cells (see Figure 2.19B). As shown in Figure 2.22B, both BCAT1-targeting cells exhibited increased autophosphorylation of MTOR and phosphorylation of RPS6K compared to control. However, while the phosphorylation of EIF2A was mildly increased in both BCAT1-targeting cells at thirty minutes, it was decreased compared to control by eight hours. The kinetic profiles of the phosphorylation of RPS6K and EIF2A were strikingly different, suggesting that the phosphorylation of EIF2A was not regulated by mTORC1 in these cells. Additionally, the rate of puromycin incorporated into protein was not noticeably different in BCAT1-targeting cells compared to the control, consistent with the concept that protein translation was not regulated by mTORC1 in SCLC cells.

There was a possibility that the parental cells, which the CRISPR cells were derived from, had been cultured for too long and had undergone secondary changes that impacted their metabolism and rendered them significantly different from cells in

A


sgScr, sgB1#3, or sgB1#4 cells treated with 0.2 µg/mL dox for 48 hours
Culture 5X10^6 cells in 2 mL RPMI-1640 with 0.1% serum in 4 wells of a 6-well plate per cell type
18 hours
Culture in 1.5 mL RPMI-1640 with 10% serum
0.5, 4, or 8 hours
10 minutes before collection, treat cells with 10 µg/mL puromycin
10 minutes
Collect cells on ice, wash once with ice-cold PBS, freeze, and lyse
Run 20 µg total protein on SDS-PAGE
Immunoblot for phosphorylated and total MTOR, EIF2A and RPS6K, puromycin, BCAT1, and ACTB

B

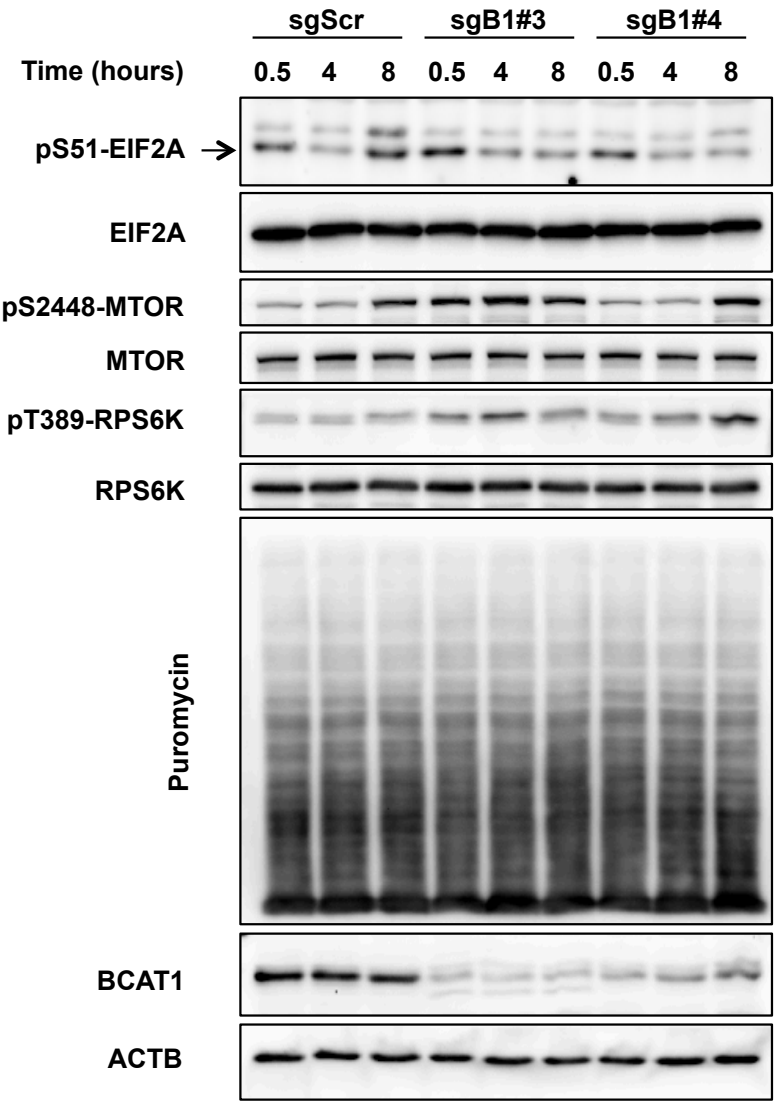
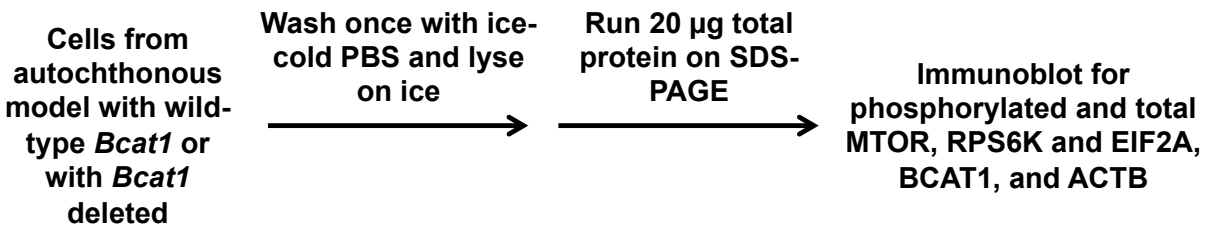

sgScr
sgB1#3
sgB1#4
Time (hours)
0.5 4 8 0.5 4 8 0.5 4 8
pS51-EIF2A
EIF2A
pS2448-MTOR
MTOR
pT389-RPS6K
RPS6K
Puromycin
BCAT1
ACTB

Figure 2.22. Conditional CRISPR-mediated knockdown of BCAT1 did not inhibit EIF2A following serum synchronization.

(A) Schematic of experiment to assess mTORC1 activity, protein translation, and EIF2A inhibition in SCLC cells. KP1-Cas9 cells transduced with LV-gRNA-zeocin-sgScr, -sgB1#3, or -sgB1#4 were treated with doxycycline (dox). They were incubated in low serum medium, followed by complete medium for the indicated times. They were then pulsed with puromycin and processed for protein extract. 20 µg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for puromycin, BCAT1, total and phosphorylated RPS6K, total or phosphorylated MTOR, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment.

tumors. To help reduce the potential impact of the length of time in cell culture, we investigated the relationship between mTORC1 and the ISR in primary cancer cells that were recently cultured from tumors excised from the autochthonous GEMM. As we had previously found that these primary knockout cells exhibited reduced incorporation of puromycin into protein compared to control cells (see Figure 2.18B), we suspected that these cells could reveal a phenotype encompassing loss of BCAT1, increased activity of mTORC1, increased phosphorylation of EIF2A, and decreased puromycin incorporation. We assessed the status of these biomarkers in cell lysates (Figure 2.23A), which were previously prepared in an effort to validate the knockout of BCAT1. Unexpectedly, the cells from BCAT1 knockout tumors had differing mTORC1 phenotypes; one had decreased autophosphorylation of MTOR, whereas the other may have had increased autophosphorylation (Figure 2.23B). The phosphorylation of RPS6K appeared to be similar between all of the cell lines. The phosphorylation of EIF2A was mixed, with cells from one knockout tumor displaying increased phosphorylation, and the other displaying reduced phosphorylation relative to the controls. Thus, the mixed phosphorylation of MTOR, the lack of effect on RPS6K, and the mixed phosphorylation of EIF2A also suggested that mTORC1 did not regulate the phosphorylation of EIF2A in SCLC cells. The fact that the overwhelming majority of our data fail to establish a link between mTORC1 and the phosphorylation of EIF2A indicated that mTORC1 did not induce the ISR in SCLC cells. Additionally, our data was consistent with the concept that the activation of mTORC1 following knockdown of BCAT1 did not increase protein translation in SCLC cells.

A


Cells from autochthonous model with wild-type *Bcat1* or with *Bcat1* deleted
Wash once with ice-cold PBS and lyse on ice
Run 20 µg total protein on SDS-PAGE
Immunoblot for phosphorylated and total MTOR, RPS6K and EIF2A, BCAT1, and ACTB

B

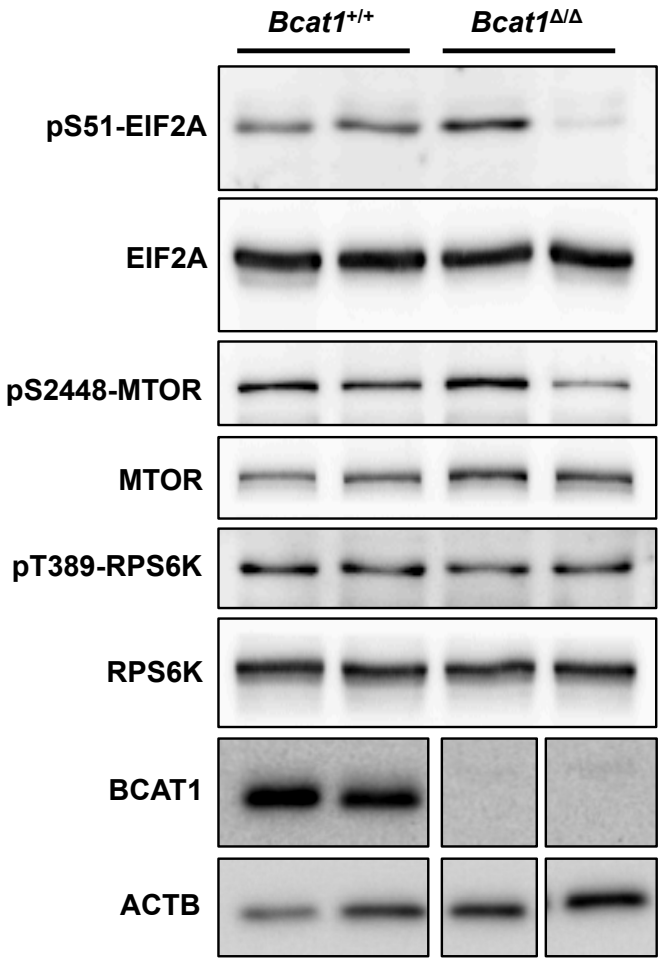

Bcat1$^{+/+}$
Bcat1$^{\Delta/\Delta}$
pS51-EIF2A
EIF2A
pS2448-MTOR
MTOR
pT389-RPS6K
RPS6K
BCAT1
ACTB

Figure 2.23. Deletion of BCAT1 in primary cancer cells did not produce a consistent pattern of mTORC1 activity and EIF2A phosphorylation.

(A) Schematic of experiment to assess mTORC1 activity and EIF2A inhibition in SCLC cells. Primary cancer cells harvested from *Bcat1*$^{+/+}$ (left two lanes) and *Bcat1*$^{\Delta/\Delta}$ (right two lanes) lung tumors (figure 2.9) were cultured in complete medium and processed for protein extract. 20 µg of total protein was run on SDS-PAGE gels. (B) Immunoblot for BCAT1, total and phosphorylated RPS6K, total or phosphorylated MTOR, and total and phosphorylated EIF2A. ACTB blot verified equal loading of total protein. The original BCAT1 and ACTB blots were produced by Kim, KB and displayed protein from more than four samples, but the EIF2A, MTOR and RPS6K blots were produced by Butler, N and only used four of those same samples. Gaps within the BCAT1 and ACTB blots were to omit some samples to allow vertical matching to the other blots. Immunoblot is representative of one experiment.

2.2.12 Knockdown of BCAT1 does not decrease autophagy in SCLC cells

Our data indicated that when BCAT1 was inhibited, SCLC cells exhibited decreased growth in soft agar (see Figures 2.3 and 2.4), decreased transamination of leucine (see Figure 2.12D), and increased activity of mTORC1 (see Figures 2.15 and 2.19-2.22). The activity of mTORC1 did not appear to induce the ISR or determine the rate of protein translation in SCLC cells (see Figures 2.19-2.23). However, mTORC1 can regulate multiple other pathways that impact cell growth, such as autophagy (Hosokawa et al., 2009; Jung et al., 2009; Kim et al., 2011; Rabanal-Ruiz et al., 2017). Autophagy is a normal housekeeping process in which a cell sequesters its own macromolecules and organelles to degrade them. This helps to maintain cellular homeostasis by recycling old and damaged components, and can relieve stress due to factors such as amino acid insufficiency and the production of reactive oxygen species. Importantly, autophagy can promote cancer cell survival. For example, in hepatocellular carcinoma cells, BCAT1 was found to induce chemoresistance to cisplatin, which was attenuated by chloroquine, i.e., an inhibitor of autophagy (Zheng et al., 2016).

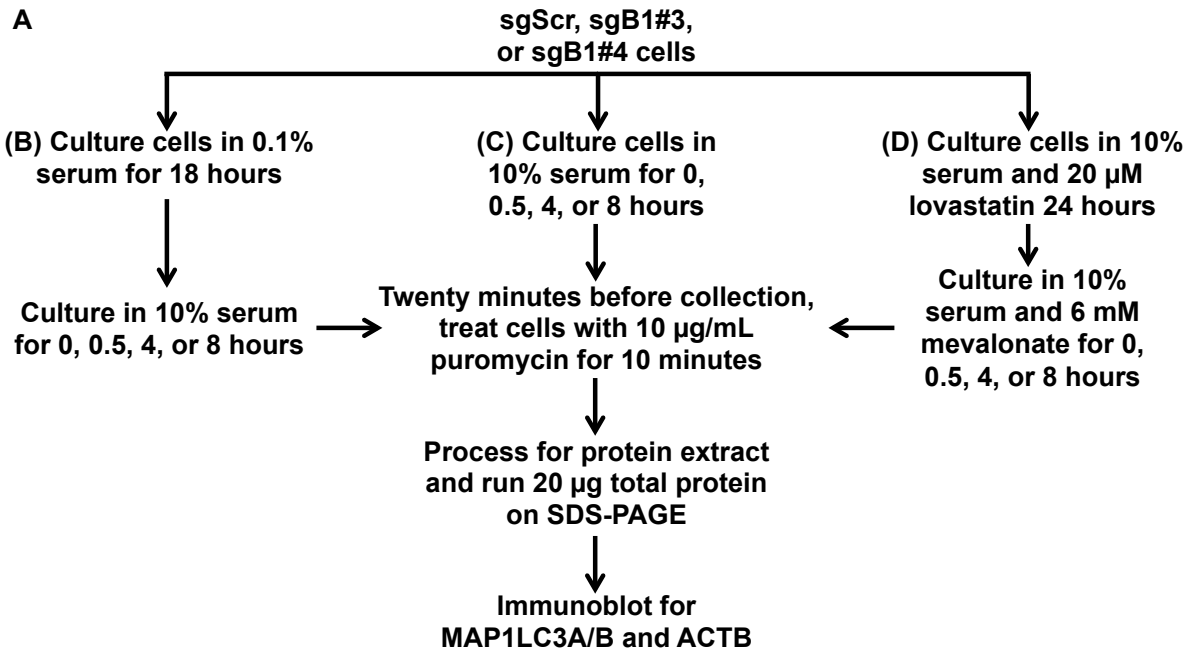
Since the inhibition of BCAT1 in SCLC cells enhanced the activity of mTORC1, and mTORC1 inhibits autophagy, we hypothesized that knockdown of BCAT1 could inhibit autophagy in SCLC. This concept was supported by the fact that ectopic expression of BCAT1 in hepatocellular carcinoma cells increased the expression of autophagy-related genes, whereas knockdown of BCAT1 reduced the expression of autophagy-related genes (Zheng et al., 2016). We investigated the relationship between

BCAT1 and autophagy in sgScr, sgB1#3, and sgB1#4 cells through immunoblot analysis of MAP1LC3A/B-II levels (Figure 2.24); measurement of MAP1LC3A/B-II is a common first step in investigating autophagy. The lysates analyzed were the same ones from the experiments described in section 2.2.10, which spanned multiple conditions of either synchronizing cells or not. The amount of MAP1LC3A/B-II in the lysates was not noticeably decreased in BCAT1-targeting cells compared to control, which indicated that the inhibition of BCAT1 did not decrease autophagy in SCLC cells.

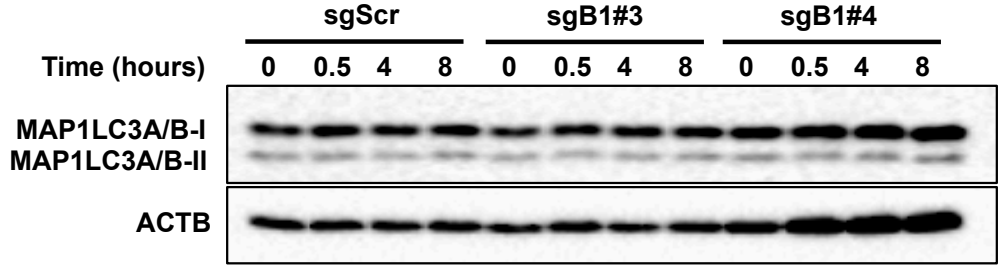
2.2.13 Knockdown of BCAT1 does not increase mitochondrial function in SCLC cells

BCAT1 has been shown to increase the activity of mTORC1 in breast cancer cells, which in turn enhanced mitochondrial biogenesis (Zhang & Han, 2017). Additionally, BCAA transamination utilizes α-KG, which is an important component of the TCA cycle that generates intermediates for oxidative phosphorylation (Zdzisińska et al., 2017). Our data indicated that the inhibition of BCAT1 reduced the transamination of leucine and activated mTORC1 in SCLC cells. We also postulated that the inhibition of BCAT1 could increase the intracellular levels of α-KG. Therefore, we hypothesized that the inhibition of BCAT1 could increase mitochondrial function in SCLC. Importantly, mitochondrial respiration is the primary source of reactive oxygen species (ROS) in cells, and while ROS have been implicated in tumorigenesis, high levels of ROS can induce cell death (Alfadda & Sallam, 2012). We postulated that high levels of ROS could inhibit the growth of SCLC cells; therefore, we also sought to determine whether

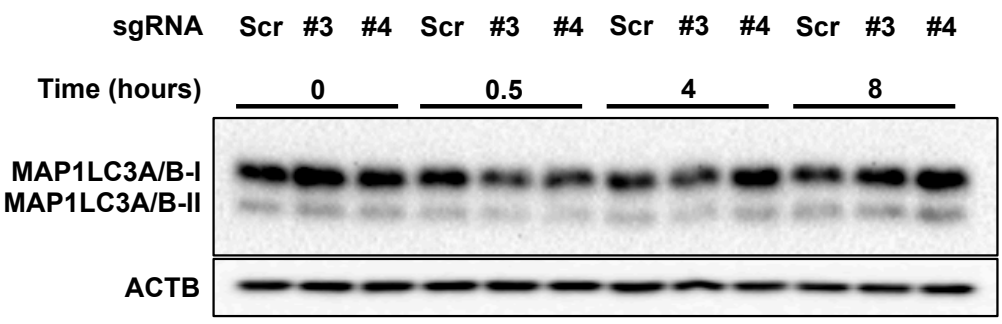
A


sgScr, sgB1#3,
or sgB1#4 cells
(B) Culture cells in 0.1%
serum for 18 hours
(C) Culture cells in
10% serum for 0,
0.5, 4, or 8 hours
(D) Culture cells in 10%
serum and 20 μM
lovastatin 24 hours
Culture in 10% serum
for 0, 0.5, 4, or 8 hours
Twenty minutes before collection,
treat cells with 10 μg/mL
puromycin for 10 minutes
Culture in 10%
serum and 6 mM
mevalonate for 0,
0.5, 4, or 8 hours
Process for protein extract
and run 20 μg total protein
on SDS-PAGE
Immunoblot for
MAP1LC3A/B and ACTB

B


sgScr
sgB1#3
sgB1#4
Time (hours)
0 0.5 4 8 0 0.5 4 8 0 0.5 4 8
MAP1LC3A/B-I
MAP1LC3A/B-II
ACTB

C


sgRNA
Scr #3 #4 Scr #3 #4 Scr #3 #4 Scr #3 #4
Time (hours)
0
0.5
4
8
MAP1LC3A/B-I
MAP1LC3A/B-II
ACTB

D

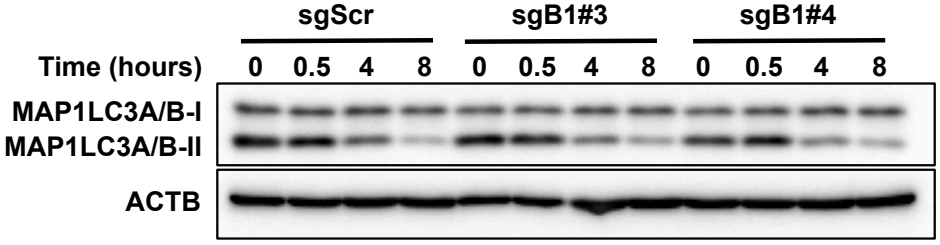

sgScr
sgB1#3
sgB1#4
Time (hours)
0 0.5 4 8 0 0.5 4 8 0 0.5 4 8
MAP1LC3A/B-I
MAP1LC3A/B-II
ACTB

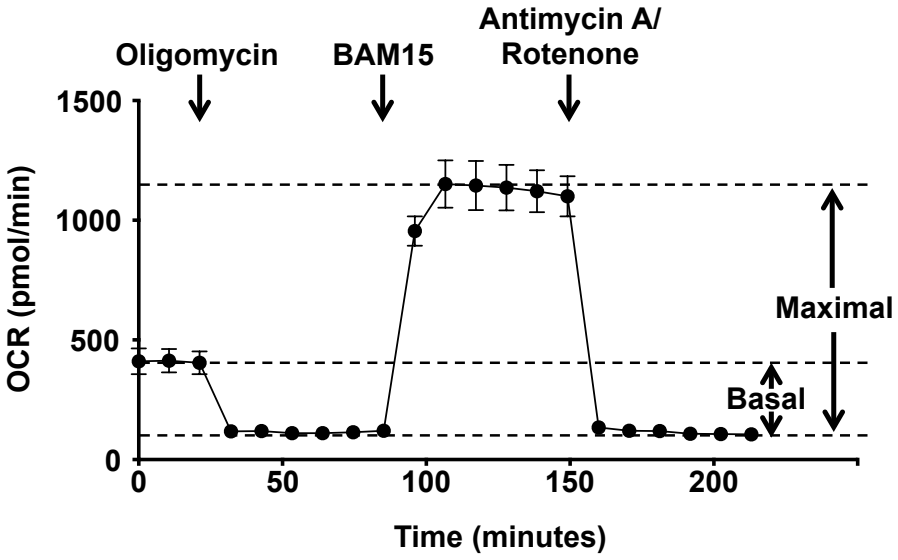
Figure 2.24. CRISPR-mediated knockdown of BCAT1 did not affect autophagy.

(A) Schematic of experiments to assess autophagy in SCLC cells. Lysates in B-D were the same as those analyzed in figures 2.19-2.21. ACTB blots were the same as in figures 2.19-2.21. 20 μg of total protein from each sample was run on SDS-PAGE gels. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. (B) Immunoblot for MAP1LC3A/B-II in cells following serum synchronization. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment. (C) Immunoblot for MAP1LC3A/B-II in cells without synchronization. ACTB blot verified equal loading of total protein. Immunoblot representative of one experiment. (D) Immunoblot for MAP1LC3A/B-II in cells following synchronization with lovastatin. ACTB blot verified equal loading of total protein. Immunoblot representative of two independent experiments.

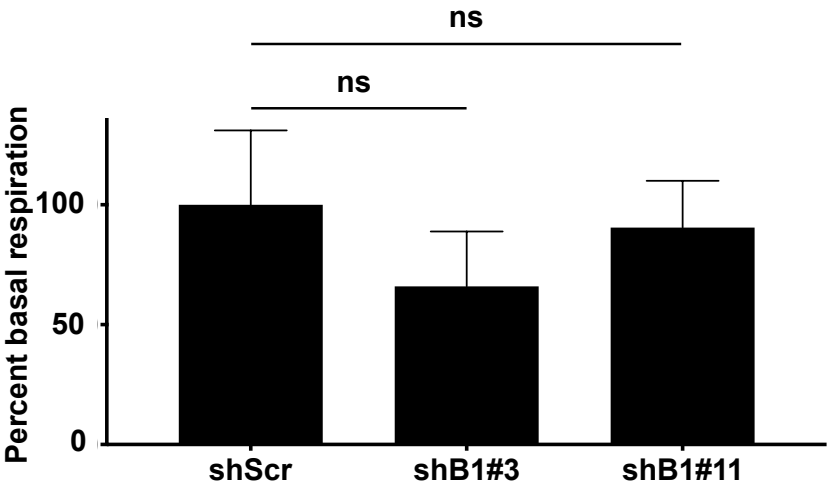
the inhibition of BCAT1 increased the production of ROS.

To test the hypothesis that inhibition of BCAT1 increased mitochondrial function, which depletes oxygen, we assessed the rate of oxygen consumption in shRNA cells using the Agilent Seahorse mito stress test (Figure 2.25A). In this assay, cells were adhered to the bottom of XF24 cell culture microplates, which were placed into an XF analyzer with probes to measure the dissolved oxygen in the medium. Over time, the cells were automatically treated with compounds, including oligomycin, which is an ATP synthase (mitochondrial complex V) inhibitor, BAM15, which is a mitochondrial protonophore uncoupler, antimycin A, which is a complex III inhibitor, and rotenone, which is a complex I inhibitor. By subtracting the lowest rate of oxygen consumption obtained following treatment with antimycin A and rotenone from the last rate obtained before treatment with oligomycin, we determined the cells' basal respiration. Neither shB1#3 nor shB1#11 cells exhibited significantly different basal respiration compared to shScr cells (shB1#3: $p=0.43$; shB1#11: $p=0.81$) (Figure 2.25B). By subtracting the lowest rate of oxygen consumption obtained following treatment with antimycin A and rotenone from the maximum rate obtained following treatment with BAM15, we determined the cells' maximal respiration. shB1#3 cells exhibited significantly decreased maximal respiration compared to shScr cells, but shB1#11 cells did not (shB1#3: $p=0.032$; shB1#11: $p=0.81$) (Figure 2.25C). Thus we were unable to conclude the effect of BCAT1 on mitochondrial function from this experiment. To further test the role of BCAT1 in SCLC mitochondrial function, we next assessed the rates of oxygen consumption in sgScr, sgB1#1, sgB1#2, sgB1#3, and sgB1#4 cells (Figure 2.26A).

A


Oligomycin
BAM15
Antimycin A/
Rotenone
1500
1000
500
0
OCR (pmol/min)
Maximal
Basal
0
50
100
150
200
Time (minutes)

B


ns
ns
Percent basal respiration
100
50
0
shScr
shB1#3
shB1#11

C

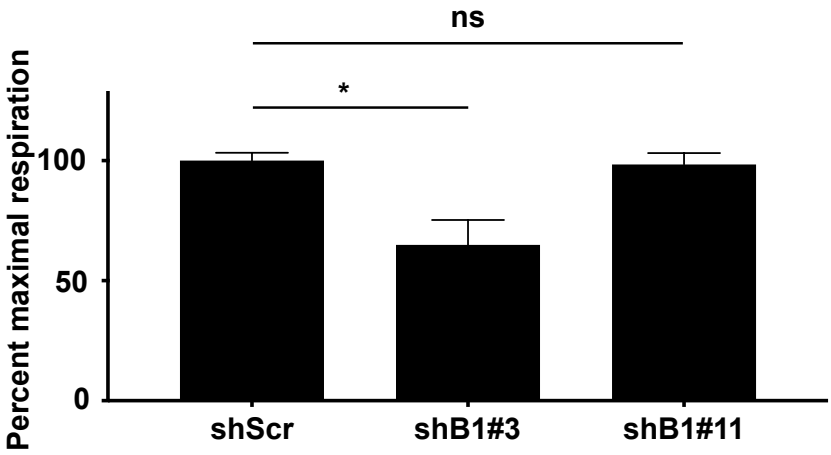
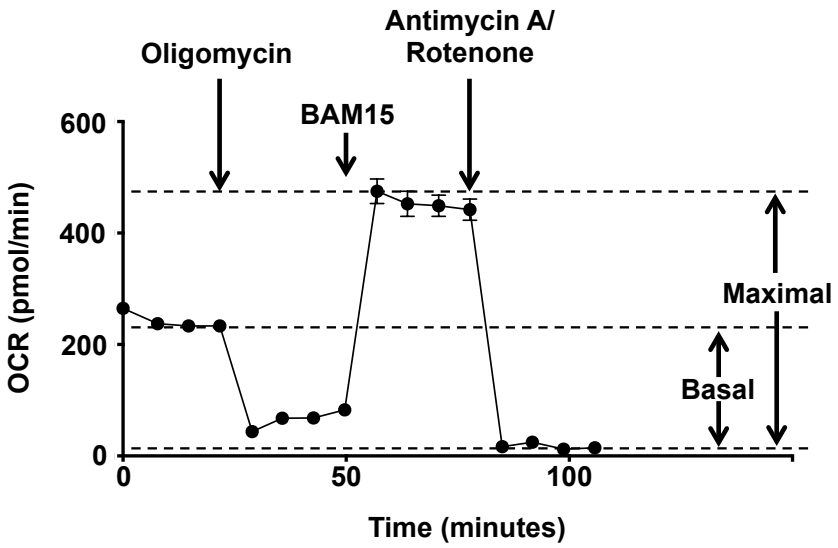

ns
*
Percent maximal respiration
100
50
0
shScr
shB1#3
shB1#11

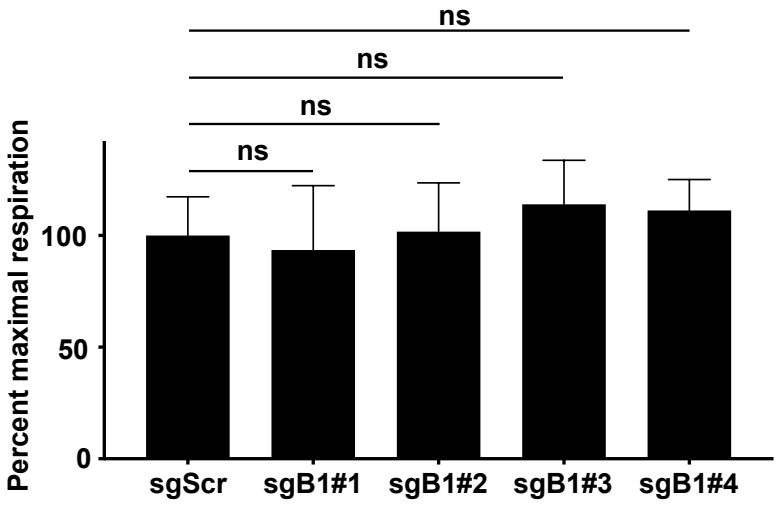
Figure 2.25. shRNA-mediated knockdown BCAT1 did not increase mitochondrial function.

(A) Mitochondrial function was assessed in KP1 cells, which were transduced with Tet-pLKO-puro-shScr, -shB1#3, or -shB1#11 and treated with doxycycline (dox). Cells were treated at the indicated time points with oligomycin, BAM15, or antimycin A and rotenone. Representative oxygen consumption rate (OCR) profile of shScr cells from one of three independent experiments. Data are mean pmol O_2 per minute ± standard error of mean. (B) Basal oxygen consumption was calculated. Data are mean pmol O_2 per minute ± standard error of mean relative to shScr and are the average of three independent experiments. (C) Maximal oxygen consumption was calculated. Data are mean pmol O_2 per minute ± standard error of mean relative to shScr and are the average of three independent experiments. shScr: scrambled shRNA control; shB1: shRNA targeting BCAT1. n=4 replicates per cell type. ns: not significant, $p>0.05$ (B) & (C); *: $p<0.05$ (C), two-tailed Student's t-test.

A


Oligomycin
Antimycin A/
Rotenone
BAM15
600
400
200
0
OCR (pmol/min)
Maximal
Basal
0
50
100
Time (minutes)

B


ns
ns
ns
ns
Percent maximal respiration
100
50
0
sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4

C

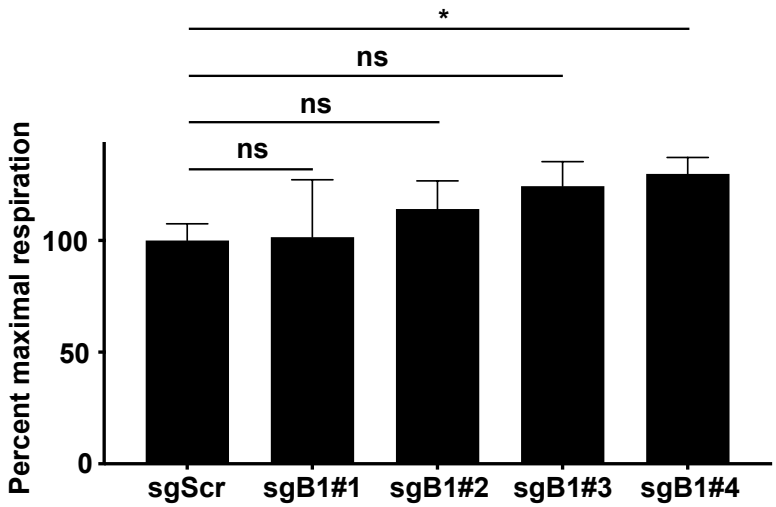

*
ns
ns
ns
Percent maximal respiration
100
50
0
sgScr
sgB1#1
sgB1#2
sgB1#3
sgB1#4

Figure 2.26. CRISPR-mediated knockdown BCAT1 did not increase mitochondrial function.

(A) Mitochondrial function was assessed in KP1 cells, which were transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, sgB1#2, sgB1#3, or sgB1#4. The cells were treated at the indicated time points with oligomycin, BAM15, or antimycin A and rotenone. Representative oxygen consumption rate (OCR) profile of sgScr cells from one of three independent experiments. Data are mean pmol O_2 per minute ± standard error of mean. (B) Basal oxygen consumption was calculated. Data are mean pmol O_2 per minute ± standard error of mean relative to sgScr and are the average of three independent experiments. (C) Maximal oxygen consumption was calculated. Data are mean pmol O_2 per minute ± standard error of mean relative to sgScr and are the average of three independent experiments. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting BCAT1. n=4 replicates per cell type. ns: not significant, $p>0.05$ (B) & (C); *: $p<0.05$ (C), two-tailed Student's t-test.

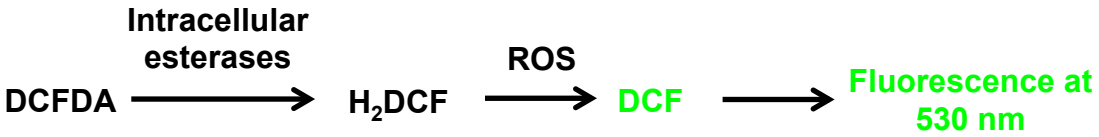
None of the BCAT1-targeting cells exhibited significantly different basal consumption compared to control (sgB1#1: p=0.86; sgB1#2: p=0.95; sgB1#3: p=0.62; sgB1#4: p=0.64) (Figure 2.26B), and only sgB1#4 cells exhibited significantly increased maximal respiration (sgB1#1: p=0.96; sgB1#2: p=0.39; sgB1#3: p=0.14; sgB1#4: p=0.047) (Figure 2.26C), which suggested that the inhibition of BCAT1 did not increase mitochondrial function.

To determine whether the inhibition of BCAT1 increased the production of ROS, which was not expected due to the insignificant effect on oxygen consumption, we treated sgScr, sgB1#3, and sgB1#4 cells with 2', 7'–dichlorofluorescin diacetate (DCFDA), which was oxidized by ROS into the fluorescent molecule 2', 7'–dichlorofluorescein (DCF) (Figure 2.27A). DCF was detected by fluorescence emitted at 535 nm. As shown in Figure 2.27B, neither of the BCAT1-targeting cells showed a significant difference in the fluorescence emitted at 535 nm compared to sgScr cells, which indicated no difference in the oxidation to DCF. This was consistent with the concept that the inhibition of BCAT1 did not increase mitochondrial function or increase the levels of ROS.

2.3 Discussion

In this chapter, we described the novel finding that BCAT1 was important for the growth of SCLC. We determined, for the first time, that the level of BCAT1 protein was

A


Intracellular
esterases
DCFDA
H2DCF
ROS
DCF
Fluorescence at
530 nm

B

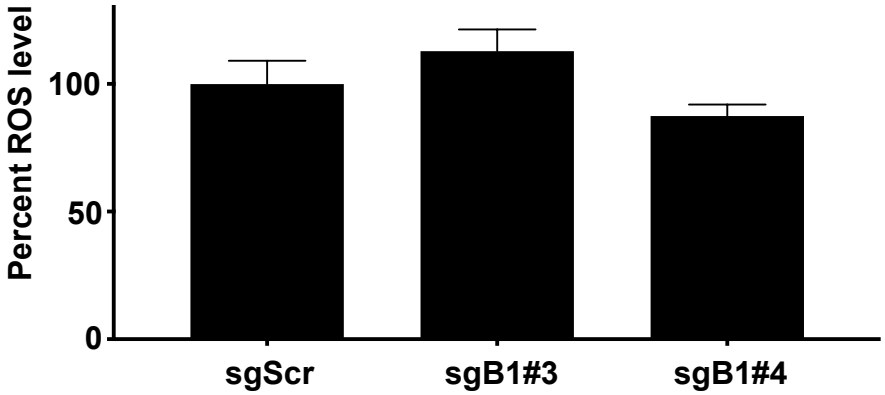

Percent ROS level
100
50
0
sgScr
sgB1#3
sgB1#4

Figure 2.27. CRISPR-mediated knockdown BCAT1 did not increase reactive oxygen species.

(A) Schematic to assess reactive oxygen species (ROS) levels in SCLC cells. KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, sgB1#2, sgB1#3, or sgB1#4 were washed, stained with DCFDA, and washed again. The fluorescence at 530 nm was used as a readout for ROS. (B) Quantification of ROS levels. n=2 replicates per cell type. Representative result from one of three independent experiments. Data are mean fluorescence ± standard error of mean relative to sgScr. sgScr: scrambled sgRNA control; sgB1: sgRNA targeting Bcat1.

increased in mouse SCLC cells relative to precancerous cells and that BCAT1 was detectable in human SCLC cell lines. We also asked whether BCAT1 regulates the growth of SCLC cells and found that knockdown of BCAT1 with multiple genetic models resulted in decreased growth of SCLC cells in soft agar. Additionally, following deletion of *Bcat1*, we observed decreased tumor burden in an autochthonous GEMM. To determine the mechanism of action of BCAT1 in SCLC, we investigated metabolites of BCAT1 as well as related signaling pathways. In those studies, we found that knockdown of BCAT1 reduced the transamination of leucine without affecting downstream oxidation of KIC. We also found that knockdown of BCAT1 resulted in decreased intracellular levels of non-essential amino acids, such as glutamate, glutamine and aspartate. Interestingly, knockdown of BCAT1 increased the activity of mTORC1 as measured by phosphorylation of RPS6K and autophosphorylation of MTOR without a corresponding increase in protein translation. Taken together, the decreased growth of SCLC in soft agar and in the autochthonous GEMM following inhibition of BCAT1 indicated that BCAT1 promoted the growth of SCLC both *in vitro* and *in vivo*. While we have not yet determined the specific mechanism of action of BCAT1 in SCLC, our current data provides possibilities to investigate further, such as production of KIC, maintenance of non-essential amino acid levels, or regulation of mTORC1.

Aberrant metabolism is a hallmark of cancer (Hanahan & Weinberg, 2011) and is an attractive target to treat tumors for which standard chemotherapy does not produce durable responses, such as SCLC (Farago & Keane, 2018). In our previous study, we

found that expression of *Myc* family members in preSC was associated with increased expression of *Bcat1* mRNA (Kim et al., 2016). Since BCAA metabolism and the role of *Bcat1* expression was previously uncharacterized in SCLC, we used SCLC as a model and set out to determine the role of *Bcat1* in tumor development and growth. We first asked what the expression of BCAT1 was in SCLC. We found that the expression of *Bcat1* transcript and BCAT1 protein was strikingly increased in mouse SCLC compared to preSC (Figure 2.1A and B). We also found that BCAT1 protein expression was detectable in human SCLC (Figure 2.1C). This raised the possibility that BCAT1 promoted the development of SCLC.

We next asked whether BCAT1 was sufficient to induce the cancerous transformation of preSC. One feature of the transformation from preSC to SCLC is increased anchorage-independent growth (Kim et al., 2016). Unexpectedly, we found ectopic expression of BCAT1 protein did not increase the number of preSC colonies that grew in soft agar (Figure 2.2), which indicated that BCAT1 alone was not sufficient to induce their anchorage-independent growth and suggested that BCAT1 alone was also not sufficient to induce the cancerous transformation of preSC. However, we did not determine whether expression of *Bcat1* was required for the cancerous transformation of preSC cells. MYC family members bind to the promoters of many genes (Fernandez et al., 2003; Li et al., 2003) and the oncogenic effects of *Mycl* might require a network of alterations in addition to aberrant expression of *Bcat1*. It is possible that inhibiting *Bcat1* gene expression could perturb the necessary network of alterations and inhibit the anchorage-independent growth of preSC cells. Thus, future studies

should compare the growth of preSC cells in soft agar with and without inhibition of BCAT1.

The elevated expression of BCAT1 in mouse SCLC relative to preSC raised the question of whether it had a role in the growth of SCLC cells. We found that genetic knockdown of BCAT1 reduced the growth of SCLC cells in soft agar (Figures 2.3 and 2.4), which indicated that BCAT1 was necessary for the growth of SCLC cells. Prior to the start of this thesis project, only a couple of studies directly tested the effect of BCAT1 expression on the growth of cancer, including glioma (Tönjes et al., 2013) and nasopharyngeal carcinoma (Zhou et al., 2013). Those studies found that BCAT1 promoted glioma and nasopharyngeal carcinoma growth, and over the course of performing the work described in this thesis, other studies published similar observations and demonstrated that BCAT1 was an important mediator of the growth of a broader set of cancers (Gu et al., 2019; Hattori et al., 2017; Mayers et al., 2016; Raffel et al., 2017; Thewes et al., 2017; Wang et al., 2018; Wang et al., 2015; Ye et al., 2020; Zhang & Han, 2017; Zheng et al., 2016; Zhu et al., 2017). Thus, the decreased growth of the BCAT1 knockdown cells in soft agar adds to a growing body of evidence that indicates increased expression of BCAT1 is critical for cancer cell growth. It also raised the possibility that BCAT1 could be important for the growth of SCLC tumors.

Due to the decreased growth of SCLC cells *in vitro* following knockdown of BCAT1, we sought to determine whether the knockdown of BCAT1 would decrease the growth of SCLC tumors *in vivo*. Unexpectedly, the growth of allograft tumors arising

from cells with shRNA or sgRNA targeting *Bcat1* was not consistently decreased compared to those arising from control cells (Figures 2.5-2.7A and B; Figure 2.8B and C), which suggested that the expression of *Bcat1* might not be required for the growth of SCLC tumors. However, we found that targeting *Bcat1* with shRNA#3 or sgRNA#4 tended to result in fewer allograft tumors (Figures 2.5A and B; Figure 2.6B, Figure 2.7B), that mice injected with cells edited with sgRNA#4 had significantly increased durations of tumor-free survival as measured by the number of days to detect raised masses under the skin (Figure 2.8A), and that cells cultured from allografts arising from sgRNA#3 or sgRNA#4 cells tended to exhibit reduced knockdown of BCAT1 protein compared to the injected parental cells (Figures 2.8D). These results were consistent with the concept that *Bcat1* was necessary for the growth of SCLC tumors and suggested that cells with high expression of BCAT1 were under selective pressure to grow in subcutaneous allografts. Furthermore, we postulated that the absence of interactions between the lung microenvironment and the SCLC cells in the allograft experiments could have prevented a potential BCAT1-knockdown phenotype. Indeed, deletion of *Bcat1* in the recently developed $Rb1^{\Delta/\Delta}/Tp53^{\Delta/\Delta}/Rbl2^{\Delta/\Delta}/Bcat1^{\Delta/\Delta}$ mice, which provided the advantage of avoiding selective pressure on the cells *in vitro* and allowed for the evaluation of SCLC growth in the lung microenvironment, resulted in reduced tumor burden compared to $Rb1^{\Delta/\Delta}/Tp53^{\Delta/\Delta}/Rbl2^{\Delta/\Delta}/Bcat1^{+/+}$ mice (Figure 2.9), providing direct evidence that *Bcat1* is required for the efficient growth of SCLC *in vivo*. The reason why we were able to observe obvious differences in tumor growth in the autochthonous model, but not the allograft models remains to be determined. A likely explanation is that the autochthonous model overcame the selective pressure to

maintain elevated levels of BCAT1. For example, cells cultured from allografts arising from sgRNA#3 or sgRNA#4 cells tended to exhibit reduced knockdown of BCAT1 protein compared to the injected parental cells (Figures 2.8D), but cells cultured from tumors in the *Rb1*$^{\Delta/\Delta}$/*Tp53*$^{\Delta/\Delta}$/*Rbl2*$^{\Delta/\Delta}$/*Bcat1*$^{\Delta/\Delta}$ mice contained extremely low levels of BCAT1 protein (Figure 2.9C). This is likely because the allografts were formed from mixed populations of cells, some of which likely did not efficiently knock down BCAT1 and thus had a growth advantage, whereas *Bcat1*$^{\Delta/\Delta}$ cells had the gene deleted, avoiding expression of BCAT1 protein altogether. Taken together, the decreased growth of subcutaneous allografts arising from shB1#3 cells, the observation that sgB1#4 cell injections tended to form fewer allografts, the possible selection for high expression of BCAT1 in CRISPR allografts, and the decreased tumor burden following genetic deletion of *Bcat1* in the autochthonous GEMM suggested that *Bcat1* was required for the growth of SCLC in vivo.

Standard chemotherapy does not produce durable responses for the majority of SCLC patients, which underscores a need for novel therapies (Demedts et al., 2010). Thus, we asked whether BCAT1 could be targeted with drugs to inhibit the growth of SCLC. Unexpectedly, treatment with 20 mM gabapentin, which was the dose used to treat glioma cells (Tönjes et al., 2013), did not reduce the number of SCLC colonies in soft agar (Figure 2.10A). However, gabapentin was not originally designed to target BCAT1 (Taylor, 1997) and might have had off-target effects in the SCLC cells. Treatment with BCATc inhibitor 2, which is a small molecule inhibitor of BCAT1 (Caballero et al., 2009; Hu et al., 2006), and BCAT2 to a much lesser extent,

significantly reduced the growth of two out of three SCLC cell types tested in soft agar (Figure 2.10B and C). This further indicates that BCAT1 contributes to the growth of at least a subset of SCLC cells and supports the concept of targeting BCAT1 to reduce the growth of SCLC. Thus, the results of our growth assays add to the increasing body of research that indicates BCAT1 promotes cancer growth. Interestingly, targeting aberrant expression of *Bcat1* mRNA in hepatocellular carcinoma (Zheng et al., 2016) reduced chemoresistance to cisplatin. It would be interesting to test the hypothesis that BCATc inhibitor 2 renders SCLC cells more sensitive to chemotherapy. Patients might benefit from combining BCATc inhibitor 2 with chemotherapy, either to use lower doses of chemotherapy initially, or to increase the effect of chemotherapy following recurrence of the tumor.

We next sought to determine the mechanism of action and asked how BCAT1 changed the metabolism of BCAAs in SCLC. Following knockdown of BCAT1 or its inhibition with BI2, we observed impaired leucine transamination (Figure 2.11 and Figure 2.12) and decreased intracellular glutamate (Figure 2.13B). This was in agreement with the majority of previous work that showed BCAT1 transaminated BCAAs toward production of BCKAs and glutamate (Mayers et al., 2016), although a small number of studies reported BCAT1 actually generated BCAAs (García-Martínez et al., 2009). Thus, while the role of BCAT1 in BCAA metabolism might depend on cell type or cell context, BCAT1 promotes the transamination of leucine in SCLC. While BCAT1 did not affect downstream oxidation of KIC in SCLC cells (Figure 2.12D), which has been observed in NSCLC (Mayers et al., 2016), it would be interesting to test the

effect of KIC on SCLC growth *in vivo*. Recent work has shown that immune function can be modulated by KIC secreted by glioma cells (Silva et al., 2017), raising the possibility that KIC secreted by SCLC cells can aid in immune evasion and promote tumor growth.

Nucleotide biosynthesis, which is critical to cell division, is dependent on amino acid metabolism (Huang et al., 2018; Jones, 1980; Pedley & Benkovic, 2017). As glutamate can be utilized toward the production of glutamine and aspartate, which can then be used for de novo purine and pyrimidine synthesis, we asked whether BCAT1 increased the levels of those amino acids in SCLC cells. Indeed, following knockdown of BCAT1, we found lower intracellular levels of glutamine and aspartate (Figure 2.13B). These results are consistent with the hypothesis that BCAT1 promoted the growth of SCLC by transaminating leucine to generate glutamate for the synthesis of glutamine and aspartate in order to fuel nucleotide biosynthesis. This mechanism has recently been reported in NSCLC (Mayers et al., 2016). As glutamine and aspartate are limiting factors for cell proliferation (Birsoy et al., 2015; Sullivan et al., 2015), the increased glutamate, glutamine, and aspartate should be investigated further as a potential mechanism of action for BCAT1 in SCLC.

Previous work from the Park lab showed that SCLC cells are dependent on increased protein translation (Kim et al., 2016), which is normally stimulated by mTORC1 in response to amino acids, e.g., leucine, and growth factors. Here, the altered metabolism of BCAAs raised the question of how BCAT1 influenced mTORC1 and protein translation. We found that CRISPR-mediated knockdown of BCAT1

increased the phosphorylation of RPS6K (Figure 2.15), which suggested that BCAT1 inhibited mTORC1. Given that leucine is a potent stimulator of mTORC1 (Jewell et al., 2015), and that we found that BCAT1 increased leucine transamination in SCLC cells, this result was expected. However, the observation that BCAT1 inhibited mTORC1 led us to expect decreased protein translation, which would be inconsistent with the concept that BCAT1 promoted the growth of SCLC. Surprisingly, there was no difference in the amount of puromycin incorporated into protein following CRISPR-mediated knockdown of BCAT1 (Figure 2.17B), which indicated that BCAT1 did not inhibit protein translation. Interestingly, shRNA-mediated knockdown of BCAT1 (Figure 2.16B), or deletion of *Bcat1* in cells cultured from the autochthonous model (Figure 2.18B), was associated with decreased protein translation, which suggested that BCAT1 might actually promote protein translation in SCLC, despite inhibiting mTORC1.

As the previously mentioned findings contradict conventional wisdom, which dictates the increased activity of mTORC1 should enhance protein translation (Showkat et al., 2014), we sought to elucidate how inhibition of BCAT1 could activate a positive regulator of translation, but not increase translation. One possibility was that inhibition of BCAT1 disrupted energy homeostasis: translation requires a significant amount of ATP and activation of mTORC1 has been shown to result in a lower ATP/AMP ratio in kidney cells (Ito et al., 2011). Also, the products of BCAT1, including α-KG and BCKAs, can be used to fuel the TCA cycle and generate ATP (Harper et al., 1984). However, oxidation of KIC was not significantly affected by inhibition of BCAT1 (Figure 2.12D) and we did not observe significant differences in mitochondrial function (Figure 2.25 and Figure

2.26), which suggested BCAT1 was not likely to regulate ATP levels. This finding was similar to that observed in pancreatic ductal adenocarcinoma and NSCLC (Mayers et al., 2016).

We next asked whether BCAT1 could regulate the integrated stress response, which would detect amino acid insufficiency through GCN2, oxidative stress through HRI, ER stress through PERK, and viral infection through PKR (Pakos-Zebrucka et al., 2016). As discussed before, we observed significant decreases in the intracellular concentrations of glutamate, glutamine, and aspartate following inhibition of BCAT1 (Figure 2.13B), raising the possibility that GCN2 could have been activated. We also measured the levels of reactive oxygen species in SCLC cells because BCAT1 was recently shown to protect against oxidative stress (Hillier et al., 2018). However, we did not observe differences in the levels of reactive oxygen species following inhibition of BCAT1 (Figure 2.27), which suggested that inhibition of BCAT1 was unlikely to increase oxidative stress. Additionally, hyperactivation of mTORC1 has been shown to contribute to endoplasmic reticulum stress and induce the unfolded protein response, which could induce the ISR and lead to cell death if unresolved (Di Nardo et al., 2009; Ito et al., 2011; Kato et al., 2012; Ozcan et al., 2008). Consistent with this concept, the inhibition of mTORC1 with rapamycin has been reported to partially suppress the phosphorylation of PERK and EIF2A during treatment with tunicamycin, an ER stress-inducing agent, resulting in decreased apoptosis and increased cell survival (Dong et al., 2015). However, while CRISPR-mediated inhibition of BCAT1 consistently activated mTORC1, it did not consistently correlate to the phosphorylation of EIF2A (Figure 2.19-2.23). This

indicated that inhibition of BCAT1 did not result in activation of the integrated stress response. How CRISPR-mediated inhibition of BCAT1 promotes mTORC1 activity, but is not sufficient to increase protein translation remains elusive, but it might be related to decreased substrates of protein translation, e.g., glutamate, glutamine, and aspartate. It might be that when BCAT1 is inhibited, the concentrations of those amino acids are low enough to inhibit protein translation, but the concentrations of overall amino acids are high enough to avoid activation of GCN2 and phosphorylation of EIF2A. Thus, future experiments should determine whether supplementing glutamate, glutamine, or aspartate can rescue the growth of SCLC cells in soft agar.

Despite the limited treatment options and poor clinical outcomes, the treatment strategy for SCLC has remained unchanged for decades (Farago & Keane, 2018). While immunotherapies are gaining traction as useful therapeutic options, first-line immunotherapy only increases the median overall survival by 2-3 months (Horn et al., 2018; Paz-Ares et al., 2019). While few studies have explored the potential for targeting tumor metabolism in SCLC (Chalishazar et al., 2019; Huang et al., 2018; Khanzada et al., 2006; Morita et al., 2018), we are the first to show that BCAT1 is important for the growth of this tumor. Interestingly, our findings suggest that BCAT1 reduces the activity of mTORC1, but does not inhibit protein translation in SCLC. While the mechanism of action remains to be determined, and our findings are far from being applied to any novel therapeutic strategy, this study is significant in demonstrating that the inhibition of a relatively unknown metabolic enzyme, i.e., BCAT1, inhibits the growth of SCLC, a NCI-designated recalcitrant tumor.

Chapter 3: Perspectives

Cancer cells require increased synthesis of a variety of macromolecules, such as non-essential amino acids, nucleic acids, lipids, and ATP, in order to generate the proteins, DNA, RNA, and cellular membranes necessary to sustain cell division (Pavlova & Thompson, 2016). To increase macromolecule biosynthesis, cancer cells frequently exhibit altered metabolism (Hanahan & Weinberg, 2011), which could be an especially valuable therapeutic target for recalcitrant tumors, such as SCLC. Despite a poor five-year survival of 6%, the standard treatment of SCLC has not changed for the past few decades, which underscores the necessity for novel therapeutic targets (Demedts et al., 2010). Previous studies of altered amino acid metabolism in SCLC have focused on few amino acids, such as arginine and methionine. For example, arginine supports the growth of SCLC by fueling polyamine biosynthesis and through activation of mTORC1 (Chalishazar et al., 2019). Until now, the role of branched chain amino acid metabolism had not been investigated. The results described in chapter 2 provide insight into the role of BCAT1 and BCAA metabolism in the growth of SCLC. Specifically, the data focus on how BCAT1 affects SCLC growth and how BCAT1 affects the metabolism of leucine and downstream signaling in SCLC cells.

We show that BCAT1 is highly expressed in SCLC compared to preSC and that its inhibition reduced the growth of SCLC *in vitro* and *in vivo*. We also show that BCAT1 promoted the transamination of leucine without affecting further oxidation. Furthermore, inhibition of BCAT1 resulted in decreased intracellular levels of glutamate, glutamine,

and aspartate. Since glutamine and aspartate are limiting factors for cell proliferation, the aforementioned result suggests that BCAT1 might support the growth of SCLC by maintaining elevated levels of glutamine and aspartate. Inhibition of BCAT1 also increased the activity of mTORC1, which would normally be expected to increase protein translation. However, protein translation was not increased following inhibition of BCAT. We hypothesized that inhibition of BCAT1 could induce the integrated stress response, which would inhibit protein translation, but the phosphorylation of EIF2A was not consistently increased following knockdown of BCAT1. Thus, activation of the integrated stress response does not explain the disconnect between mTORC1 and protein translation in SCLC.

While this work provides a better understanding of how BCAT1 affects SCLC growth and provides potential mechanisms of action to investigate further, several questions remain about the role of BCAT1 in the cancerous transformation of preSC cells, the mechanism of action in SCLC cells, and the potential for SCLC therapy. For example, it is unclear whether the BCAT1-mediated transamination of leucine is important for the growth of SCLC. It would be interesting to investigate whether leucine is used for the synthesis of glutamate, glutamine, and aspartate in these cells, and whether the decreased levels of glutamate, glutamine, and aspartate are responsible for the decreased growth of SCLC cells following knockdown of BCAT1. Additionally, we still have not been able to explain the unexpected observation that inhibition of BCAT1 resulted in increased activity of mTORC1 without a corresponding increase in protein translation. This chapter will present hypotheses that could potentially answer these

questions, as well as experiments to test them. Finally, we will explore the potential of BCAT1 as a therapeutic target for SCLC.

3.1 What is the role of BCAT1 in the cancerous transformation of preSC cells?

In chapter 2, we found that the expression of BCAT1 was increased in SCLC compared to preSC. Based on that, we hypothesized that BCAT1 could promote the development of SCLC, which we investigated using precancerous cells with or without ectopic expression of BCAT1. We found that ectopic expression of BCAT1 did not increase the anchorage-independent growth of preSC cells, which suggested that BCAT1 was not sufficient for their cancerous transformation. However, there may still be a role for BCAT1 in preSC cells. In this section, we discuss some of those possibilities.

3.1.1 Is BCAT1 required for the transformation of preSC?

Expression of a single oncogene does not always result in cancerous transformation. For some oncogenes, even extremely potent ones such as RAS, high expression can actually result in premature senescence (Serrano et al., 1997). While we found that overexpression of BCAT1 was not sufficient for the cancerous transformation of preSC, it might participate in a network of alterations that are required for the transformation. Supporting the concept that BCAT1 is necessary, preSC cells

transformed with *Mycl* exhibited increased expression of BCAT1 (Kim et al., 2016). However, some human SCLC cells express significantly less BCAT1 than others, suggesting that some SCLC cells may find alternate routes to transformation. To test whether BCAT1 is necessary for the transformation of SCLC, we propose to knock down BCAT1 in preSC cells and assess their growth in soft agar. If knockdown cells grow in soft agar, then BCAT1 is not required for the cancerous transformation. However, if they do not grow in soft agar, then BCAT1 is required. These experiments could give insight into the mechanisms underlying the early development of SCLC, which is critical given the fact that the majority of SCLC patients present with late-stage disease.

3.1.2 How does BCAT1 promote protein synthesis in preSC cells?

In chapter 2, we found that ectopic expression of BCAT1 in preSC cells increased mTORC1 activity and increased protein translation. However, we found the opposite phenotype in SCLC cells; knockdown of BCAT1 in SCLC cells increased mTORC1 activity, which made sense, given that inhibition of BCAT1 also reduced the transamination of leucine, although it did not increase protein translation. It would be interesting to explore the mechanism behind the observation in preSC cells because it might give insight into the early development of SCLC and because there are currently no previous reports showing BCAT1 to have opposite functions in different stages of the same cancer. How BCAT1 activates mTORC1 in preSC cells may also be related to leucine. While BCAT1 usually transaminates leucine to KIC, some cells with abnormally

high expression of BCAT1 exhibit transamination of BCKAs back to BCAAs (García-Martínez et al., 2009; Hattori et al., 2017). Thus, it is possible that aberrant expression of BCAT1 in preSC cells increases the transamination of BCKAs to BCAAs, which would be predicted to activate mTORC1. To test this, we could use stable isotope tracing. For example, we could give the cells [^{13}C]-BCKA for twenty-four hours and measure the amount of [^{13}C]-BCAA in the cells by liquid chromatography/mass spectroscopy. Additionally, since the transamination would require glutamate as a nitrogen donor, we could give the cells [^{15}N]-glutamate and measure the amount of [^{15}N]-BCAA in the cells. These methods have recently been used in a report studying the function of BCAT1 in leukemia (García-Martínez et al., 2009). If preSC cells ectopically expressing BCAT1 have more [^{13}C]-BCAA or [^{15}N]-BCAA than control cells, that would suggest that BCAT1 promotes the transamination of BCKAs to BCAAs in preSC cells, leading to increased activity of mTORC1.

To determine whether the increased activity of mTORC1 was responsible for the increased protein translation, we could treat cells with rapamycin to attenuate the activation of mTORC1 back to that of control cells. In this experiment, we would expect that without rapamycin, preSC cells with BCAT1 overexpression would exhibit more incorporation of puromycin into protein than control cells, but when treated with rapamycin, the amount of puromycin incorporated would be similar to that of control cells. The results of the experiments proposed in this section could give novel insight into the development of SCLC. If inhibition of BCAT1 prevents preSC cells from forming colonies in soft agar, that would suggest that BCAT1 is required for the transformation

to preSC. If ectopic expression of BCAT1 increases production of BCAAs and if increased activity of mTORC1 promotes protein translation, then that would suggest a model whereby BCAT1 is required for the cancerous transformation of preSC cells at least in part by promoting mTORC1 activity and increasing protein translation.

3.2 What is the mechanism of action of BCAT1 in SCLC cells?

In chapter 2, we showed that inhibition of BCAT1 reduced the growth of SCLC cells, which indicated that BCAT1 promotes their growth. A major unanswered question is how BCAT1 promotes the growth of SCLC cells; so far, we have yet to determine the specific mechanism of action. We measured some of the direct metabolites of BCAT1 and investigated a subset of pathways relevant to the metabolites, such as protein translation, the integrated stress response, autophagy, and mitochondrial respiration. While we found that those pathways were either not significantly altered or not consistently altered, there are other interesting potential mechanisms that should be explored. For example, glutamine and aspartate might be limiting factors for cell growth (Birsoy et al., 2015; Sullivan et al., 2015), hyper activation of mTORC1 could induce negative feedback loops (Elghazi et al., 2010; Takano et al., 2001; Tremblay et al., 2001), and BCKAs might modulate immune function in the native tumor microenvironment (Silva et al., 2017). This section explores potential mechanisms of action of BCAT1 in SCLC, which are informed by changes in the metabolites of BCAT1 and changes in downstream signaling.

3.2.1 Are increased levels of glutamate, glutamine, or aspartate important for SCLC growth?

In chapter 2, we found that the intracellular levels of glutamate, glutamine, and aspartate where decreased following knockdown of BCAT1. As glutamine and glutamate are important for the synthesis of nucleic acids, and they are limiting factors for cell proliferation, investigating the role of these non-essential amino acids could provide novel insight into the metabolic needs of SCLC (Birsoy et al., 2015; Sullivan et al., 2015, 2018). One question is whether BCAAs are transaminated by BCAT1 to sustain high levels of glutamate, glutamine, and aspartate, or whether BCAAs indirectly sustain those amino acids. To investigate this, we could give SCLC cells a known concentration of [^{15}N]-leucine for some time and then measure the concentration of [^{15}N]-leucine remaining in the medium after the assay, which would allow us to determine specifically how much [^{15}N]-leucine the cells have taken up. We would also measure the intracellular and extracellular concentrations of [^{15}N]-glutamate, [^{15}N]-glutamine, and [^{15}N]-aspartate to determine how much of the [^{15}N]-leucine was transaminated for the synthesis of those amino acids. If we detect labeled glutamate as well as labeled glutamine or aspartate within the cells, then that would suggest that BCAT1 transaminates leucine to fuel the biosynthesis of those amino acids and possibly nucleotides. In that case, we could then determine if BCAAs fuel nucleotide biosynthesis by giving SCLC cells [^{15}N]-leucine and measuring the amount of labeled DNA. If only glutamate is labeled, that would suggest that BCAT1 does not increase the

levels of glutamine and aspartate. We would then focus on the contribution of glutamate alone. To determine whether the increased levels of glutamate, glutamine, and aspartate are important for the growth of SCLC, we could culture BCAT1 knockdown cells in soft agar and supplement with those amino acids. We would expect that giving those amino acids to the cells would rescue their growth in soft agar. Supplementing with each amino acid alone would determine whether one of them is more important than the others. If single amino acids alone do not rescue the growth in soft agar but multiple amino acids do, it would suggest that increased levels of multiple amino acids are required for the cell growth, which could be tested by supplementing with a combination of the three.

3.2.2 What is the effect of increased activity of mTORC1?

In chapter 2, we showed that inhibition of BCAT1 reduced the growth of SCLC cells in soft agar and also increased the activity of mTORC1. As mTORC1 is normally thought to promote cell growth (Kim & Guan, 2019), and mutations in the mTORC1 pathway are frequently observed in SCLC (George et al., 2015), the observation of decreased SCLC cell growth despite increased mTORC1 activity was surprising. However, hyper activation of mTORC1 has been shown to inhibit PI3K/AKT signaling through a negative feedback loop involving activation of RPS6K and inhibition of insulin receptor substrates (Manning, 2004). As AKT promotes cell survival, a reasonable hypothesis is that high expression of BCAT1 serves to limit the activity of mTORC1 so that mTORC1 does not induce the negative feedback loop that inhibits AKT and

decreases cell survival. To test this hypothesis, we could compare the levels of phosphorylated MTOR, phosphorylated RPS6K, IRS1 and/or IRS2, and phosphorylated AKT in SCLC cells with or without knockdown of BCAT1 and with or without rapamycin. If the aforementioned hypothesis were true, then we would expect that knockdown of BCAT1 would produce increased phosphorylation of MTOR and RPS6K, decreased levels of IRS1 and/or IRS2, and decreased phosphorylation of AKT. If this were due to activation of mTORC1, then treatment with rapamycin would be expected to attenuate those effects. To determine whether mTORC1-mediated negative feedback reduces the viability of SCLC cells, we could use flow cytometry to measure the number of cells stained that are positive for Annexin V/7AAD with or without rapamycin. In that experiment, we would expect that knockdown of BCAT1 would increase the number of cells stained with of Annexin V/7AAD, and that treatment would rapamycin would attenuate that effect.

3.2.3 Are BCKAs important for SCLC growth *in vivo*?

BCKAs can be oxidized to fuel cell growth. For example, KIC can be oxidized to acetyl-CoA, supporting the synthesis of fatty acids and facilitating expansion of the cell membrane (Lee et al., 2019). However, we found that inhibition of BCAT1 did not affect downstream oxidation of KIC, making it unlikely that BCAT1 promotes the synthesis of lipids in SCLC. Alternatively, other cell types in the tumor microenvironment, such as immune cells, can be affected by cancer cell-derived BCKAs. For example, a recent report showed that BCKAs secreted by glioma cells can be taken up by macrophages

and that this reduces the phagocytic activity of the macrophages (Silva et al., 2017). Thus, it is possible that the KIC secreted by SCLC cells modulates the phagocytic activity of macrophages in order to facilitate immune evasion, which is a hallmark of cancer (Hanahan & Weinberg, 2011). To test this hypothesis, we could determine whether conditioned medium from SCLC cells affects the ability of macrophages to take up BCKAs. We would give SCLC cells [^{13}C]-BCAA and collect the medium after some time, which would contain [^{13}C]-BCKA. We would then culture macrophages in that medium and determine whether they have detectable levels of [^{13}C]-BCKA or [^{13}C]-BCAA. If they do, then that would indicate that BCKAs secreted from SCLC could be taken up by macrophages. To determine whether factors secreted by SCLC can alter the function of macrophages, we could assess the engulfment of fluorescent beads by macrophages in the presence of fresh medium or SCLC-conditioned medium. If engulfment is decreased in the conditioned medium, then that would suggest that SCLC cells secrete something that decreases phagocytic activity. To investigate the role of BCKAs, specifically, we could assess the engulfment of fluorescent beads in the presence or absence of BCKAs. If the presence of BCKAs reduces the engulfment of the beads, then that would suggest that BCAT1 facilitates immune evasion by producing KIC and negatively regulating the phagocytic activity of macrophages. If the SCLC conditioned medium reduces the phagocytic activity, but BCKAs do not, then it would be interesting to explore what other factors can be secreted by SCLC that can inhibit macrophage function.

3.3 What is the therapeutic potential for SCLC therapy?

Although SCLC only represents about 15% of lung cancer cases (Howlader et al., 2020), it is highly metastatic and often recurs with resistance to chemotherapy. Additionally, SCLC has an extremely low 5-year survival rate (Wang et al., 2017) and has been designated as a recalcitrant cancer (Gazdar & Minna, 2016), underscoring the lack of progress made toward therapeutic improvement. The identification and characterization of novel therapeutic targets is urgently needed in order to improve patient outcomes. Interestingly, the metabolism of BCAAs is gaining traction as an important mediator of tumor growth; over the past decade, BCAT1 has been shown to promote the growth of several types of cancer cells (Mayers et al., 2016; Tönjes et al., 2013; Wang et al., 2015; Zhang & Han, 2017; Zhou et al., 2013). However, the role of BCAT1 in SCLC growth had not been investigated previously. In this dissertation, we showed that targeting BCAT1, genetically or pharmacologically, reduced the growth of SCLC cells in soft agar. We have also shown that deletion of BCAT1 reduced the growth of SCLC tumors *in vivo* under some conditions. Those results suggest that elevated expression of BCAT1 is important for the growth of SCLC and underscore the potential utility of BCAT1 as an attractive candidate to treat SCLC. This section explores the potential for BCAT1 to be targeted pharmacologically for future treatment of SCLC.

3.3.1 Does pharmacological inhibition of BCAT1 reduce SCLC growth in mice?

An ideal drug target for cancer is one that is not widely expressed in non-cancerous cells, one that significantly reduces cancer growth when inhibited, and one that can be inhibited without severe toxicity. Unlike BCAT2, BCAT1 is restricted to a few tissue types, especially the brain and gonads (Suryawan et al., 1998). Previous reports have shown that BCAT1 can be targeted with drugs to inhibit the growth of cancers such as glioma and leukemia (García-Martínez et al., 2009; Tönjes et al., 2013). In chapter 2, we showed that treating SCLC cells with the same concentration of gabapentin used to treat glioma cells was not effective in reducing cell growth *in vitro*. However, gabapentin has recently been shown to suppress cell proliferation independently of BCAT1 (Grankvist et al., 2018). Therefore, we postulated that gabapentin could have had unforeseen effects on SCLC cells regardless of the effect on BCAT1.

In chapter 2, we also showed that treatment with BCATc inhibitor 2 (BI2), whose only known targets are BCAT1 and BCAT2 (Goto et al., 2005), reduced the growth of SCLC cells *in vitro*. Further, we showed that knockdown of BCAT1 reduced the growth of SCLC *in vivo*. These observations raise the question of whether treatment with BI2 can reduce the growth of SCLC in the *Trp53*/*Rb*/*Rbl2* preclinical model, which is a necessary step toward investigating BI2 in humans. We propose to further investigate whether inhibition of BCAT1 could be useful for the treatment of SCLC by determining

the efficacy and toxicity of BI2 in mice. To determine the efficacy of BI2, we could treat tumor-bearing mice with BI2 or vehicle and then compare tumor burden between those two groups. If BI2 were to reduce tumor burden, then that would give further evidence that BCAT1 could be a useful target for SCLC. To assess the toxicity of BI2, we could include two groups of non-tumor-bearing mice, one group treated with vehicle and the other treated with BI2, and measure weights of the mice throughout the course of treatment. If BI2 were not toxic to non-tumor-bearing mice at the chosen dose, then the group treated with BI2 would maintain weight comparable to the group given the vehicle. However, if BI2 were toxic to non-tumor-bearing mice, then we would expect the group treated with BI2 to lose weight over time compared to the group given vehicle. Taken together, if we were to find that treatment with BI2 significantly reduced the tumor burden in tumor-bearing mice, but did not significantly reduce the weight of non-tumor-bearing mice, then that would suggest that BCAT1 could be inhibited pharmacologically to reduce the growth of SCLC and that targeting BCAT1 with BI2 would be unlikely to result in severe toxicity. As chemotherapy can produce severe toxicity, the results of this experiment could be significant in working toward an effective and safer alternative to chemotherapy.

3.3.2 Can targeting BCAT1 improve the efficacy of chemotherapy?

After initial treatment with standard chemotherapy, SCLC often recurs with resistance to the same drugs. Further, the only approved second-line chemotherapy is topotecan, which only produces a response rate of about 22% and a median overall

survival of 5 months (Hagmann et al., 2015). Thus, second-line chemotherapeutic options are limited for the majority of patients. It would benefit patients if their tumors could be made more sensitive to chemotherapy. Interestingly, BCAT1 has been shown to increase the chemoresistance of hepatocellular carcinoma cells to cisplatin (Zheng et al., 2016). Thus, it would be interesting to investigate whether treatment with BI2 could sensitize SCLC cells resistant to standard chemotherapy, such as cisplatin and etoposide. To test this possibility, we could generate chemoresistant cells by treating them with cisplatin and culturing the cells that survive. Then we could treat those cells with vehicle, chemotherapy plus vehicle, BI2 plus vehicle, or BI2 plus chemotherapy and assess their growth in soft agar. In testing this, we would expect chemotherapy plus vehicle to produce similar numbers of colonies to vehicle alone. If BI2 sensitizes cells to chemotherapy, then we would expect chemotherapy plus BI2 to produce fewer colonies. However, if BI2 plus chemotherapy produces similar numbers of colonies to chemotherapy or vehicle, then that would suggest BCAT1 does not promote chemoresistance in SCLC under the conditions of the experiment.

The data presented in this dissertation emphasize the progress made in understanding the role of BCAA metabolism in SCLC. In chapter 2, we determined that BCAT1 was not sufficient to transform preSC cells to SCLC. Future work on the role of BCAT1 in preSC should explore whether BCAT1 is required for their transformation to SCLC and determine how BCAT1 promotes protein translation in preSC cells. Importantly, our data suggested that the elevated expression of BCAT1 was important for the robust growth of SCLC. We showed that BCAT1 promoted a shift toward

increased transamination of leucine in SCLC and increased intracellular concentrations of non-essential amino acids, such as glutamate, glutamine, and aspartate. Surprisingly, our data suggest that BCAT1 reduced the activation of mTORC1 without reducing protein translation. Future studies on the mechanism of action of BCAT1 in SCLC should explore the importance of KIC in the tumor microenvironment, explore the importance of elevated levels of glutamate, glutamine, and aspartate, and determine whether mTORC1 induces a negative feedback loop through inhibition of AKT. In chapter 2, we also found that treatment with BI2 reduced the growth of SCLC *in vitro*. Future studies should assess the efficacy and safety of treating SCLC tumors with BI2 *in vivo*. While there are still gaps in our understanding of the role of BCAT1 in the development and maintenance of SCLC, it is clear that BCAA catabolism is increased in SCLC and that BCAT1 can be inhibited to reduce the growth of SCLC. Future studies will explore the possibility of using BCAT1 as a novel therapeutic target for SCLC, which is urgently needed to improve patient outcomes.

Chapter 4: Materials and methods

4.1 Cell culture

Murine precancerous neuroendocrine cells (preSC) and SCLC cells were derived from early-stage neuroendocrine lesion and primary lung tumor, respectively, developed in *Rb1/Trp53*-mutant GEMM as previously described (Schaffer et al. 2010; Kim et al, 2016). Human SCLC lines, NCI-H1184, NCI-H209, NCI-H2141, NCI-H2171, NCI-H69 and NCI-H82, were obtained from the ATCC. Human glioblastoma cell line U-87 MG was a gift from Roger Abounader at University of Virginia. PreSC and SCLC cells were routinely dissociated using 0.5% trypsin EDTA (Gibco 15400-054) and cultured in RPMI-1640 medium (Hyclone SH30096.01) supplemented to 1% penicillin/streptomycin/glutamine (Gibco 10378-016) and 10% bovine growth serum (BGS) (Hyclone SH3054.03). 293T cells, which were a gift from Amy Bouton, were routinely cultured in DMEM (Corning 10-013-CV) supplemented with 10% BGS.

4.2 Cloning and lentivirus production and concentration

A cDNA fragment encoding mouse *Bcat1* (ENSMUST00000111742.7) was generated by PCR using primers that included 5'- EcoRI and 3'-NotI sites. The cDNA was inserted between the same sites of the pENTR4 vector (Invitrogen) and then transferred to the pCW57.1 vector (a gift from David Root, Addgene plasmid # 41393)

using the LR Clonase II enzyme mix (Invitrogen). Empty vector or the pCW57.1-Bcat1 plasmid was co-transfected into 293T cells, along with psPAX2 (a gift from Didier Trono, Addgene plasmid # 12260) and pMD2.G (a gift from Didier Trono, Addgene plasmid # 12259), using polyethylenimine (Sigma Aldrich). Forty-eight hours after transfection, the viral supernatants were collected into 50 mL conical tubes and stored at 4˚C. Seventy-two hours after transfection, viral supernatants were collected again and pooled with the first collection. The pools were filtered (0.45 μm) into new 50 mL tubes and mixed with a 4X concentrator solution (40% PEG-8000 and 1.2M NaCl in PBS pH adjusted to 7.1 and filtered through 0.2 μm) at 3:1. The tubes were rotated at 4˚C overnight and centrifuged at 3220 x g for one hour. The supernatant was aspirated and the pellet was re-suspended in 0.5 mL serum-free RPMI.

Bcat1-targeting CRISPR guide RNAs were designed using the Dharmacon CRISPR design tool and are as follows; sgBcat1#1: 5'-GGATGCTCCGCGCCGTTTGC and 5'-GCAAACGGCGCGGAGCATCC, sgBcat1#2: 5'-GTCTTTCTCCAGCAAACGGCG and 5'-CGCCGTTTGCTGGAGAAAGAC, sgBcat1#3: 5'-GCGCCGTTTGCTGGAGAAAG and 5'-CTTTCTCCAGCAAACGGCGC, sgBcat1#4: 5'-GAAGACCAGCGAATCCGGGTC and 5'-GACCCGGATTCGCTGGTCTTC. As control, a scramble sequence was generated from guide #1 using the Invivogen Scramble siRNA tool: 5'-GCGGTGTCCCGTCCGTAGCT and 5'-AGCTACGGACGGGACACCGC. The oligos were synthesized by Invitrogen, annealed in a BioRad MyCycler, and inserted into the pL-CRISPR.EFS.tRFP vector (a gift from

Benjamin Ebert, Addgene plasmid # 57819) following the published protocol. Lentivirus production and concentration was as described above.

4.3 Generation of BCAT1 over-expression and knockdown cells

To generate over-expression cells, $1x10^6$ preSC cells were plated in each of 6-cm dishes the evening before concentrating and adding virus. Following addition of virus, the medium was changed to fresh RPMI-1640 containing 0.2 mM glutamine, 10% BGS and 5 µg/mL polybrene. Following an overnight incubation in virus, the cells were cultured in complete RPMI for another day. Forty-eight hours after infection, the cells were selected with 0.25 µg/mL puromycin, which was refreshed every two days until cells on the uninfected control plate were completely killed, which takes six days. To induce expression of BCAT1, cells were treated with 0.5 µg/mL doxycycline for forty-eight hours. The third plate was left without virus to be used as a control for puromycin selection.

To generate CRISPR-based knockout cells, $1x10^6$ KP1 mouse SCLC cells were seeded in wells of a 6-well plate the evening before concentrating and adding virus. The cells were incubated in RPMI1640, glutamine, BGS, polybrene and scramble or Bcat1-targeting virus overnight. The next day, expression of RFP was confirmed by fluorescent microscope and the cells were FACS sorted based on RFP to obtain only the positive populations.

4.4 Immunoblot analyses and antibodies

Whole cell lysates used in Western blot analyses were prepared using RIPA buffer with Pierce protease inhibitor mini tablet added. Lysates were normalized using the Micro BCA Protein Assay Kit from Thermo Scientific and analyzed using 10, 12.5 or 15% SDS-PAGE gels. Proteins separated in these gels were then transferred to Amersham Hybond PVDF membranes (GE). Membranes were blocked for one hour in 5% non-fat dry milk in TBST and probed overnight at 4˚C with primary antibodies diluted in 3% milk in TBST. After washing three times for five minutes in TBST, secondary antibodies were diluted in 3% milk in TBST and transferred on top of each membrane. After a two hour room temperature incubation in secondary antibody and washing with TBST, each membrane was briefly incubated in Pierce ECL Western Blotting Substrate and imaged using the chemiluminescent function of the BioRad Chemidoc XRS. Primary antibodies specific to phospho-Thr389-RPS6K (#9234), RPS6K (#9202), phospho-Ser65-EIF4EBP1 (#9451), EIF4EBP1 (#9452), phospho-Ser51-EIF2A (#9721), EIF2A (#9722), and MAP1LC3A/B (#12741) were obtained from Cell Signaling Technologies. Primary antibody specific to phospho-MTOR (sc-293133), MTOR (sc-517464) ACTB (sc-8432), and GAPDH (sc-166574) was obtained from Santa Cruz Biotechnology. Primary antibodies specific for BCAT1 were obtained from Invitrogen (MA5-25892) and Origene (TA504360). Primary antibody specific for puromycin (MABE343) was obtained from EMD Millipore. Secondary anti-mouse (sc-516102) and anti-rabbit (sc-2357) antibodies were obtained from Santa Cruz Biotechnology.

4.5 Soft agar colony formation assays

UltraPure LMP Agarose (Invitrogen 16520-100) was dissolved at 0.8% and 1% in water and autoclaved. 2X RPMI was prepared by mixing one packet of RMPI Medium 1640 (Gibco 31800-022) with 500 mL H_2O and two grams $NaHCO_3$ and adjusted to pH 7.4. The RPMI was filtered (0.2 μm) and just prior to plating, it was pre-warmed and supplemented with 20% BGS and 0.4 mM glutamine. For the bottom layer, 1% agarose and 2X RPMI was mixed 1:1 and 0.5 mL was transferred to wells of a 12-well plate. After letting solidify for one hour, each cell line was dissociated, suspended in 2X RPMI and mixed 1:1 with 0.8% agarose.

To test the effect of BI2 on colony formation, 0.5 mL ($1x10^4$ cells) of each cell/agarose mixture was transferred on top of the bottom layer of six wells of 12-well plates. After letting solidify for two hours, three wells of each cell line were given complete RPMI with DMSO and three wells were given complete RPMI with 10 μM BI2. The medium was replenished every day and after three weeks the colonies were stained with nitro blue tetrazolium chloride. Each well was individually imaged with a macroscope-mounted camera and ImageJ was used to convert each image to 8-bit. Nikon NIS-Elements software was used to determine scale and measure all colonies ≥50 μm. The stained percentage of each DMSO well was averaged and the stained percentage of each BI2 well was normalized to this average to yield the percent area.

To test the effect of Bcat1 knockout on colony formation, 0.5 mL ($1x10^4$ cells) of each cell/agarose mixture was transferred on top of the bottom layer of three wells of 12-well plates. After letting solidify for two hours, each well was given complete RPMI, which was replenished every day for three weeks. After three weeks, the wells were stained and the stained percentage was determined as described above. The stained percentage of the sgScramble wells was averaged and the stained percentage of each BCAT1-targeting well was normalized to that average to yield the percent area.

4.6 Subcutaneous allografts

sgScramble, sgBCAT1#3 and sgBCAT1#4 cells were dissociated and suspended in serum-free RPMI. Subsequently, the single-cell suspensions were loaded into syringes and $0.5x10^6$ cells were injected into the right flank of 6 or 7 nude mice, which were anesthetized with 2,2,2-tibromoethanol. To induce knockout, the mice were given doxycycline chow to ingest ad libitum. To determine tumor-free survival, the mice were closely monitored each day for tumor formation. Once tumors were apparent, they were measured with calipers every day until the length grew to 1.5 cm, at which time the mouse was euthanized by 2,2,2-tibromoethanol overdose and cervical dislocation. Following euthanasia, tumors were excised from the mouse, weighed and snap-frozen on dry ice. To compare knockout in tumors to knockout in cell lines, the tumors were chopped with a razor and lysed with RIPA buffer. The protein concentrations of tumor

lysates and of cell line lysates, which were prepared at the time of injection, and the separation of protein was performed as described above. The membranes were probed for BCAT1 and ACTB and the density of each band was determined using Image Lab. The density of BCAT1 was normalized to ACTB to determine the knockout efficiency.

4.7 Assessment of leucine catabolism

$6x10^7$ preSC and SCLC cells were pre-incubated in complete RPMI with DMSO or 10 μM BI2 for twenty-four hours before assessment. Afterward, they were counted again and in duplicate, $3x10^7$ were washed twice by 5-minute centrifugation in pre-warmed Krebs buffer (128 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 5 mM D-glucose, 2 mM $CaCl_2$, 20 mM HEPES pH7.4, and 0.25 mM glutamine in H_2O) with DMSO or BI2. The cell pellets were then suspended in 2 mL Krebs buffer with ~650 CPM [U-14C] leucine, 1 mM unlabeled leucine and DMSO or BI2. The cells were transferred to 20 mL scintillation vials, which were then sealed with rubber stoppers holding a center chamber above the cells, which was subsequently filled with 0.3 mL of 2M NaOH. Additionally, two blank vials contained buffer without cells. The vials were incubated on a shaker in a 37˚C incubator for two hours and then 0.5 mL of 3% $HClO_4$ was injected into the cell solution. After a one hour incubation, 0.5 mL of 30% H_2O_2 was injected and the vials were shaken for another hour. Following the last incubation, the NaOH was pipetted out of the center chamber and into new scintillation vials containing 8 mL of scintillation solution (National Diagnostics LS-273). To determine the specific activity, 10 μL samples of the 2 mL blank solution were

transferred into new vials with scintillation fluid. CPMs were obtained using a Beckman LS 6500 and after blanking, the pmols of [14C] CO_2 from the experimental vials were calculated from the specific activity. Assessment of Bcat1 knockout cells was essentially the same, except without incubation in DMSO or BI2.

4.8 Measurement of intracellular amino acids

$2x10^6$ preSC and SCLC cells were starved of leucine by washing once in pre-warmed PBS and then incubating for one hour in DMEM with dialyzed serum and without leucine (Crystalgen 226-024). They were then centrifuged and given DMEM supplemented with dialyzed serum and 0.4 mM leucine. After a thirty minute incubation, they were washed once in ice-cold PBS, split into two eppendorf tubes and snap-frozen on dry ice. The pellets were processed and intracellular amino acids were analyzed by mass spectroscopy in the Biomolecular Analysis Facility at University of Virginia. This procedure was also used to analyze BCAAs in BCAT1 knockout cells.

4.9 Puromycin incorporation assays

After inducing BCAT1 expression or knockdown by treating cells with 0.5 µg/mL or 0.2 µg/mL doxycycline for 48 hours, $0.5x10^6$ cells were incubated in 150 µL of growth medium with 10 µg/mL puromycin for ten minutes. Afterward, cells were collected from each well, centrifuged at 300 x g for three minutes, washed once with ice-cold PBS and

lysed with RIPA buffer. 20 µg of total protein was run on two 12.5% SDS-PAGE gels and probed for puromycin or ACTB as described above. Subsequently, the ACTB membrane was stripped and probed for Bcat1.

4.10 Time course analysis of mTORC1 activity, ER stress and puromycin incorporation

sgScramble, sgBCAT1#3 and sgBCAT1#4 cells were incubated in four 6-cm dishes, each, containing low-serum (0.1%) RPMI for seventeen hours. Afterward, three plates per cell line were given growth medium with 10% BGS and incubated for half, four and eight hours; the fourth plate was left with low-serum medium as a baseline and cells were collected immediately after giving the other plates complete RPMI (zero hours). At each time point, cells were collected, washed once with ice-cold PBS and snap-frozen on dry ice. Once the last time point was collected, all pellets were lysed with RIPA buffer and protein was quantified and separated as described above. Membranes were then probed for BCAT1, ACTB, puromycin, and phosphorylated and total EIF2A, RPS6K, and EIF4EBP1.

4.11 Assessment of mitochondrial function

Mitochondrial function was assessed using the Agilent Seahorse mito stress test. Wells of a XF24 cell culture microplate were coated in Cell-Tak adhesive and allowed to

air-dry. $2.75x10^5$ cells were adhered to four wells per cell type after incubating at 37˚C for thirty minutes in 100 µL RPMI with 2 mM glutamine. The medium was changed to 675 µL unbuffered DMEM with 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose, which was brought to pH 7.35-7.4 at 37˚C. The plate was incubated at 37˚C for thirty minutes while the sensor cartridge was loaded. The plate was transferred to an XF analyzer and at the wells were treated at the indicated time points with 2 µM oligomycin, 10 µM BAM15, 2 µM antimycin A, or 0.1 µM rotenone. Basal oxygen consumption was calculated by subtracting the lowest OCR obtained following treatment with antimycin A and rotenone from the last OCR obtained before treatment with oligomycin. Maximal oxygen consumption was calculated by subtracting the lowest OCR obtained following treatment with antimycin A and rotenone from the maximum OCR obtained following treatment with BAM15.

4.12 Measurement of Reactive Oxygen Species

The levels of reactive oxygen species were determined using abcam's DCFDA cellular ROS assay kit. $7.7X10^5$ KP1 cells transduced with pL-CRISPR.EFS.tRFP-sgScr, -sgB1#1, sgB1#2, sgB1#3, or sgB1#4 were washed once with 770 µL PBS warmed to 37˚C. Cells were then stained with 770 µL DCFDA by incubating at 37˚C for thirty minutes in the dark. Cells were washed with 770 µL 1X buffer and resuspended in 220 µL 1X buffer supplemented with 10% serum. For each cell type, 100 µL ($3.5X10^5$ cells) were transferred to two wells of a black, clear-bottom ninety-six well plate. The fluorescence at 530 nm was used as a readout for ROS.

List of References

Ævarsson, A., Chuang, J. L., Max Wynn, R., Turley, S., Chuang, D. T., & Hol, W. G. J. (2000). Crystal structure of human branched-chain α-ketoacid dehydrogenase and the molecular basis of multienzyme complex deficiency in maple syrup urine disease. *Structure*, *8*(3), 277–291. https://doi.org/10.1016/S0969-2126(00)00105-2

Agarwal, M. L., Agarwal, A., Taylor, W. R., & Stark, G. R. (1995). p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(18), 8493–8497. https://doi.org/10.1073/pnas.92.18.8493

Akiyama, T., Ohuchi, T., Sumida, S., Matsumoto, K., & Toyoshima, K. (1992). Phosphorylation of the retinoblastoma protein by cdk2. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(17), 7900–7904. https://doi.org/10.1073/pnas.89.17.7900

Alain, T., Morita, M., Fonseca, B. D., Yanagiya, A., Siddiqui, N., Bhat, M., Zammit, D., Marcus, V., Metrakos, P., Voyer, L. A., Gandin, V., Liu, Y., Topisirovic, I., & Sonenberg, N. (2012). eIF4E/4E-BP ratio predicts the efficacy of mTOR targeted therapies. *Cancer Research*, *72*(24), 6468–6476. https://doi.org/10.1158/0008-5472.CAN-12-2395

Alberg, A. J., & Samet, J. M. (2003). Epidemiology of lung cancer. *Chest*, *123*(1 SUPPL.), 21S-49S. https://doi.org/10.1378/chest.123.1_suppl.21S

Alfadda, A. A., & Sallam, R. M. (2012). Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology*, *2012*, 936486.

https://doi.org/10.1155/2012/936486

Amati, B., Dalton, S., Brooks, M. W., Littlewood, T. D., Evan, G. I., & Land, H. (1992). Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max. *Nature*, *359*(6394), 423–426. https://doi.org/10.1038/359423a0

Amin, C., Wagner, A. J., & Hay, N. (1993). Sequence-specific transcriptional activation by Myc and repression by Max. *Molecular and Cellular Biology*, *13*(1), 383–390. https://doi.org/10.1128/mcb.13.1.383

Ananieva, E. A., & Wilkinson, A. C. (2018). Branched-chain amino acid metabolism in cancer. In *Current Opinion in Clinical Nutrition and Metabolic Care* (Vol. 21, Issue 1, pp. 64–70). Lippincott Williams and Wilkins. https://doi.org/10.1097/MCO.0000000000000430

Ananieva, E. A., Patel, C. H., Drake, C. H., Powell, J. D., & Hutson, S. M. (2014). Cytosolic branched chain aminotransferase (BCATc) regulates mTORC1 signaling and glycolytic metabolism in CD4- T cells. *Journal of Biological Chemistry*, *289*(27), 18793–18804. https://doi.org/10.1074/jbc.M114.554113

Antonia, S. J., López-Martin, J. A., Bendell, J., Ott, P. A., Taylor, M., Eder, J. P., Jäger, D., Pietanza, M. C., Le, D. T., de Braud, F., Morse, M. A., Ascierto, P. A., Horn, L., Amin, A., Pillai, R. N., Evans, J., Chau, I., Bono, P., Atmaca, A., … Calvo, E. (2016). Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. *The Lancet Oncology*, *17*(7), 883–895. https://doi.org/10.1016/S1470-2045(16)30098-5

Aoki, M., Blazek, E., & Vogt, P. K. (2001). A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 136–141. https://doi.org/10.1073/pnas.98.1.136

Appenzeller-Herzog, C., & Hall, M. N. (2012). Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling. In *Trends in Cell Biology* (Vol. 22, Issue 5, pp. 274–282). Elsevier. https://doi.org/10.1016/j.tcb.2012.02.006

Ardizzoni, A., Hansen, H., Dombernowsky, P., Gamucci, T., Kaplan, S., Postmus, P., Giaccone, G., Schaefer, B., Wanders, J., & Verweij, J. (1997). Topotecan, a new active drug in the second-line treatment of small-cell lung cancer: A phase II study in patients with refractory and sensitive disease. *Journal of Clinical Oncology*, *15*(5), 2090–2096. https://doi.org/10.1200/JCO.1997.15.5.2090

Asano, S., Kameyama, M., Oura, A., Morisato, A., Sakai, H., Tabuchi, Y., Chairoungdua, A., Endou, H., & Kanai, Y. (2007). L-type amino acid transporter-1 expressed in human astrocytomas, U343MGa. *Biological and Pharmaceutical Bulletin*, *30*(3), 415–422. https://doi.org/10.1248/bpb.30.415

Ashcroft, M., & Vousden, K. H. (1999). Regulation of p53 stability. In *Oncogene* (Vol. 18, Issue 53, pp. 7637–7643). Nature Publishing Group. https://doi.org/10.1038/sj.onc.1203012

Atkins, M. B., Hidalgo, M., Stadler, W. M., Logan, T. F., Dutcher, J. P., Hudes, G. R., Park, Y., Liou, S. H., Marshall, B., Boni, J. P., Dukart, G., & Sherman, M. L. (2004). Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian

target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *Journal of Clinical Oncology*, *22*(5), 909–918. https://doi.org/10.1200/JCO.2004.08.185

Augert, A., Zhang, Q., Bates, B., Cui, M., Wang, X., Wildey, G., Dowlati, A., & MacPherson, D. (2017). Small Cell Lung Cancer Exhibits Frequent Inactivating Mutations in the Histone Methyltransferase KMT2D/MLL2: CALGB 151111 (Alliance). *Journal of Thoracic Oncology*, *12*(4), 704–713. https://doi.org/10.1016/j.jtho.2016.12.011

Avrahami, L., Paz, R., Dominko, K., Hecimovic, S., Bucci, C., & Eldar-Finkelman, H. (2020). GSK-3-TSC axis governs lysosomal acidification through autophagy and endocytic pathways. *Cellular Signalling*, *71*, 109597. https://doi.org/10.1016/j.cellsig.2020.109597

Awan, F. T., Gore, L., Gao, L., Sharma, J., Lager, J., & Costa, L. J. (2016). Phase Ib trial of the PI3K/mTOR inhibitor voxtalisib (SAR245409) in combination with chemoimmunotherapy in patients with relapsed or refractory B-cell malignancies. *British Journal of Haematology*, *175*(1), 55–65. https://doi.org/10.1111/bjh.14181

Bae, Y. S., Cantley, L. G., Chen, C. S., Kim, S. R., Kwon, K. S., & Rhee, S. G. (1998). Activation of phospholipase C-γ by phosphatidylinositol 3,4,5- trisphosphate. *Journal of Biological Chemistry*, *273*(8), 4465–4469. https://doi.org/10.1074/jbc.273.8.4465

Ballester, M., Sentandreu, E., Luongo, G., Santamaria, R., Bolonio, M., Alcoriza-Balaguer, M. I., Palomino-Schätzlein, M., Pineda-Lucena, A., Castell, J., Lahoz, A.,

& Bort, R. (2019). Glutamine/glutamate metabolism rewiring in reprogrammed human hepatocyte-like cells. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-54357-x

Bar-Peled, L., Chantranupong, L., Cherniack, A. D., Chen, W. W., Ottina, K. A., Grabiner, B. C., Spear, E. D., Carter, S. L., Meyerson, M., & Sabatini, D. M. (2013). A tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science*, *340*(6136), 1100–1106. https://doi.org/10.1126/science.1232044

Bar-Peled, L., & Sabatini, D. M. (2014). Regulation of mTORC1 by amino acids. In *Trends in Cell Biology* (Vol. 24, Issue 7, pp. 400–406). Elsevier Ltd. https://doi.org/10.1016/j.tcb.2014.03.003

Bar-Peled, L., Schweitzer, L. D., Zoncu, R., & Sabatini, D. M. (2012). Ragulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. *Cell*, *150*(6), 1196–1208. https://doi.org/10.1016/j.cell.2012.07.032

Barna, M., Pusic, A., Zollo, O., Costa, M., Kondrashov, N., Rego, E., Rao, P. H., & Ruggero, D. (2008). Suppression of Myc oncogenic activity by ribosomal protein haploinsufficiency. *Nature*, *456*(7224), 971–975. https://doi.org/10.1038/nature07449

Basu, H. S., Sturkenboom, M. C. J. M., Delcros, J. G., Csokan, P. P., Szollosi, J., Feuerstein, B. G., & Marton, L. J. (1992). Effect of polyamine depletion on chromatin structure in U-87 MG human brain tumour cells. *Biochemical Journal*, *282*(3), 723–727. https://doi.org/10.1042/bj2820723

Bates, S., Ryan, K. M., Phillips, A. C., & Vousden, K. H. (1998). Cell cycle arrest and DNA endoreduplication following p2l(Waf1/Cip1) expression. *Oncogene*, *17*(13), 1691–1703. https://doi.org/10.1038/sj.onc.1202104

Bauer, C., Brass, N., Diesinger, I., Kayser, K., Grässer, F. A., & Meese, E. (2002). Overexpression of the eukaryotic translation initiation factor 4G (eIF4G-1) in squamous cell lung carcinoma. *International Journal of Cancer*, *98*(2), 181–185. https://doi.org/10.1002/ijc.10180

Bee, A., Ke, Y., Forootan, S., Lin, K., Beesley, C., Forrest, S. E., & Foster, C. S. (2006). Ribosomal protein L19 is a prognostic marker for human prostate cancer. *Clinical Cancer Research*, *12*(7 I), 2061–2065. https://doi.org/10.1158/1078-0432.CCR-05-2445

Ben-Sahra, I., Hoxhaj, G., Ricoult, S. J. H., Asara, J. M., & Manning, B. D. (2016). mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science*, *351*(6274), 728–733. https://doi.org/10.1126/science.aad0489

Ben-Shem, A., De Loubresse, N. G., Melnikov, S., Jenner, L., Yusupova, G., & Yusupov, M. (2011). The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science*, *334*(6062), 1524–1529. https://doi.org/10.1126/science.1212642

Benne, R., Brown Luedi, M. L., & Hershey, J. W. B. (1978). Purification and characterization of protein synthesis initiation factors eIF-1, eIF-4C, eIF-4D, and eIF-5 from rabbit reticulocytes. *Journal of Biological Chemistry*, *253*(9), 3070–3077.

Benne, R., & Hershey, J. W. B. (1978). The mechanism of action of protein synthesis

initiation factors from rabbit reticulocytes. *Journal of Biological Chemistry*, *253*(9), 3078–3087.

Berdis, A. J. (2017). Inhibiting DNA polymerases as a therapeutic intervention against cancer. In *Frontiers in Molecular Biosciences* (Vol. 4, p. 78). Frontiers Media S.A. https://doi.org/10.3389/fmolb.2017.00078

Bertagnolli, M. M., Yang, L., Herrmann, S. H., & Kirkman, R. L. (1994). Evidence that rapamycin inhibits interleukin-12-induced proliferation of activated T lymphocytes. *Transplantation*, *58*(10), 1091–1096. http://www.ncbi.nlm.nih.gov/pubmed/7974715

Bierer, B. E., Mattila, P. S., Standaert, R. F., Herzenberg, L. A., Burakoff, S. J., Crabtree, G., & Schreiber, S. L. (1990). Two distinct signal transmission pathways in T lymphocytes are inhibited by complexes formed between an immunophilin and either FK506 or rapamycin. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(23), 9231–9235. https://doi.org/10.1073/pnas.87.23.9231

Birsoy, K., Wang, T., Chen, W. W., Freinkman, E., Abu-Remaileh, M., & Sabatini, D. M. (2015). An Essential Role of the Mitochondrial Electron Transport Chain in Cell Proliferation Is to Enable Aspartate Synthesis. *Cell*, *162*(3), 540–551. https://doi.org/10.1016/j.cell.2015.07.016

Blazar, B. R., Taylor, P. A., Snover, D. C., Sehgal, S. N., & Vallera, D. A. (1993). Murine recipients of fully mismatched donor marrow are protected from lethal graft-versus-host disease by the in vivo administration of rapamycin but develop an autoimmune-like syndrome. *Journal of Immunology (Baltimore, Md. : 1950)*,

151(10), 5726–5741.

Blazar, B. R., Taylor, P. A., Panoskaltsis-Mortari, A., & Vallera, D. A. (1998). Rapamycin inhibits the generation of graft-versus-host disease- and graft-versus-leukemia-causing T cells by interfering with the production of Th1 or Th1 cytotoxic cytokines. *Journal of Immunology*, *160*(11), 5355–5365.

Blenis, J., Kuo, C. J., & Erikson, R. L. (1987). Identification of a ribosomal protein S6 kinase regulated by transformation and growth-promoting stimuli. *The Journal of Biological Chemistry*, *262*(30), 14373–14376.

Bommer, U. A., Noll, F., Lutsch, G., & Bielka, H. (1980). Immunochemical detection of proteins in the small subunit of rat liver ribosomes involved in binding of the ternary initiation complex. *FEBS Letters*, *111*(1), 171–174. https://doi.org/10.1016/0014-5793(80)80785-X

Bonvini, A., Coqueiro, A. Y., Tirapegui, J., Calder, P. C., & Rogero, M. M. (2018). Immunomodulatory role of branched-chain amino acids. *Nutrition Reviews*, *76*(11), 840–856. https://doi.org/10.1093/nutrit/nuy037

Bookstein, R., Shew, J. Y., Chen, P. L., Scully, P., & Lee, W. H. (1990). Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated RB gene. *Science*, *247*(4943), 712–715. https://doi.org/10.1126/science.2300823

Boon, K., Caron, H. N., Van Asperen, R., Valentijn, L., Hermus, M. C., Van Sluis, P., Roobeek, I., Weis, I., Voûte, P. A., Schwab, M., & Versteeg, R. (2001). N-myc enhances the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis. *EMBO Journal*, *20*(6), 1383–1393.

https://doi.org/10.1093/emboj/20.6.1383

Borges, M., Linnoila, R. I., Van De Velde, H. J. K., Chen, H., Nelkin, B. D., Mabry, M., Baylin, S. B., & Ball, D. W. (1997). An achaete-scute homologue essential for neuroendocrine differentiation in the lung. *Nature*, *386*(6627), 852–855. https://doi.org/10.1038/386852a0

Boros, L. G., Puigjaner, J., Cascante, M., Lee, W. N. P., Brandes, J. L., Bassilian, S., Yusuf, F. I., Williams, R. D., Muscarella, P., Melvin, W. S., & Schirmer, W. J. (1997). Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Research*, *57*(19), 4242–4248.

Borromeo, M. D., Savage, T. K., Kollipara, R. K., He, M., Augustyn, A., Osborne, J. K., Girard, L., Minna, J. D., Gazdar, A. F., Cobb, M. H., & Johnson, J. E. (2016). ASCL1 and NEUROD1 Reveal Heterogeneity in Pulmonary Neuroendocrine Tumors and Regulate Distinct Genetic Programs. *Cell Reports*, *16*(5), 1259–1272. https://doi.org/10.1016/j.celrep.2016.06.081

Bosotti, R., Isacchi, A., & Sonnhammer, E. L. L. (2000). FAT: A novel domain in PIK-related kinases. *Trends in Biochemical Sciences*, *25*(5), 225–227. https://doi.org/10.1016/S0968-0004(00)01563-2

Bowman, L. H., Rabin, B., & Schlessinger, D. (1981). Multiple ribosomal RNA cleavage pathways in mammalian cells. *Nucleic Acids Research*, *9*(19), 4951–4966. https://doi.org/10.1093/nar/9.19.4951

Brana, I., LoRusso, P., Baselga, J., Heath, E. I., Patnaik, A., Gendreau, S., Laird, A., & Papadopoulos, K. (2010). A phase I dose-escalation study of the safety,

pharmacokinetics (PK), and pharmacodynamics of XL765 (SAR245409), a PI3K/TORC1/TORC2 inhibitor administered orally to patients (pts) with advanced malignancies. *Journal of Clinical Oncology*, *28*(15_suppl), 3030–3030. https://doi.org/10.1200/jco.2010.28.15_suppl.3030

Bremner, R., Cohen, B. L., Sopta, M., Hamel, P. A., Ingles, C. J., Gallie, B. L., & Phillips, R. A. (1995). Direct transcriptional repression by pRB and its reversal by specific cyclins. *Molecular and Cellular Biology*, *15*(6), 3256–3265. https://doi.org/10.1128/mcb.15.6.3256

Brooks, C. L., & Gu, W. (2003). Ubiquitination, phosphorylation and acetylation: The molecular basis for p53 regulation. In *Current Opinion in Cell Biology* (Vol. 15, Issue 2, pp. 164–171). Elsevier Ltd. https://doi.org/10.1016/S0955-0674(03)00003-6

Brown, J. R., Hamadani, M., Arnason, J., Karlin, L., Hayslip, J., Wagner-Johnston, N., Cartron, G., Ribrag, V., de Guibert, S., Opat, S., Tilly, H., Cannell, P., Janssens, A., Offner, F., Ganguly, S., Ailawadhi, S., Millenson, M., Bron, D., Xu, Y., … Kersten, M. J. (2013). SAR245409 Monotherapy In Relapsed/Refractory Follicular Lymphoma: Preliminary Results From The Phase II ARD12130 Study. *Blood*, *122*(21), 86–86. https://doi.org/10.1182/blood.v122.21.86.86

Brown, J. R., Hamadani, M., Hayslip, J., Janssens, A., Wagner-Johnston, N., Ottmann, O., Arnason, J., Tilly, H., Millenson, M., Offner, F., Gabrail, N. Y., Ganguly, S., Ailawadh, S., Kasar, S., Kater, A. P., Doorduijn, J. K., Gao, L., Lager, J. J., Wu, B., … Kersten, M. J. (2018). Voxtalisib (XL765) in patients with relapsed or refractory non-Hodgkin lymphoma or chronic lymphocytic leukaemia: an open-label, phase 2

trial. *The Lancet Haematology*, *5*(4), e170–e180. https://doi.org/10.1016/S2352-3026(18)30030-9

Brown, N. C., Canellakis, Z. N., Lundin, B., Reichard, P., & Thelander, L. (1969). Ribonucleoside Diphosphate Reductase. Purification of the two Subunits, Proteins B1 and B2. *European Journal of Biochemistry*, *9*(4), 561–573. https://doi.org/10.1111/j.1432-1033.1969.tb00646.x

Brugarolas, J., Lei, K., Hurley, R. L., Manning, B. D., Reiling, J. H., Hafen, E., Witters, L. A., Ellisen, L. W., & Kaelin, W. G. (2004). Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes and Development*, *18*(23), 2893–2904. https://doi.org/10.1101/gad.1256804

Brunn, G. J., Fadden, P., Haystead, T. A. J., & Lawrence, J. C. (1997). The mammalian target of rapamycin phosphorylates sites having a (Ser/Thr)-Pro motif and is activated by antibodies to a region near its COOH terminus. *Journal of Biological Chemistry*, *272*(51), 32547–32550. https://doi.org/10.1074/jbc.272.51.32547

Brunn, G. J., Hudson, C. C., Sekulić, A., Williams, J. M., Hosoi, H., Houghton, P. J., Lawrence, J. C., & Abraham, R. T. (1997). Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. *Science*, *277*(5322), 99–101. https://doi.org/10.1126/science.277.5322.99

Burnett, P. E., Barrow, R. K., Cohen, N. A., Snyder, S. H., & Sabatini, D. M. (1998). RAFT1 phosphorylation of the translational regulators p70 S6 kinase and 4E-BP1. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(4), 1432–1437. https://doi.org/10.1073/pnas.95.4.1432

Caballero, J., Vergara-Jaque, A., Fernández, M., Coll, D., Caballero, J., Vergara-Jaque, · A, Fernández, M., & Coll, D. (2009). Docking and quantitative structure-activity relationship studies for sulfonyl hydrazides as inhibitors of cytosolic human branched-chain amino acid aminotransferase. *Mol Divers*, *13*, 493–500. https://doi.org/10.1007/s11030-009-9140-1

Cai, J., Fang, L., Huang, Y., Li, R., Yuan, J., Yang, Y., Zhu, X., Chen, B., Wu, J., & Li, M. (2013). miR-205 targets PTEN and PHLPP2 to augment AKT signaling and drive malignant phenotypes in non-small cell lung cancer. *Cancer Research*, *73*(17), 5402–5415. https://doi.org/10.1158/0008-5472.CAN-13-0297

Calles, A., Aguado, G., Sandoval, C., & Álvarez, R. (2019). The role of immunotherapy in small cell lung cancer. In *Clinical and Translational Oncology* (Vol. 21, Issue 8, pp. 961–976). Springer-Verlag Italia s.r.l. https://doi.org/10.1007/s12094-018-02011-9

Cano-Crespo, S., Chillarón, J., Junza, A., Fernández-Miranda, G., García, J., Polte, C., R. de la Ballina, L., Ignatova, Z., Yanes, Ó., Zorzano, A., Stephan-Otto Attolini, C., & Palacín, M. (2019). CD98hc (SLC3A2) sustains amino acid and nucleotide availability for cell cycle progression. *Scientific Reports*, *9*(1), 1–19. https://doi.org/10.1038/s41598-019-50547-9

Cao, T. T., Lin, S. H., Fu, L., Tang, Z., Che, C. M., Zhang, L. Y., Ming, X. Y., Liu, T. F., Tang, X. M., Tan, B. Bin, Xiang, D., Li, F., Chan, O. Y., Xie, D., Cai, Z., & Guan, X. Y. (2017). Eukaryotic translation initiation factor 5A2 promotes metabolic reprogramming in hepatocellular carcinoma cells. *Carcinogenesis*, *38*(1), 94–104. https://doi.org/10.1093/carcin/bgw119

Carabet, L. A., Rennie, P. S., & Cherkasov, A. (2019). Therapeutic inhibition of myc in cancer. Structural bases and computer-aided drug discovery approaches. In *International Journal of Molecular Sciences* (Vol. 20, Issue 1). MDPI AG. https://doi.org/10.3390/ijms20010120

Carl Cori, B. F., & Cori, G. T. (1925). THE CARBOHYDRATE METABOLISM OF TUMORS II. CHANGES IN THE SUGAR, LACTIC ACID, AND CO2-COMBINING POWER OF BLOOD PASSING THROUGH A TUMOR. *Journal of Biological Chemistry*, *65*(2), 397–405. http://www.jbc.org/

Carney, D. N., Gazdar, A. F., Bepler, G., Guccion, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H., & Minna, J. D. (1985). Establishment and Identification of Small Cell Lung Cancer Cell Lines Having Classic and Variant Features. *Cancer Research*, *45*(6).

Carracedo, A., Ma, L., Teruya-Feldstein, J., Rojo, F., Salmena, L., Alimonti, A., Egia, A., Sasaki, A. T., Thomas, G., Kozma, S. C., Papa, A., Nardella, C., Cantley, L. C., Baselga, J., Pandolfi, P. P., Wullschleger, S., Loewith, R., Hall, M. N., Guertin, D. A., … Chen, Z. (2008). Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *The Journal of Clinical Investigation*, *118*(9), 3065–3074. https://doi.org/10.1172/JCI34739

Carrasco, D. R., Sukhdeo, K., Protopopova, M., Sinha, R., Enos, M., Carrasco, D. E. E., Zheng, M., Mani, M., Henderson, J., Pinkus, G. S., Munshi, N., Horner, J., Ivanova, E. V, Protopopov, A., Anderson, K. C., Tonon, G., & DePinho, R. A. (2007). The Differentiation and Stress Response Factor XBP-1 Drives Multiple Myeloma Pathogenesis. *Cancer Cell*, *11*(4), 349–360.

https://doi.org/10.1016/j.ccr.2007.02.015

Castro, A. F., Rebhun, J. F., Clark, G. J., & Quilliam, L. A. (2003). Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin- and farnesylation-dependent manner. *Journal of Biological Chemistry*, *278*(35), 32493–32496. https://doi.org/10.1074/jbc.C300226200

Cazzalini, O., Scovassi, A. I., Savio, M., Stivala, L. A., & Prosperi, E. (2010). Multiple roles of the cell cycle inhibitor p21CDKN1A in the DNA damage response. In *Mutation Research - Reviews in Mutation Research* (Vol. 704, Issues 1–3, pp. 12–20). Elsevier. https://doi.org/10.1016/j.mrrev.2010.01.009

Cerella, C., Grandjenette, C., Dicato, M., & Diederich, M. (2016). Roles of Apoptosis and Cellular Senescence in Cancer and Aging. *Current Drug Targets*, *17*(4), 405–415. https://doi.org/10.2174/1389450116666150202155915

Cha, J. H., Bae, S. H., Kim, H. L., Park, N. R., Choi, E. S., Jung, E. S., Choi, J. Y., & Yoon, S. K. (2013). Branched-Chain Amino Acids Ameliorate Fibrosis and Suppress Tumor Growth in a Rat Model of Hepatocellular Carcinoma with Liver Cirrhosis. *PLoS ONE*, *8*(11), e77899. https://doi.org/10.1371/journal.pone.0077899

Chalishazar, M. D., Wait, S. J., Huang, F., Ireland, A. S., Mukhopadhyay, A., Lee, Y., Schuman, S. S., Guthrie, M. R., Berrett, K. C., Vahrenkamp, J. M., Hu, Z., Kudla, M., Modzelewska, K., Wang, G., Ingolia, N. T., Gertz, J., Lum, D. H., Cosulich, S. C., Bomalaski, J. S., … Oliver, T. G. (2019). MYC-driven small-cell lung cancer is metabolically distinct and vulnerable to arginine depletion. *Clinical Cancer Research*, *25*(16), 5107–5121. https://doi.org/10.1158/1078-0432.CCR-18-4140

Chan, D. A., Sutphin, P. D., Nguyen, P., Turcotte, S., Lai, E. W., Banh, A., Reynolds, G. E., Chi, J. T., Wu, J., Solow-Cordero, D. E., Bonnet, M., Flanagan, J. U., Bouley, D. M., Graves, E. E., Denny, W. A., Hay, M. P., & Giaccia, A. J. (2011). Targeting GLUT1 and the Warburg effect in renal cell carcinoma by chemical synthetic lethality. *Science Translational Medicine*, *3*(94), 94ra70-94ra70. https://doi.org/10.1126/scitranslmed.3002394

Chaneton, B., Hillmann, P., Zheng, L., Martin, A. C. L., Maddocks, O. D. K., Chokkathukalam, A., Coyle, J. E., Jankevics, A., Holding, F. P., Vousden, K. H., Frezza, C., O'Reilly, M., & Gottlieb, E. (2012). Serine is a natural ligand and allosteric activator of pyruvate kinase M2. *Nature*, *491*(7424), 458–462. https://doi.org/10.1038/nature11540

Chang, I. W., Wu, W. J., Wang, Y. H., Wu, T. F., Liang, P. I., He, H. L., Yeh, B. W., & Li, C. F. (2016). BCAT1 overexpression is an indicator of poor prognosis in patients with urothelial carcinomas of the upper urinary tract and urinary bladder. *Histopathology*, *68*(4), 520–532. https://doi.org/10.1111/his.12778

Chantranupong, L., Scaria, S. M., Saxton, R. A., Gygi, M. P., Shen, K., Wyant, G. A., Wang, T., Harper, J. W., Gygi, S. P., & Sabatini, D. M. (2016). The CASTOR Proteins Are Arginine Sensors for the mTORC1 Pathway. *Cell*, *165*(1), 153–164. https://doi.org/10.1016/j.cell.2016.02.035

Chauhan, D., Tian, Z., Nicholson, B., Kumar, K. G. S., Zhou, B., Carrasco, R., McDermott, J. L., Leach, C. A., Fulcinniti, M., Kodrasov, M. P., Weinstock, J., Kingsbury, W. D., Hideshima, T., Shah, P. K., Minvielle, S., Altun, M., Kessler, B. M., Orlowski, R., Richardson, P., … Anderson, K. C. (2012). A Small Molecule

Inhibitor of Ubiquitin-Specific Protease-7 Induces Apoptosis in Multiple Myeloma Cells and Overcomes Bortezomib Resistance. *Cancer Cell*, *22*(3), 345–358. https://doi.org/10.1016/j.ccr.2012.08.007

Chee, N. T., Lohse, I., & Brothers, S. P. (2019). MRNA-to-protein translation in hypoxia. In *Molecular Cancer* (Vol. 18, Issue 1, pp. 1–13). BioMed Central Ltd. https://doi.org/10.1186/s12943-019-0968-4

Chellappan, S. P., Hiebert, S., Mudryj, M., Horowitz, J. M., & Ft Nevins, J. (1991). The E2F Transcription Factor Is a Cellular Target for the RB Protein. In *Cell* (Vol. 65).

Chen, J. J., & Jones, M. E. (1976). The cellular location of dihydroorotate dehydrogenase: Relation to de novo biosynthesis of pyrimidines. *Archives of Biochemistry and Biophysics*, *176*(1), 82–90. https://doi.org/10.1016/0003-9861(76)90143-0

Chen, L., & Flies, D. B. (2013). Molecular mechanisms of T cell co-stimulation and co-inhibition. In *Nature Reviews Immunology* (Vol. 13, Issue 4, pp. 227–242). NIH Public Access. https://doi.org/10.1038/nri3405

Chen, P. L., Scully, P., Shew, J. Y., Wang, J. Y. J., & Lee, W. H. (1989). Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. *Cell*, *58*(6), 1193–1198. https://doi.org/10.1016/0092-8674(89)90517-5

Cheng, L., Rossi, F., Fang, W., Mori, T., & Cobrinik, D. (2000). Cdk2-dependent phosphorylation and functional inactivation of the pRB-related p130 protein in pRB(-), p16(INK4A)(+) tumor cells. *Journal of Biological Chemistry*, *275*(39),

30317–30325. https://doi.org/10.1074/jbc.M005707200

Chesney, J., Mitchell, R., Benigni, F., Bacher, M., Spiegel, L., Al-Abed, Y., Han, J. H., Metz, C., & Bucala, R. (1999). An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: Role in tumor cell glycolysis and the Warburg effect. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 3047–3052. https://doi.org/10.1073/pnas.96.6.3047

Chiduza, G. N., Johnson, R. M., Wright, G. S. A., Antonyuk, S. V., Muench, S. P., & Hasnain, S. S. (2019). LAT1 (SLC7A5) and CD98hc (SLC3A2) complex dynamics revealed by single-particle cryo-EM. *Acta Crystallographica Section D: Structural Biology*, *75*(Pt 7), 660–669. https://doi.org/10.1107/S2059798319009094

Chin, R. M., Fu, X., Pai, M. Y., Vergnes, L., Hwang, H., Deng, G., Diep, S., Lomenick, B., Meli, V. S., Monsalve, G. C., Hu, E., Whelan, S. A., Wang, J. X., Jung, G., Solis, G. M., Fazlollahi, F., Kaweeteerawat, C., Quach, A., Nili, M., … Huang, J. (2014). The metabolite α-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. *Nature*, *510*(7505), 397–401. https://doi.org/10.1038/nature13264

Chiu, M. I., Katz, H., & Berlin, V. (1994). RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(26), 12574–12578. https://doi.org/10.1073/pnas.91.26.12574

Cho, H. R., Jeon, H., Park, C. K., Park, S. H., Kang, K. M., & Choi, S. H. (2017). BCAT1 is a New MR Imaging-related Biomarker for Prognosis Prediction in IDH1-wildtype Glioblastoma Patients. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-

17062-1

Chresta, C. M., Davies, B. R., Hickson, I., Harding, T., Cosulich, S., Critchlow, S. E., Vincent, J. P., Ellston, R., Jones, D., Sini, P., James, D., Howard, Z., Dudley, P., Hughes, G., Smith, L., Maguire, S., Hummersone, M., Malagu, K., Menear, K., … Pass, M. (2010). AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. *Cancer Research*, *70*(1), 288–298. https://doi.org/10.1158/0008-5472.CAN-09-1751

Christensen, C. L., Kwiatkowski, N., Abraham, B. J., Carretero, J., Al-Shahrour, F., Zhang, T., Chipumuro, E., Herter-Sprie, G. S., Akbay, E. A., Altabef, A., Zhang, J., Shimamura, T., Capelletti, M., Reibel, J. B., Cavanaugh, J. D., Gao, P., Liu, Y., Michaelsen, S. R., Poulsen, H. S., … Wong, K. K. (2014). Targeting Transcriptional Addictions in Small Cell Lung Cancer with a Covalent CDK7 Inhibitor. *Cancer Cell*, *26*(6), 909–922. https://doi.org/10.1016/j.ccell.2014.10.019

Chuang, J. L., Davie, J. R., Chinsky, J. M., Wynn, R. M., Cox, R. P., & Chuang, D. T. (1995). Molecular and Biochemical Basis of Intermediate Maple Syrup Urine Disease: Occurrence of Homozygous G245R and F364C Mutations at the E1α Locus of Hispanic-Mexican Patients. *Journal of Clinical Investigation*, *95*(3), 954–963. https://doi.org/10.1172/JCI117804

Chung, H. C., Piha-Paul, S. A., Lopez-Martin, J., Schellens, J. H. M., Kao, S., Miller, W. H., Delord, J. P., Gao, B., Planchard, D., Gottfried, M., Zer, A., Jalal, S. I., Penel, N., Mehnert, J. M., Matos, I., Bennouna, J., Kim, D. W., Xu, L., Krishnan, S., … Ott, P. A. (2020). Pembrolizumab After Two or More Lines of Previous Therapy in

Patients With Recurrent or Metastatic SCLC: Results From the KEYNOTE-028 and KEYNOTE-158 Studies. *Journal of Thoracic Oncology*, *15*(4), 618–627. https://doi.org/10.1016/j.jtho.2019.12.109

Clarke, A. R., Maandag, E. R., Van Roon, M., Van Der Lugt, N. M. T., Van Der Valk, M., Hooper, M. L., Berns, A., & Te Rielef, H. (1992). Requirement for a functional Rb-1 gene in murine development. *Nature*, *359*(6393), 328–330. https://doi.org/10.1038/359328a0

Clausen, D. M., Guo, J., Parise, R. A., Beumer, J. H., Egorin, M. J., Lazo, J. S., Prochownik, E. V., & Eiseman, J. L. (2010). In vitro cytotoxicity and in vivo efficacy, pharmacokinetics, and metabolism of 10074-G5, a novel small-molecule inhibitor of c-Myc/Max dimerization. *Journal of Pharmacology and Experimental Therapeutics*, *335*(3), 715–727. https://doi.org/10.1124/jpet.110.170555

Coleman, P. F., Suttle, P., & Stark, G. R. (1977). *Purification from Hamster Cells of the Multifunctional Protein That Initiates de Nouo Synthesis of Pyrimidine Nucleotides** (Vol. 252, Issue 18).

Cook, D., Fry, M. J., Hughes, K., Sumathipala, R., Woodgett, J. R., & Dale, T. C. (1996). Wingless inactivates glycogen synthase kinase-3 via an intracellular signalling pathway which involves a protein kinase C. *The EMBO Journal*, *15*(17), 4526–4536. https://doi.org/10.1002/j.1460-2075.1996.tb00830.x

Costanzo, A., Merlo, P., Pediconi, N., Fulco, M., Sartorelli, V., Cole, P. A., Fontemaggi, G., Fanciulli, M., Schiltz, L., Blandino, G., Balsano, C., & Levrero, M. (2002). DNA damage-dependent acetylation of p73 dictates the selective activation of apoptotic

target genes. *Molecular Cell*, *9*(1), 175–186. https://doi.org/10.1016/S1097-2765(02)00431-8

Cristea, S., Coles, G. L., Hornburg, D., Gershkovitz, M., Arand, J., Cao, S., Sen, T., Williamson, S. C., Kim, J. W., Drainas, A. P., He, A., Le Cam, L., Byers, L. A., Snyder, M. P., Contrepois, K., & Sage, J. (2020). The MEK5-ERK5 kinase axis controls lipid metabolism in small-cell lung cancer. *Cancer Research*, *80*(6), 1293–1303. https://doi.org/10.1158/0008-5472.CAN-19-1027

Cupp, J. R., & McAlister-Henn, L. (1991). NAD+-dependent isocitrate dehydrogenase: Cloning, nucleotide sequence, and disruption of the IDH2 gene from Saccharomyces cerevisiae. *Journal of Biological Chemistry*, *266*(33), 22199–22205.

Curtis, D. R., Phillis, J. W., & Watkins, J. C. (1960). The chemical excitation of spinal neurones by certain acidic amino acids. *The Journal of Physiology*, *150*(3), 656–682. https://doi.org/10.1113/jphysiol.1960.sp006410

Dahabreh, J., Stathopoulos, G. P., Koutantos, J., & Rigatos, S. (2009). Lung carcinoid tumor biology: Treatment and survival. *Oncology Reports*, *21*(3), 757–760. https://doi.org/10.3892/or_00000281

Dahiya, A., Wong, S., Gonzalo, S., Gavin, M., & Dean, D. C. (2001). Linking the Rb and Polycomb pathways. *Molecular Cell*, *8*(3), 557–568. https://doi.org/10.1016/S1097-2765(01)00346-X

Dancis, J., Levitz, M., & Westall, R. G. (1960). Maple syrup urine disease: branched-chain keto-aciduria. *Pediatrics*, *25*, 72–79.

Dancis, J., Levitz, M., Miller, S., & Westall, R. G. (1959). Maple syrup urine disease. *British Medical Journal*, *1*(5114), 91–93. https://doi.org/10.1136/bmj.1.5114.91

Demedts, I. K., Vermaelen, K. Y., & Van Meerbeeck, J. P. (2010). Treatment of extensive-stage small cell lung carcinoma: Current status and future prospects. *European Respiratory Journal*, *35*(1), 202–215. https://doi.org/10.1183/09031936.00105009

Dennis, M. D., McGhee, N. K., Jefferson, L. S., & Kimball, S. R. (2013). Regulated in DNA damage and development 1 (REDD1) promotes cell survival during serum deprivation by sustaining repression of signaling through the mechanistic target of rapamycin in complex 1 (mTORC1). *Cellular Signalling*, *25*(12), 2709–2716. https://doi.org/10.1016/j.cellsig.2013.08.038

DeVita, V. T., Lawrence, T. S., & Rosenberg, S. A. (2018). DeVita, Hellman, and Rosenberg's cancer: Principles & practice of oncology. In A. Chiang, F. Detterbeck, T. Stewart, R. Decker, & L. Tanoue (Eds.), *DeVita, Hellman, and Rosenberg's Cancer: Principles & Practice of Oncology* (11th ed.). Lippincott Williams & Wilkins.

Deyoung, M. P., Horak, P., Sofer, A., Sgroi, D., & Ellisen, L. W. (2008). Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes and Development*, *22*(2), 239–251. https://doi.org/10.1101/gad.1617608

Di Nardo, A., Kramvis, I., Cho, N., Sadowski, A., Meikle, L., Kwiatkowski, D. J., & Sahin, M. (2009). Tuberous sclerosis complex activity is required to control neuronal stress responses in an mTOR-dependent manner. *Journal of Neuroscience*,

29(18), 5926–5937. https://doi.org/10.1523/JNEUROSCI.0778-09.2009

Dibble, C. C., & Cantley, L. C. (2015). Regulation of mTORC1 by PI3K signaling. In *Trends in Cell Biology* (Vol. 25, Issue 9, pp. 545–555). Elsevier Ltd. https://doi.org/10.1016/j.tcb.2015.06.002

Dibble, C. C., Elis, W., Menon, S., Qin, W., Klekota, J., Asara, J. M., Finan, P. M., Kwiatkowski, D. J., Murphy, L. O., & Manning, B. D. (2012). TBC1D7 Is a Third Subunit of the TSC1-TSC2 Complex Upstream of mTORC1. *Molecular Cell*, *47*(4), 535–546. https://doi.org/10.1016/j.molcel.2012.06.009

Dilling, M. B., Dias, P., Shapiro, D. N., Germain, G. S., Johnson, R. K., & Houghton, P. J. (1994). Rapamycin Selectively Inhibits the Growth of Childhood Rhabdomyosarcoma Cells through Inhibition of Signaling via the Type I Insulin-like Growth Factor Receptor. *Cancer Research*, *54*(4), 903–907.

Dong, G., Liu, Y., Zhang, L., Huang, S., Ding, H. F., & Dong, Z. (2015). mTOR contributes to ER stress and associated apoptosis in renal tubular cells. *American Journal of Physiology - Renal Physiology*, *308*(3), F267–F274. https://doi.org/10.1152/ajprenal.00629.2014

Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., Roche, P. C., Lu, J., Zhu, G., Tamada, K., Lennon, V. A., Cells, E., & Chen, L. (2002). Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*, *8*(8), 793–800. https://doi.org/10.1038/nm730

Douros, J., & Suffness, M. (1981). New antitumor substances of natural origin. *Cancer Treatment Reviews*, *8*(1), 63–87. https://doi.org/10.1016/S0305-7372(81)80006-0

Drapkin, B. J., George, J., Christensen, C. L., Mino-Kenudson, M., Dries, R., Sundaresan, T., Phat, S., Myers, D. T., Zhong, J., Igo, P., Hazar-Rethinam, M. H., Licausi, J. A., Gomez-Caraballo, M., Kem, M., Jani, K. N., Azimi, R., Abedpour, N., Menon, R., Lakis, S., … Farago, A. F. (2018). Genomic and functional fidelity of small cell lung cancer patient-derived xenografts. *Cancer Discovery*, *8*(5), 600–615. https://doi.org/10.1158/2159-8290.CD-17-0935

Dryja, T. P., Rapaport, J. M., Joyce, J. M., & Petersen, R. A. (1986). Molecular detection of deletions involving band q14 of chromosome 13 in retinoblastomas. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(19), 7391–7394. https://doi.org/10.1073/pnas.83.19.7391

Duan, Y., Li, F., Li, Y., Tang, Y., Kong, X., Feng, Z., Anthony, T. G., Watford, M., Hou, Y., Wu, G., & Yin, Y. (2015). The role of leucine and its metabolites in protein and energy metabolism. *Amino Acids*, *48*(1), 41–51. https://doi.org/10.1007/s00726-015-2067-1

Dupage, M., Dooley, A. L., & Jacks, T. (2009). *Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase*. https://doi.org/10.1038/nprot.2009.95

Durán, R. V, Oppliger, W., Robitaille, A. M., Heiserich, L., Skendaj, R., Gottlieb, E., & Hall, M. N. (2012). Glutaminolysis activates Rag-mTORC1 signaling. *Molecular Cell*, *47*(3), 349–358. https://doi.org/10.1016/j.molcel.2012.05.043

Efeyan, A., Zoncu, R., Chang, S., Gumper, I., Snitkin, H., Wolfson, R. L., Kirak, O., Sabatini, D. D., & Sabatini, D. M. (2013). Regulation of mTORC1 by the Rag

GTPases is necessary for neonatal autophagy and survival. *Nature*, *493*(7434), 679–683. https://doi.org/10.1038/nature11745

Eghtesad, S., Jhunjhunwala, S., Little, S. R., & Clemens, P. R. (2012). Effect of rapamycin on immunity induced by vector-mediated dystrophin expression in mdx skeletal muscle. *Scientific Reports*, *2*(399). https://doi.org/10.1038/srep00399

Eischen, C. M. (2016). Genome stability requires p53. *Cold Spring Harbor Perspectives in Medicine*, *6*(6). https://doi.org/10.1101/cshperspect.a026096

Elghazi, L., Balcazar, N., Blandino-Rosano, M., Cras-Méneur, C., Fatrai, S., Gould, A. P., Chi, M. M., Moley, K. H., & Bernal-Mizrachi, E. (2010). Decreased IRS signaling impairs β-cell cycle progression and survival in transgenic mice overexpressing S6K in β-cells. *Diabetes*, *59*(10), 2390–2399. https://doi.org/10.2337/db09-0851

Elmore, S. P., Qian, T., Grissom, S. F., & Lemasters, J. J. (2001). The mitochondrial permeability transition initiates autophagy in rat hepatocytes. *The FASEB Journal*, *15*(12), 1–17. https://doi.org/10.1096/fj.01-0206fje

Enomoto, K., Sato, F., Tamagawa, S., Gunduz, M., Onoda, N., Uchino, S., Muragaki, Y., & Hotomi, M. (2019). A novel therapeutic approach for anaplastic thyroid cancer through inhibition of LAT1. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-51144-6

Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D., & Hunt, T. (1983). Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*, *33*(2), 389–396. https://doi.org/10.1016/0092-8674(83)90420-8

Ewen, M. E., Sluss, H. K., Sherr, C. J., Matsushime, H., Kato, J. ya, & Livingston, D. M. (1993). Functional interactions of the retinoblastoma protein with mammalian D-type cyclins. *Cell*, *73*(3), 487–497. https://doi.org/10.1016/0092-8674(93)90136-E

Faiena, I., Cummings, A. L., Crosetti, A. M., Pantuck, A. J., Chamie, K., & Drakaki, A. (2018). Durvalumab: An investigational anti-pd-l1 monoclonal antibody for the treatment of urothelial carcinoma. In *Drug Design, Development and Therapy* (Vol. 12, pp. 209–215). Dove Medical Press Ltd. https://doi.org/10.2147/DDDT.S141491

Farago, A. F., & Keane, F. K. (2018). Current standards for clinical management of small cell lung cancer. In *Translational Lung Cancer Research* (Vol. 7, Issue 1, pp. 69–79). AME Publishing Company. https://doi.org/10.21037/tlcr.2018.01.16

Feldman, M. E., Apsel, B., Uotila, A., Loewith, R., Knight, Z. A., Ruggero, D., & Shokat, K. M. (2009). Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biology*, *7*(2), 0371–0383. https://doi.org/10.1371/journal.pbio.1000038

Feng, Z., Hu, W., De Stanchina, E., Teresky, A. K., Jin, S., Lowe, S., & Levine, A. J. (2007). The regulation of AMPK β1, TSC2, and PTEN expression by p53: Stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Research*, *67*(7), 3043–3053. https://doi.org/10.1158/0008-5472.CAN-06-4149

Feng, Z., Zhang, H., Levine, A. J., & Jin, S. (2005). The coordinate regulation of the p53 and mTOR pathways in cells. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(23), 8204–8209.

https://doi.org/10.1073/pnas.0502857102

Fernandez, P. C., Frank, S. R., Wang, L., Schroeder, M., Liu, S., Greene, J., Cocito, A., & Amati, B. (2003). *Genomic targets of the human c-Myc protein*. https://doi.org/10.1101/gad.1067003

Ferraresso, M., Tian, L., Ghobrial, R., Stepkowski, S. M., & Kahan, B. D. (1994). Rapamycin inhibits production of cytotoxic but not noncytotoxic antibodies and preferentially activates T helper 2 cells that mediate long-term survival of heart allografts in rats. *Journal of Immunology*, *153*(7), 3307–3318.

Flemington, E. K., Speck, S. H., & Kaelin, W. G. (1993). E2F-1-mediated transactivation is inhibited by complex formation with the retinoblastoma susceptibility gene product. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(15), 6914–6918. https://doi.org/10.1073/pnas.90.15.6914

Flores, E. R., Tsai, K. Y., Crowley, D., Sengupta, S., Yang, A., McKeon, F., & Jacks, T. (2002). p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature*, *416*(6880), 560–564. https://doi.org/10.1038/416560a

Florey, E. (1954). An inhibitory and an excitatory factor of mammalian central nervous system, and their action on a single sensory neuron. *Archives of Physiology and Biochemistry*, *62*(1), 33–53. https://doi.org/10.3109/13813455409145367

Freedman, D. A., Wu, L., & Levine, A. J. (1999). Functions of the MDM2 oncoprotein. In *Cellular and Molecular Life Sciences* (Vol. 55, Issue 1, pp. 96–107). https://doi.org/10.1007/s000180050273

Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., Fitz, L.

J., Malenkovich, N., Okazaki, T., Byrne, M. C., Horton, H. F., Fouser, L., Carter, L., Ling, V., Bowman, M. R., Carreno, B. M., Collins, M., Wood, C. R., & Honjo, T. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *Journal of Experimental Medicine*, *192*(7), 1027–1034. https://doi.org/10.1084/jem.192.7.1027

Frias, M. A., Thoreen, C. C., Jaffe, J. D., Schroder, W., Sculley, T., Carr, S. A., & Sabatini, D. M. (2006). mSin1 Is Necessary for Akt/PKB Phosphorylation, and Its Isoforms Define Three Distinct mTORC2s. *Current Biology*, *16*(18), 1865–1870. https://doi.org/10.1016/j.cub.2006.08.001

Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M., & Dryja, T. P. (1986). A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature*, *323*(6089), 643–646. https://doi.org/10.1038/323643a0

Fuchs, B. C., & Bode, B. P. (2005). Amino acid transporters ASCT2 and LAT1 in cancer: partners in crime? *Seminars in Cancer Biology*, *15*(4), 254–266. https://doi.org/10.1016/j.semcancer.2005.04.005

Fujino, K., Motooka, Y., Hassan, W. A., Ali Abdalla, M. O., Sato, Y., Kudoh, S., Hasegawa, K., Niimori-Kita, K., Kobayashi, H., Kubota, I., Wakimoto, J., Suzuki, M., & Ito, T. (2015). Insulinoma-associated protein 1 is a crucial regulator of neuroendocrine differentiation in lung cancer. *American Journal of Pathology*, *185*(12), 3164–3177. https://doi.org/10.1016/j.ajpath.2015.08.018

Fung, Y. K. T., Linn Murphree, A., T'Ang, A., Qian, J., Hinrichs, S. H., & Benedict, W. F.

(1987). Structural evidence for the authenticity of the human retinoblastoma gene. *Science*, *236*(4809), 1657–1661. https://doi.org/10.1126/science.2885916

Garami, A., Zwartkruis, F. J. T., Nobukuni, T., Joaquin, M., Roccio, M., Stocker, H., Kozma, S. C., Hafen, E., Bos, J. L., & Thomas, G. (2003). Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. *Molecular Cell*, *11*(6), 1457–1466. https://doi.org/10.1016/S1097-2765(03)00220-X

García-Martínez, J. M., Moran, J., Clarke, R. G., Gray, A., Cosulich, S. C., Chresta, C. M., & Alessi, D. R. (2009). Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *Biochemical Journal*, *421*(1), 29–42. https://doi.org/10.1042/BJ20090489

García-Yuste, M., Matilla, J. M., Cueto, A., Paniagua, J. M. R., Ramos, G., Cañizares, M. A., & Muguruza, I. (2007). Typical and atypical carcinoid tumours: analysis of the experience of the Spanish Multi-centric Study of Neuroendocrine Tumours of the Lung. *European Journal of Cardio-Thoracic Surgery*, *31*(2), 192–197. https://doi.org/10.1016/j.ejcts.2006.11.031

Gatica, D., Lahiri, V., & Klionsky, D. J. (2018). Cargo recognition and degradation by selective autophagy. In *Nature Cell Biology* (Vol. 20, Issue 3, pp. 233–242). Nature Publishing Group. https://doi.org/10.1038/s41556-018-0037-z

Gazdar, A. F., Carney, D. N., Nau, M. M., & Minna, J. D. (1985). Characterization of Variant Subclasses of Cell Lines Derived from Small Cell Lung Cancer Having Distinctive Biochemical, Morphological, and Growth Properties. *Cancer Research*, *45*(6).

Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baylin, S. B., Bunn, P. A., Guccion, J. G., & Minna, J. D. (1980). Establishment of Continuous, Clonable Cultures of Small-Cell Carcinoma of the Lung Which Have Amine Precursor Uptake and Decarboxylation Cell Properties. *Cancer Research*, *40*(10).

Gazdar, A. F., Hirsch, F. R., & Minna, J. D. (2016). From mice to men and back: An assessment of preclinical model systems for the study of lung cancers. In *Journal of Thoracic Oncology* (Vol. 11, Issue 3, pp. 287–299). Lippincott Williams and Wilkins. https://doi.org/10.1016/j.jtho.2015.10.009

Gazdar, A. F., & Minna, J. D. (2016). Developing New, Rational Therapies for Recalcitrant Small Cell Lung Cancer. *Journal of the National Cancer Institute*, *108*(10). https://doi.org/10.1093/jnci/djw119

Gazdar, A. F., Savage, T. K., Johnson, J. E., Berns, A., Sage, J., Linnoila, R. I., Macpherson, D., Mcfadden, D. G., Farago, A., Jacks, T., Travis, W. D., & Brambilla, E. (2015). The comparative pathology of genetically engineered mouse models for neuroendocrine carcinomas of the lung. *Journal of Thoracic Oncology*, *10*(4), 553–564. https://doi.org/10.1097/JTO.0000000000000459

George, J., Lim, J. S., Jang, S. J., Cun, Y., Ozretia, L., Kong, G., Leenders, F., Lu, X., Fernández-Cuesta, L., Bosco, G., Müller, C., Dahmen, I., Jahchan, N. S., Park, K. S., Yang, D., Karnezis, A. N., Vaka, D., Torres, A., Wang, M. S., … Thomas, R. K. (2015). Comprehensive genomic profiles of small cell lung cancer. *Nature*, *524*(7563), 47–53. https://doi.org/10.1038/nature14664

Gierl, M. S., Karoulias, N., Wende, H., Strehle, M., & Birchmeier, C. (2006). The Zinc-

finger factor Insm1 (IA-1) is essential for the development of pancreatic β cells and intestinal endocrine cells. *Genes and Development*, *20*(17), 2465–2478. https://doi.org/10.1101/gad.381806

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: Cellular and molecular mechanisms. In *Journal of Pathology* (Vol. 221, Issue 1, pp. 3–12). John Wiley & Sons, Ltd. https://doi.org/10.1002/path.2697

Goldthwaitt, D. A. (1956). 5-Phosphoribosylamine, a precursor of glycinamide ribotide. *Journal of Biological Chemistry*, *222*, 1051–1068. http://www.jbc.org/

Gosain, R., Mukherjee, S., Yendamuri, S. S., & Iyer, R. (2018). Management of typical and atypical pulmonary carcinoids based on different established guidelines. In *Cancers* (Vol. 10, Issue 12). MDPI AG. https://doi.org/10.3390/cancers10120510

Goto, M., Miyahara, I., Hirotsu, K., Conway, M., Yennawar, N., Islam, M. M., & Hutson, S. M. (2005). Structural determinants for branched-chain aminotransferase isozyme-specific inhibition by the anticonvulsant drug gabapentin. *Journal of Biological Chemistry*, *280*(44), 37246–37256. https://doi.org/10.1074/jbc.M506486200

Gozuacik, D., & Kimchi, A. (2004). Autophagy as a cell death and tumor suppressor mechanism. In *Oncogene* (Vol. 23, Issue 16 REV. ISS. 2, pp. 2891–2906). Nature Publishing Group. https://doi.org/10.1038/sj.onc.1207521

Gran, P., & Cameron-Smith, D. (2011). The actions of exogenous leucine on mTOR signalling and amino acid transporters in human myotubes. *BMC Physiology*, *11*(1), 10. https://doi.org/10.1186/1472-6793-11-10

Grandori, C., Gomez-Roman, N., Felton-Edkins, Z. A., Ngouenet, C., Galloway, D. A., Eisenman, R. N., & White, R. J. (2005). c-Myc binds to human ribosomal DNA and stimulates transcription of rRNA genes by RNA polymerase I. *Nature Cell Biology*, *7*(3), 311–318. https://doi.org/10.1038/ncb1224

Grankvist, N., Lagerborg, K. A., Jain, M., & Nilsson, R. (2018). Gabapentin can suppress cell proliferation independent of the cytosolic branched-chain amino acid transferase 1 (BCAT1) Graphical Abstract HHS Public Access. *Biochemistry*, *57*(49), 6762–6766. https://doi.org/10.1021/acs.biochem.8b01031

Green, C. R., Wallace, M., Divakaruni, A. S., Phillips, S. A., Murphy, A. N., Ciaraldi, T. P., & Metallo, C. M. (2016). Branched-chain amino acid catabolism fuels adipocyte differentiation and lipogenesis. *Nature Chemical Biology*, *12*(1), 15–21. https://doi.org/10.1038/nchembio.1961

Gregersen, N., Bross, P., Vang, S., & Christensen, J. H. (2006). Protein Misfolding and Human Disease. *Annual Review of Genomics and Human Genetics*, *7*(1), 103–124. https://doi.org/10.1146/annurev.genom.7.080505.115737

Grewe, M., Gansauge, F., Schmid, R. M., Adler, G., & Seufferlein, T. (1999). Regulation of cell growth and cyclin D1 expression by the constitutively active FRAP-p70(s6K) pathway in human pancreatic cancer cells. *Cancer Research*, *59*(15), 3581–3587.

Gu, Z., Liu, Y., Cai, F., Patrick, M., Zmajkovic, J., Cao, H., Zhang, Y., Tasdogan, A., Chen, M., Qi, L., Liu, X., Li, K., Lyu, J., Dickerson, K. E., Chen, W., Ni, M., Merritt, M. E., Morrison, S. J., Skoda, R. C., … Xu, J. (2019). Loss of EZH2 reprograms BCAA metabolism to drive leukemic transformation. *Cancer Discovery*, *9*(9), 1228–

1247. https://doi.org/10.1158/2159-8290.CD-19-0152

Guan, X. Y., Sham, J. S. T., Tang, T. C. M., Fang, Y., Huo, K. K., & Yang, J. M. (2001). Isolation of a novel candidate oncogene within a frequently amplified region at 3q26 in ovarian cancer. *Cancer Research*, *61*(9), 3806–3809.

Guertin, D. A., & Sabatini, D. M. (2007). Defining the Role of mTOR in Cancer. In *Cancer Cell* (Vol. 12, Issue 1, pp. 9–22). https://doi.org/10.1016/j.ccr.2007.05.008

Guimaraes, J. C., & Zavolan, M. (2016). Patterns of ribosomal protein expression specify normal and malignant human cells. *Genome Biology*, *17*(1). https://doi.org/10.1186/s13059-016-1104-z

Guinee, D. G., Fishback, N. F., Koss, M. N., Abbondanzo, S. L., & Travis, W. D. (1994). The Spectrum of Immunohistochemical Staining of Small-cell Lung Carcinoma in Specimens From Transbronchial and Open-lung Biopsies. *American Journal of Clinical Pathology*, *102*(4), 406–414. https://doi.org/10.1093/AJCP/102.4.406

Guo, L., Zhang, H., & Chen, B. (2017). Nivolumab as Programmed Death-1 (PD-1) Inhibitor for Targeted Immunotherapy in Tumor. In *Journal of Cancer* (Vol. 8, Issue 3, pp. 410–416). Ivyspring International Publisher. https://doi.org/10.7150/jca.17144

Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E., & Shaw, R. J. (2008). AMPK Phosphorylation of Raptor Mediates a Metabolic Checkpoint. *Molecular Cell*, *30*(2), 214–226. https://doi.org/10.1016/j.molcel.2008.03.003

Haar, E. V., Lee, S. il, Bandhakavi, S., Griffin, T. J., & Kim, D. H. (2007). Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. *Nature Cell*

Biology, *9*(3), 316–323. https://doi.org/10.1038/ncb1547

Hackstein, H., Taner, T., Zahorchak, A. F., Morelli, A. E., Logar, A. J., Gessner, A., & Thomson, A. W. (2003). Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. *Blood*, *101*(11), 4457–4463. https://doi.org/10.1182/blood-2002-11-3370

Hagmann, R., Hess, V., Zippelius, A., & Rothschild, S. I. (2015). Second-line therapy of small-cell lung cancer: Topotecan compared to a combination treatment with adriamycin, cyclophosphamide and vincristine (ACO) - A single center experience. *Journal of Cancer*, *6*(11), 1148–1154. https://doi.org/10.7150/jca.13080

Halama, A., Horsch, M., Kastenmüller, G., Möller, G., Kumar, P., Prehn, C., Laumen, H., Hauner, H., Hrabě de Angelis, M., Beckers, J., Suhre, K., & Adamski, J. (2015). Metabolic switch during adipogenesis: From branched chain amino acid catabolism to lipid synthesis. *Archives of Biochemistry and Biophysics*. https://doi.org/10.1016/j.abb.2015.09.013

Hall, T. R., Wallin, R., Reinhart, G. D., & Hutson, S. M. (1993). Branched chain aminotransferase isoenzymes. Purification and characterization of the rat brain isoenzyme. *Journal of Biological Chemistry*.

Hamilton, G., & Rath, B. (2019). Immunotherapy for small cell lung cancer: mechanisms of resistance. In *Expert Opinion on Biological Therapy* (Vol. 19, Issue 5, pp. 423–432). Taylor and Francis Ltd. https://doi.org/10.1080/14712598.2019.1592155

Han, J. M., Jeong, S. J., Park, M. C., Kim, G., Kwon, N. H., Kim, H. K., Ha, S. H., Ryu, S. H., & Kim, S. (2012). Leucyl-tRNA synthetase is an intracellular leucine sensor

for the mTORC1-signaling pathway. *Cell*, *149*(2), 410–424. https://doi.org/10.1016/j.cell.2012.02.044

Hanahan, D., & Coussens, L. M. (2012). Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. In *Cancer Cell* (Vol. 21, Issue 3, pp. 309–322). Elsevier. https://doi.org/10.1016/j.ccr.2012.02.022

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*(5), 646–674. http://www.ncbi.nlm.nih.gov/pubmed/21376230

Harada, K., Saheki, S., Wada, K., & Tanaka, T. (1978). Purification of four pyruvate kinase isozymes of rats by affinity elution chromatography. *Biochimica et Biophysica Acta (BBA) - Enzymology*, *524*(2), 327–339. https://doi.org/10.1016/0005-2744(78)90169-9

Harbour, J. W., Lai, S.-L., Whang-Peng, J., Gazdar, A. F., Minna, J. D., & Kaye, F. J. (1988). Abnormalities in Structure and Expression of the Human Retinoblastoma Gene in SCLC HHS Public Access. In *Science* (Vol. 241, Issue 4863).

Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., & Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Molecular Cell*, *6*(5), 1099–1108. https://doi.org/10.1016/S1097-2765(00)00108-8

Harding, H. P., Zhang, Y., Bertolotti, A., Zeng, H., & Ron, D. (2000). Perk is essential for translational regulation and cell survival during the unfolded protein response. *Molecular Cell*, *5*(5), 897–904. https://doi.org/10.1016/S1097-2765(00)80330-5

Harding, H. P., Zhang, Y., & Ron, D. (1999). Protein translation and folding are coupled

by an endoplasmic- reticulum-resident kinase. *Nature*, *397*(6716), 271–274. https://doi.org/10.1038/16729

Harper, A. E., Miller, R. H., & Block, K. P. (1984). Branched-Chain Amino Acid Metabolism. *Annual Review of Nutrition*, *4*(1), 409–454. https://doi.org/10.1146/annurev.nu.04.070184.002205

Harper, J. W., Elledge, S. J., Keyomarsi, K., Dynlacht, B., Tsai, L. H., Zhang, P., Dobrowolski, S., Bai, C., Connell-Crowley, L., Swindell, E., Fox, M. P., & Wei, N. (1995). Inhibition of cyclin-dependent kinases by p21. *Molecular Biology of the Cell*, *6*(4), 387–400. https://doi.org/10.1091/mbc.6.4.387

Harris, R. A., Zhang, B., Goodwin, G. W., Kuntz, M. J., Shimomura, Y., Rougraff, P., Dexter, P., Zhao, Y., Gibson, R., & Crabb, D. W. (1990). Regulation of the branched-chain α-ketoacid dehydrogenase and elucidation of a molecular basis for maple syrup urine disease. *Advances in Enzyme Regulation*, *30*(C), 245–256. https://doi.org/10.1016/0065-2571(90)90021-S

Hattori, A., Tsunoda, M., Konuma, T., Kobayashi, M., Nagy, T., Glushka, J., Tayyari, F., McSkimming, D., Kannan, N., Tojo, A., Edison, A. S., & Ito, T. (2017). Cancer progression by reprogrammed BCAA metabolism in myeloid leukaemia. *Nature*, *545*(7655), 500–504. https://doi.org/10.1038/nature22314

Heidelberger, C., Chaudhuri, N. K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R. J., Pleven, E., & Scheiner, J. (1957). Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature*, *179*(4561), 663–666. https://doi.org/10.1038/179663a0

Heiden, M. G. V., Cantley, L. C., & Thompson, C. B. (2009). Understanding the warburg effect: The metabolic requirements of cell proliferation. In *Science* (Vol. 324, Issue 5930, pp. 1029–1033). https://doi.org/10.1126/science.1160809

Heitman, J., Movva, N. R., & Hall, M. N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science*, *253*(5022), 905–909. https://doi.org/10.1126/science.1715094

Helin, K., Harlow, E., & Fattaey, A. (1993). Inhibition of E2F-1 transactivation by direct binding of the retinoblastoma protein. *Molecular and Cellular Biology*, *13*(10), 6501–6508. https://doi.org/10.1128/mcb.13.10.6501

Henras, A. K., Soudet, J., Gérus, M., Lebaron, S., Caizergues-Ferrer, M., Mougin, A., & Henry, Y. (2008). The post-transcriptional steps of eukaryotic ribosome biogenesis. In *Cellular and Molecular Life Sciences* (Vol. 65, Issue 15, pp. 2334–2359). Springer. https://doi.org/10.1007/s00018-008-8027-0

Hillier, J., Wadley, A. J., Morgan, R. G., Cherry, A., Conway, M. E., & Coles, S. J. (2018). Identification and Characterisation of a Novel Antioxidant Activity for the BCAT1 Cxxc Motif: Implications for Myeloid Leukaemia Development. *Blood*, *132*(Supplement 1), 1473–1473. https://doi.org/10.1182/blood-2018-99-118823

Hinds, P. W., Mittnacht, S., Dulic, V., Arnold, A., Reed, S. I., & Weinberg, R. A. (1992). Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. *Cell*, *70*(6), 993–1006. https://doi.org/10.1016/0092-8674(92)90249-C

Hinnebusch, A. G. (2014). The Scanning Mechanism of Eukaryotic Translation Initiation. *Annual Review of Biochemistry*, *83*(1), 779–812. https://doi.org/10.1146/annurev-

biochem-060713-035802

Hobbs, C. A., Paul, B. A., & Gilmour, S. K. (2002). Deregulation of polyamine biosynthesis alters intrinsic histone acetyltransferase and deacetylase activities in murine skin and tumors. *Cancer Research*, *62*(1), 67–74.

Hodgkinson, C. L., Morrow, C. J., Li, Y., Metcalf, R. L., Rothwell, D. G., Trapani, F., Polanski, R., Burt, D. J., Simpson, K. L., Morris, K., Pepper, S. D., Nonaka, D., Greystoke, A., Kelly, P., Bola, B., Krebs, M. G., Antonello, J., Ayub, M., Faulkner, S., … Dive, C. (2014). Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. *Nature Medicine*, *20*(8), 897–903. https://doi.org/10.1038/nm.3600

Holeček, M. (2018). Branched-chain amino acids in health and disease: Metabolism, alterations in blood plasma, and as supplements. In *Nutrition and Metabolism* (Vol. 15, Issue 1, p. 33). BioMed Central Ltd. https://doi.org/10.1186/s12986-018-0271-1

Honda, R., Tanaka, H., & Yasuda, H. (1997). Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Letters*, *420*(1), 25–27. https://doi.org/10.1016/S0014-5793(97)01480-4

Horie, M., Saito, A., Ohshima, M., Suzuki, H. I., & Nagase, T. (2016). YAP and TAZ modulate cell phenotype in a subset of small cell lung cancer. *Cancer Science*, *107*(12), 1755–1766. https://doi.org/10.1111/cas.13078

Horn, L., Mansfield, A. S., Szczęsna, A., Havel, L., Krzakowski, M., Hochmair, M. J., Huemer, F., Losonczy, G., Johnson, M. L., Nishio, M., Reck, M., Mok, T., Lam, S., Shames, D. S., Liu, J., Ding, B., Lopez-Chavez, A., Kabbinavar, F., Lin, W., … Liu,

S. V. (2018). First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. *New England Journal of Medicine*, *379*(23), 2220–2229. https://doi.org/10.1056/NEJMoa1809064

Horton, L. E., Bushell, M., Barth-Baus, D., Tilleray, V. J., Clemens, M. J., & Hensold, J. O. (2002). p53 activation results in rapid dephosphorylation of the eIF4E-binding protein 4E-BP1, inhibition of ribosomal protein S6 kinase and inhibition of translation initiation. *Oncogene*, *21*(34), 5325–5334. https://doi.org/10.1038/sj.onc.1205662

Hosoi, H., Dilling, M. B., Shikata, T., Liu, L. N., Shu, L., Ashmun, R. A., Germain, G. S., Abraham, R. T., & Houghton, P. J. (1999). Rapamycin causes poorly reversible inhibition of mTOR and induces p53- independent apoptosis in human rhabdomyosarcoma cells. *Cancer Research*, *59*(4), 886–894.

Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S. I., Natsume, T., Takehana, K., Yamada, N., Guan, J. L., Oshiro, N., & Mizushima, N. (2009). Nutrient-dependent mTORCl association with the ULK1-Atg13-FIP200 complex required for autophagy. *Molecular Biology of the Cell*, *20*(7), 1981–1991. https://doi.org/10.1091/mbc.E08-12-1248

Houchens, D. P., Ovejera, A. A., Riblet, S. M., & Slagel, D. E. (1983). Human brain tumor xenografts in nude mice as a chemotherapy model. *European Journal of Cancer and Clinical Oncology*, *19*(6), 799–805. https://doi.org/10.1016/0277-5379(83)90012-3

Howlader N., Noone A. M., Krapcho M., Garshell J., Miller D., Altekruse S. F., Kosary C.

L., Yu M., Ruhl J., Tatalovich Z., Mariotto A., Lewis D. R., Chen H. S., Feuer E. J., Cronin, K. A. (eds). (2020). SEER Cancer Statistics Review, 1975-2017. In *SEER Cancer Statistics Review*. https://seer.cancer.gov/csr/1975_2017/

Hresko, R. C., & Mueckler, M. (2005). mTOR·RICTOR is the Ser473 kinase for Akt/protein kinase B in 3T3-L1 adipocytes. *Journal of Biological Chemistry*, *280*(49), 40406–40416. https://doi.org/10.1074/jbc.M508361200

Hu, L. Y., Boxer, P. A., Kesten, S. R., Lei, H. J., Wustrow, D. J., Moreland, D. W., Zhang, L., Ahn, K., Ryder, T. R., Liu, X., Rubin, J. R., Fahnoe, K., Carroll, R. T., Dutta, S., Fahnoe, D. C., Probert, A. W., Roof, R. L., Rafferty, M. F., Kostlan, C. R., … Ohren, J. (2006). The design and synthesis of human branched-chain amino acid aminotransferase inhibitors for treatment of neurodegenerative diseases. *Bioorganic and Medicinal Chemistry Letters*, *16*(9), 2337–2340. https://doi.org/10.1016/j.bmcl.2005.07.058

Hu, W., Zhang, C., Wu, R., Sun, Y., Levine, A., & Feng, Z. (2010). Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(16), 7455–7460. https://doi.org/10.1073/pnas.1001006107

Huang, F., Ni, M., Chalishazar, M. D., Huffman, K. E., Kim, J., Cai, L., Shi, X., Cai, F., Zacharias, L. G., Ireland, A. S., Li, K., Gu, W., Kaushik, A. K., Liu, X., Gazdar, A. F., Oliver, T. G., Minna, J. D., Hu, Z., & DeBerardinis, R. J. (2018). Inosine Monophosphate Dehydrogenase Dependence in a Subset of Small Cell Lung Cancers. *Cell Metabolism*, *28*(3), 369-382.e5. https://doi.org/10.1016/j.cmet.2018.06.005

Huang, Y. H., Klingbeil, O., He, X. Y., Wu, X. S., Arun, G., Lu, B., Somerville, T. D. D., Milazzo, J. P., Wilkinson, J. E., Demerdash, O. E., Spector, D. L., Egeblad, M., Shi, J., & Vakoc, C. R. (2018). POU2F3 is a master regulator of a tuft cell-like variant of small cell lung cancer. *Genes and Development*, *32*(13–14), 915–928. https://doi.org/10.1101/gad.314815.118

Hutson, S. M., Fenstermacher, D., & Mahar, C. (1988). Role of mitochondrial transamination in branched chain amino acid metabolism. *Journal of Biological Chemistry*, *263*(8), 3618–3625.

Hutson, S. M. (1989). Regulation of Substrate Availability for the Branched‐Chain α‐Keto Acid Dehydrogenase Enzyme Complex. *Annals of the New York Academy of Sciences*, *573*(1), 230–239. https://doi.org/10.1111/j.1749-6632.1989.tb15000.x

Hutson, S. M, Sweatt, A. J., & Lanoue, K. F. (2005). Branched-chain Amino Acid Metabolism: Implications for Establishing Safe Intakes. *The Journal of Nutrition*, *135*(6), 1557–1564.

Imanaka, K., Ohkawa, K., Tatsumi, T., Katayama, K., Inoue, A., Imai, Y., Oshita, M., Iio, S., Mita, E., Fukui, H., Yamada, A., Nakanishi, F., Inada, M., Doi, Y., Suzuki, K., Kaneko, A., Marubashi, S., Ito, Y., Fukui, K., … Takehara, T. (2016). Impact of branched-chain amino acid supplementation on survival in patients with advanced hepatocellular carcinoma treated with sorafenib: A multicenter retrospective cohort study. *Hepatology Research*, *46*(10), 1002–1010. https://doi.org/10.1111/hepr.12640

Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb GTpase is a direct target of TSC2

GAP activity and regulates mTOR signaling. *Genes and Development*, *17*(15), 1829–1834. https://doi.org/10.1101/gad.1110003

Inoki, K., Li, Y., Zhu, T., Wu, J., & Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, *4*(9), 648–657. https://doi.org/10.1038/ncb839

Inoki, K., Mori, H., Wang, J., Suzuki, T., Hong, S. K., Yoshida, S., Blattner, S. M., Ikenoue, T., Rüegg, M. A., Hall, M. N., Kwiatkowski, D. J., Rastaldi, M. P., Huber, T. B., Kretzler, M., Holzman, L. B., Wiggins, R. C., & Guan, K. L. (2011). mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *Journal of Clinical Investigation*, *121*(6), 2181–2196. https://doi.org/10.1172/JCI44771

Inoki, K., Ouyang, H., Zhu, T., Lindvall, C., Wang, Y., Zhang, X., Yang, Q., Bennett, C., Harada, Y., Stankunas, K., Wang, C. yu, He, X., MacDougald, O. A., You, M., Williams, B. O., & Guan, K. L. (2006). TSC2 Integrates Wnt and Energy Signals via a Coordinated Phosphorylation by AMPK and GSK3 to Regulate Cell Growth. *Cell*, *126*(5), 955–968. https://doi.org/10.1016/j.cell.2006.06.055

Inoki, K., Zhu, T., & Guan, K. L. (2003). TSC2 Mediates Cellular Energy Response to Control Cell Growth and Survival. *Cell*, *115*(5), 577–590. https://doi.org/10.1016/S0092-8674(03)00929-2

Ishizuka, T., Sakata, N., Johnson, G. L., Gelfand, E. W., & Terada, N. (1997). Rapamycin potentiates dexamethasone-induced apoptosis and inhibits JNK activity in lymphoblastoid cells. *Biochemical and Biophysical Research Communications*,

230(2), 386–391. https://doi.org/10.1006/bbrc.1996.5967

Islam, K. M., Anggondowati, T., Deviany, P. E., Ryan, J. E., Fetrick, A., Bagenda, D., Copur, M. S., Tolentino, A., Vaziri, I., McKean, H. A., Dunder, S., Gray, J. E., Huang, C., & Ganti, A. K. (2019). Patient preferences of chemotherapy treatment options and tolerance of chemotherapy side effects in advanced stage lung cancer. *BMC Cancer*, *19*(1). https://doi.org/10.1186/s12885-019-6054-x

Ito, N., Nishibori, Y., Ito, Y., Takagi, H., Akimoto, Y., Kudo, A., Asanuma, K., Sai, Y., Miyamoto, K. I., Takenaka, H., & Yan, K. (2011). MTORC1 activation triggers the unfolded protein response in podocytes and leads to nephrotic syndrome. In *Laboratory Investigation* (Vol. 91, Issue 11, pp. 1584–1595). Nature Publishing Group. https://doi.org/10.1038/labinvest.2011.135

Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., & Minato, N. (2002). Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(19), 12293–12297. https://doi.org/10.1073/pnas.192461099

Jacinto, E., Loewith, R., Schmidt, A., Lin, S., Rüegg, M. A., Hall, A., & Hall, M. N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nature Cell Biology*, *6*(11), 1122–1128. https://doi.org/10.1038/ncb1183

Jacks, T., Fazeli, A., Schmitt, E. M., Bronson, R. T., Goodell, M. A., & Weinberg, R. A. (1992). Effects of an Rb mutation in the mouse. *Nature*, *359*(6393), 295–300.

https://doi.org/10.1038/359295a0

Jenö, P., Ballou, L. M., Novak-Hofer, I., & Thomas, G. (1988). Identification and characterization of a mitogen-activated S6 kinase. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(2), 406–410. https://doi.org/10.1073/pnas.85.2.406

Jewell, J. L., Kim, Y. C., Russell, R. C., Yu, F. X., Park, H. W., Plouffe, S. W., Tagliabracci, V. S., & Guan, K. L. (2015). Differential regulation of mTORC1 by leucine and glutamine. *Science*, *347*(6218), 194–198. https://doi.org/10.1126/science.1259472

Jewell, J. L., Russell, R. C., & Guan, K.-L. (2013). Amino acid signalling upstream of mTOR. *Nature Reviews Molecular Cell Biology*, *14*(3), 133–139. https://doi.org/10.1038/nrm3522

Jia, D., Augert, A., Kim, D. W., Eastwood, E., Wu, N., Ibrahim, A. H., Kim, K. B., Dunn, C. T., Pillai, S. P. S., Gazdar, A. F., Bolouri, H., Park, K. S., & Macpherson, D. (2018). Crebbp loss drives small cell lung cancer and increases sensitivity to HDAC inhibition. *Cancer Discovery*, *8*(11), 1422–37. https://doi.org/10.1158/2159-8290.CD-18-0385

Johnson, B. E., Russell, E., Simmons, A. M., Phelps, R., Steinberg, S. M., Ihde, D. C., & Gazdar, A. F. (1996). MYC family DNA amplification in 126 tumor cell lines from patients with small cell lung cancer. *Journal of Cellular Biochemistry*, *63*(S24), 210–217. https://doi.org/10.1002/jcb.240630516

Jones, M. E. (1980). Pyrimidine Nucleotide Biosynthesis in Animals: Genes, Enzymes,

and Regulation of UMP Biosynthesis. *Annual Review of Biochemistry*, *49*(1), 253–279. https://doi.org/10.1146/annurev.bi.49.070180.001345

Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M. J., & Thompson, C. B. (2005). AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. *Molecular Cell*, *18*(3), 283–293. https://doi.org/10.1016/j.molcel.2005.03.027

Jorgensen, P., Rupeš, I., Sharom, J. R., Schneper, L., Broach, J. R., & Tyers, M. (2004). A dynamic transcriptional network communicates growth potential to ribosome synthesis and critical cell size. *Genes and Development*, *18*(20), 2491–2505. https://doi.org/10.1101/gad.1228804

Jung, C. H., Jun, C. B., Ro, S. H., Kim, Y. M., Otto, N. M., Cao, J., Kundu, M., & Kim, D. H. (2009). ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Molecular Biology of the Cell*, *20*(7), 1992–2003. https://doi.org/10.1091/mbc.E08-12-1249

Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J. C., Valent, A., Minty, A., Chalon, P., Lelias, J. M., Dumont, X., Ferrara, P., McKeon, F., & Caput, D. (1997). Monoallelically expressed gene related to p53 at 1p36, a region frequently deleted in neuroblastoma and other human cancers. *Cell*, *90*(4), 809–819. https://doi.org/10.1016/S0092-8674(00)80540-1

Kahan, B. D., Julian, B. A., Pescovitz, M. D., Vanrenterghem, Y., & Neylan, J. (1999). Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts:

A phase II trial. *Transplantation*, *68*(10), 1526–1532. https://doi.org/10.1097/00007890-199911270-00016

Kaira, K., Oriuchi, N., Imai, H., Shimizu, K., Yanagitani, N., Sunaga, N., Hisada, T., Kawashima, O., Iijima, H., Ishizuka, T., Kanai, Y., Endou, H., Nakajima, T., & Mori, M. (2008). Expression of L-type amino acid transporter 1 (LAT1) in neuroendocrine tumors of the lung. *Pathology, Research and Practice*, *204*(8), 553–561. https://doi.org/10.1016/j.prp.2008.02.003

Kaira, K., Oriuchi, N., Imai, H., Shimizu, K., Yanagitani, N., Sunaga, N., Hisada, T., Kawashima, O., Kamide, Y., Ishizuka, T., Kanai, Y., Nakajima, T., & Mori, M. (2010). Prognostic significance of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (CD98) expression in surgically resectable stage III non-small cell lung cancer. *Experimental and Therapeutic Medicine*, *1*(5), 799–808. https://doi.org/10.3892/etm.2010.117

Kalender, A., Selvaraj, A., Kim, S. Y., Gulati, P., Brûlé, S., Viollet, B., Kemp, B. E., Bardeesy, N., Dennis, P., Schlager, J. J., Marette, A., Kozma, S. C., & Thomas, G. (2010). Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. *Cell Metabolism*, *11*(5), 390–401. https://doi.org/10.1016/j.cmet.2010.03.014

Kato, H., Nakajima, S., Saito, Y., Takahashi, S., Katoh, R., & Kitamura, M. (2012). MTORC1 serves ER stress-triggered apoptosis via selective activation of the IRE1-JNK pathway. *Cell Death and Differentiation*, *19*(2), 310–320. https://doi.org/10.1038/cdd.2011.98

Kato, J., Matsushime, H., Hiebert, S. W., Ewen, M. E., & Sherr, C. J. (1993). Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes and Development*, *7*(3), 331–342. https://doi.org/10.1101/gad.7.3.331

Kawaguchi, T., Shiraishi, K., Ito, T., Suzuki, K., Koreeda, C., Ohtake, T., Iwasa, M., Tokumoto, Y., Endo, R., Kawamura, N. hiro, Shiraki, M., Habu, D., Tsuruta, S., Miwa, Y., Kawaguchi, A., Kakuma, T., Sakai, H., Kawada, N., Hanai, T., … Suzuki, K. (2014). Branched-Chain Amino Acids Prevent Hepatocarcinogenesis and Prolong Survival of Patients With Cirrhosis. *Clinical Gastroenterology and Hepatology*, *12*(6), 1012-1018.e1. https://doi.org/10.1016/j.cgh.2013.08.050

Kaye, F. J., Kratzke, R. A., Gerster, J. L., & Horowitz, J. M. (1990). A single amino acid substitution results in a retinoblastoma protein defective in phosphorylation and oncoprotein binding. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(17), 6922–6926. https://doi.org/10.1073/pnas.87.17.6922

Keith, C. T., & Schreiber, S. L. (1995). PIK-Related Kinases: DNA Repair, Recombination, and Cell Cycle Checkpoints. *Science*, *270*(5233), 50–50. https://doi.org/10.1126/science.270.5233.50

Keller, K. E., Tan, I. S., & Lee, Y. S. (2012). SAICAR stimulates pyruvate kinase isoform M2 and promotes cancer cell survival in glucose-limited conditions. *Science*, *338*(6110), 1069–1072. https://doi.org/10.1126/science.1224409

Keyomarsi, K. (1996). Synchronization of mammalian cells by Lovastatin. *Methods in*

Cell Science, *18*(2), 109–114. https://doi.org/10.1007/BF00122161

Khan, M., Biswas, D., Ghosh, M., Mandloi, S., Chakrabarti, S., & Chakrabarti, P. (2015). mTORC2 controls cancer cell survival by modulating gluconeogenesis. *Cell Death Discovery*, *1*(1), 1. https://doi.org/10.1038/cddiscovery.2015.16

Khanzada, U. K., Pardo, O. E., Meier, C., Downward, J., Seckl, M. J., & Arcaro, A. (2006). Potent inhibition of small-cell lung cancer cell growth by simvastatin reveals selective functions of Ras isoforms in growth factor signalling. *Oncogene*, *25*(6), 877–887. https://doi.org/10.1038/sj.onc.1209117

Kiess, M., Gill, R. M., & Hamel, P. A. (1995). Expression and activity of the retinoblastoma protein (pRB)-family proteins, p107 and p130, during L6 myoblast differentiation. *Cell Growth and Differentiation*, *6*(10), 1287–1298. http://cgd.aacrjournals.org/cgi/content/abstract/6/10/1287

Kim, D. W., Wu, N., Kim, Y. C., Cheng, P. F., Basom, R., Kim, D., Dunn, C. T., Lee, A. Y., Kim, K., Lee, C. S., Singh, A., Gazdar, A. F., Harris, C. R., Eisenman, R. N., Park, K. S., & MacPherson, D. (2016). Genetic requirement for Mycl and efficacy of RNA Pol I inhibition in mouse models of small cell lung cancer. *Genes & Development*, *30*(11), 1289–1299. https://doi.org/10.1101/gad.279307.116

Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., & Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell*, *110*(2), 163–175. https://doi.org/10.1016/S0092-8674(02)00808-5

Kim, D. H., Sarbassov, D. D., Ali, S. M., Latek, R. R., Guntur, K. V. P., Erdjument-

Bromage, H., Tempst, P., & Sabatini, D. M. (2003). GβL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. *Molecular Cell*, *11*(4), 895–904. https://doi.org/10.1016/S1097-2765(03)00114-X

Kim, E., Goraksha-Hicks, P., Li, L., Neufeld, T. P., & Guan, K. L. (2008). Regulation of TORC1 by Rag GTPases in nutrient response. *Nature Cell Biology*, *10*(8), 935–945. https://doi.org/10.1038/ncb1753

Kim, J., & Guan, K. L. (2019). mTOR as a central hub of nutrient signalling and cell growth. In *Nature Cell Biology* (Vol. 21, Issue 1, pp. 63–71). Nature Publishing Group. https://doi.org/10.1038/s41556-018-0205-1

Kim, J. H., You, K. R., Kim, I. H., Cho, B. H., Kim, C. Y., & Kim, D. G. (2004). Over-Expression of the Ribosomal Protein L36a Gene Is Associated with Cellular Proliferation in Hepatocellular Carcinoma. *Hepatology*, *39*(1), 129–138. https://doi.org/10.1002/hep.20017

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature Cell Biology*, *13*(2), 132–141. https://doi.org/10.1038/ncb2152

Kim, S., Li, Q., Dang, C. V., & Lee, L. A. (2000). Induction of ribosomal genes and hepatocyte hypertrophy by adenovirus-mediated expression of c-Myc in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(21), 11198–11202. https://doi.org/10.1073/pnas.200372597

Kim, Y. H., Girard, L., Giacomini, C. P., Wang, P., Hernandez-Boussard, T., Tibshirani,

R., Minna, J. D., & Pollack, J. R. (2006). Combined microarray analysis of small cell lung cancer reveals altered apoptotic balance and distinct expression signatures of MYC family gene amplification. *Oncogene*, *25*, 130–138. https://doi.org/10.1038/sj.onc.1208997

Kim, Y. J., Keam, B., Ock, C. Y., Song, S., Kim, M., Kim, S. H., Kim, K. H., Kim, J. S., Kim, T. M., Kim, D. W., Lee, J. S., & Heo, D. S. (2019). A phase II study of pembrolizumab and paclitaxel in patients with relapsed or refractory small-cell lung cancer. *Lung Cancer*, *136*, 122–128. https://doi.org/10.1016/j.lungcan.2019.08.031

Klinge, S., Voigts-Hoffmann, F., Leibundgut, M., Arpagaus, S., & Ban, N. (2011). Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6. *Science*, *334*(6058), 941–948. https://doi.org/10.1126/science.1211204

Knecht, W., Bergjohann, U., Gonski, S., Kirschbaum, B., & Lóffler, M. (1996). Functional Expression of a Fragment of Human Dihydroorotate Dehydrogenase by Means of the Baculovirus Expression Vector System, and Kinetic Investigation of the Purified Recombinant Enzyme. *European Journal of Biochemistry*, *240*(1), 292–301. https://doi.org/10.1111/j.1432-1033.1996.0292h.x

Kondo, M., Yamaoka, T., Honda, S., Miwa, Y., Katashima, R., Moritani, M., Yoshimoto, K., Hayashi, Y., & Itakura, M. (2000). The rate of cell growth is regulated by purine biosynthesis via ATP production and G1 to S phase transition. *Journal of Biochemistry*, *128*(1), 57–64. https://doi.org/10.1093/oxfordjournals.jbchem.a022730

Koss, K., Harrison, R. F., Gregory, J., Darnton, S. J., Anderson, M. R., & Jankowski, J.

A. Z. (2004). The metabolic marker tumour pyruvate kinase type M2 (tumour M2-PK) shows increased expression along the metaplasia-dysplasia-adenocarcinoma sequence in Barrett's oesophagus. *Journal of Clinical Pathology*, *57*(11), 1156–1159. https://doi.org/10.1136/jcp.2004.018150

Kozutsumi, Y., Segal, M., Normington, K., Gething, M. J., & Sambrook, J. (1988). The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature*, *332*(6163), 462–464. https://doi.org/10.1038/332462a0

Krafts, K. P. (2010). Tissue repair: The hidden drama. In *Organogenesis* (Vol. 6, Issue 4, pp. 225–233). Taylor & Francis. https://doi.org/10.4161/org.6.4.12555

Kressler, D., Linder, P., & de la Cruz, J. (1999). Protein trans-Acting Factors Involved in Ribosome Biogenesis in Saccharomyces cerevisiae. *Molecular and Cellular Biology*, *19*(12), 7897–7912. https://doi.org/10.1128/mcb.19.12.7897

Krishnamurthy, A., & Jimeno, A. (2017). Atezolizumab: A novel PD-L1 inhibitor in cancer therapy with a focus in bladder and non-small cell lung cancers. *Drugs of Today*, *53*(4), 217–237. https://doi.org/10.1358/dot.2017.53.4.2589163

Labuschagne, C. F., van den Broek, N. J. F., Mackay, G. M., Vousden, K. H., & Maddocks, O. D. K. (2014). Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. *Cell Reports*, *7*(4), 1248–1258. https://doi.org/10.1016/j.celrep.2014.04.045

Lackey, A., & Donington, J. (2013). Surgical management of lung cancer. *Seminars in Interventional Radiology*, *30*(2), 133–140. https://doi.org/10.1055/s-0033-1342954

Lawley, P. D. (1980). DNA as a target of alkylating carcinogens. *British Medical Bulletin*, *36*(1), 19–24. https://doi.org/10.1093/oxfordjournals.bmb.a071608

Lee, A. H., Iwakoshi, N. N., & Glimcher, L. H. (2003). XBP-1 Regulates a Subset of Endoplasmic Reticulum Resident Chaperone Genes in the Unfolded Protein Response. *Molecular and Cellular Biology*, *23*(21), 7448–7459. https://doi.org/10.1128/mcb.23.21.7448-7459.2003

Lee, D. F., Kuo, H. P., Chen, C. Te, Hsu, J. M., Chou, C. K., Wei, Y., Sun, H. L., Li, L. Y., Ping, B., Huang, W. C., He, X., Hung, J. Y., Lai, C. C., Ding, Q., Su, J. L., Yang, J. Y., Sahin, A. A., Hortobagyi, G. N., Tsai, F. J., … Hung, M. C. (2007). IKKβ Suppression of TSC1 Links Inflammation and Tumor Angiogenesis via the mTOR Pathway. *Cell*, *130*(3), 440–455. https://doi.org/10.1016/j.cell.2007.05.058

Lee, D. F., Kuo, H. P., Chen, C. Te, Wei, Y., Chou, C. K., Hung, J. Y., Yen, C. J., & Hung, M. C. (2008). IKKβ suppression of TSC1 function links the mTOR pathway with insulin resistance. *International Journal of Molecular Medicine*, *22*(5), 633–638. https://doi.org/10.3892/ijmm_00000065

Lee, E. Y. H. P., To, H., Shew, J. Y., Bookstein, R., Scully, P., & Lee, W. H. (1988). Inactivation of the retinoblastoma susceptibility gene in human breast cancers. *Science*, *241*(4862), 218–221. https://doi.org/10.1126/science.3388033

Lee, J. H., Cho, Y. ra, Kim, J. H., Kim, J., Nam, H. Y., Kim, S. W., & Son, J. (2019). Branched-chain amino acids sustain pancreatic cancer growth by regulating lipid metabolism. *Experimental and Molecular Medicine*, *51*(11), 1–11. https://doi.org/10.1038/s12276-019-0350-z

Lee, W. N. P., Byerley, L. O., Bassilian, S., Ajie, H. O., Clark, I., Edmond, J., & Bergner, E. A. (1995). Isotopomer study of lipogenesis in human hepatoma cells in culture: Contribution of carbon and hydrogen atoms from glucose. *Analytical Biochemistry*, *226*(1), 100–112. https://doi.org/10.1006/abio.1995.1197

Lee, Y. J., Cho, A., Cho, B. C., Yun, M., Kim, S. K., Chang, J., Moon, J. W., Park, I. K., Choi, H. J., & Kim, J.-H. (2009). High Tumor Metabolic Activity as Measured by Fluorodeoxyglucose Positron Emission Tomography Is Associated with Poor Prognosis in Limited and Extensive Stage Small-Cell Lung Cancer. *Clinical Cancer Research*, *15*(7), 2426–2432. https://doi.org/10.1158/1078-0432.CCR-08-2258

Lemasters, J. J., Nieminen, A. L., Qian, T., Trost, L. C., Elmore, S. P., Nishimura, Y., Crowe, R. A., Cascio, W. E., Bradham, C. A., Brenner, D. A., & Herman, B. (1998). The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis and autophagy. *Biochimica et Biophysica Acta - Bioenergetics*, *1366*(1–2), 177–196. https://doi.org/10.1016/S0005-2728(98)00112-1

Leone, G., DeGregori, J., Yan, Z., Jakoi, L., Ishida, S., Williams, R. S., & Nevins, J. R. (1998). E2F3 activity is regulated during the cell cycle and is required for the induction of S phase. *Genes and Development*, *12*(14), 2120–2130. https://doi.org/10.1101/gad.12.14.2120

Levin, N. A., Brzoska, P., Gupta, N., Minna, J. D., Gray, J. W., & Christman, M. F. (1994). Identification of Frequent Novel Genetic Alterations in Small Cell Lung Carcinoma. *Cancer Research*, *54*(19).

Li, J. T., Yin, M., Wang, D., Wang, J., Lei, M. Z., Zhang, Y., Liu, Y., Zhang, L., Zou, S.

W., Hu, L. P., Zhang, Z. G., Wang, Y. P., Wen, W. Y., Lu, H. J., Chen, Z. J., Su, D., & Lei, Q. Y. (2020). BCAT2-mediated BCAA catabolism is critical for development of pancreatic ductal adenocarcinoma. In *Nature Cell Biology* (Vol. 22, Issue 2, pp. 167–174). Nature Research. https://doi.org/10.1038/s41556-019-0455-6

Li, L., Ng, S. R., Colón, C. I., Drapkin, B. J., Hsu, P. P., Li, Z., Nabel, C. S., Lewis, C. A., Romero, R., Mercer, K. L., Bhutkar, A., Phat, S., Myers, D. T., Muzumdar, M. D., Westcott, P. M. K., Beytagh, M. C., Farago, A. F., Heiden, M. G. V., Dyson, N. J., & Jacks, T. (2019). Identification of DHODH as a therapeutic target in small cell lung cancer. *Science Translational Medicine*, *11*(517). https://doi.org/10.1126/scitranslmed.aaw7852

Li, M., Brooks, C. L., Kon, N., & Gu, W. (2004). A dynamic role of HAUSP in the p53-Mdm2 pathway. *Molecular Cell*, *13*(6), 879–886. https://doi.org/10.1016/S1097-2765(04)00157-1

Li, Z., Van Calcar, S., Qu, C., Cavenee, W. K., Zhang, M. Q., & Ren, B. (2003). *A global transcriptional regulatory role for c-Myc in Burkitt's lymphoma cells*. www.pnas.org.

Liu, Y. C., Li, F., Handler, J., Huang, C. R. L., Xiang, Y., Neretti, N., Sedivy, J. M., Zeller, K. I., & Dang, C. V. (2008). Global regulation of nucleotide biosynthetic genes by c-myc. *PLoS ONE*, *3*(7). https://doi.org/10.1371/journal.pone.0002722

Liu, Y., Du, F., Chen, W., Yao, M., Lv, K., & Fu, P. (2015). EIF5A2 is a novel chemoresistance gene in breast cancer. *Breast Cancer*, *22*(6), 602–607. https://doi.org/10.1007/s12282-014-0526-2

Loewith, R., Jacinto, E., Wullschleger, S., Lorberg, A., Crespo, J. L., Bonenfant, D.,

Oppliger, W., Jenoe, P., & Hall, M. N. (2002). Two TOR complexes, only one of which is rapamycin sensitive, have distinct roles in cell growth control. *Molecular Cell*, *10*(3), 457–468. https://doi.org/10.1016/S1097-2765(02)00636-6

Lohka, M. J., Hayes, M. K., & Maller, J. L. (1988). Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(9), 3009–3013. https://doi.org/10.1073/pnas.85.9.3009

Lu, G., Sun, H., She, P., Youn, J. Y., Warburton, S., Ping, P., Vondriska, T. M., Cai, H., Lynch, C. J., & Wang, Y. (2009). Protein phosphatase 2Cm is a critical regulator of branched-chain amino acid catabolism in mice and cultured cells. *Journal of Clinical Investigation*, *119*(6), 1678–1687. https://doi.org/10.1172/JCI38151

Lu, T., Yang, X., Huang, Y., Zhao, M., Li, M., Ma, K., Yin, J., Zhan, C., & Wang, Q. (2019). Trends in the incidence, treatment, and survival of patients with lung cancer in the last four decades. *Cancer Management and Research*, *11*, 943–953. https://doi.org/10.2147/CMAR.S187317

Luo, R. X., Postigo, A. A., & Dean, D. C. (1998). Rb interacts with histone deacetylase to repress transcription. *Cell*, *92*(4), 463–473. https://doi.org/10.1016/S0092-8674(00)80940-X

Ma, J., Martin, J. D., Xue, Y., Lor, L. A., Kennedy-Wilson, K. M., Sinnamon, R. H., Ho, T. F., Zhang, G., Schwartz, B., Tummino, P. J., & Lai, Z. (2010). C-terminal region of USP7/HAUSP is critical for deubiquitination activity and contains a second mdm2/p53 binding site. *Archives of Biochemistry and Biophysics*, *503*(2), 207–212.

https://doi.org/10.1016/j.abb.2010.08.020

Ma, L., Chen, Z., Erdjument-Bromage, H., Tempst, P., & Pandolfi, P. P. (2005). Phosphorylation and functional inactivation of TSC2 by Erk: Implications for tuberous sclerosis and cancer pathogenesis. *Cell*, *121*(2), 179–193. https://doi.org/10.1016/j.cell.2005.02.031

Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J. P., Troalen, F., Trouche, D., & Harel-Bellan, A. (1998). Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature*, *391*(6667), 601–605. https://doi.org/10.1038/35410

Mahoney, C. E., Pirman, D., Chubukov, V., Sleger, T., Hayes, S., Fan, Z. P., Allen, E. L., Chen, Y., Huang, L., Liu, M., Zhang, Y., McDonald, G., Narayanaswamy, R., Choe, S., Chen, Y., Gross, S., Cianchetta, G., Padyana, A. K., Murray, S., … Smolen, G. A. (2019). A chemical biology screen identifies a vulnerability of neuroendocrine cancer cells to SQLE inhibition. *Nature Communications*, *10*(1), 1–14. https://doi.org/10.1038/s41467-018-07959-4

Malynn, B. A., De Alboran, I. M., O'Hagan, R. C., Bronson, R., Davidson, L., DePinho, R. A., & Alt, F. W. (2000). N-myc can functionally replace c-myc in murine development, cellular growth, and differentiation. *Genes and Development*, *14*(11), 1390–1399. https://doi.org/10.1101/gad.14.11.1390

Manning, B. D. (2004). Balancing Akt with S6K: Implications for both metabolic diseases and tumorigenesis. In *Journal of Cell Biology* (Vol. 167, Issue 3, pp. 399–403). The Rockefeller University Press. https://doi.org/10.1083/jcb.200408161

Manning, B. D., Tee, A. R., Logsdon, M. N., Blenis, J., & Cantley, L. C. (2002). Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/Akt pathway. *Molecular Cell*, *10*(1), 151–162. https://doi.org/10.1016/S1097-2765(02)00568-3

Mastroberardino, L., Spindler, B., Pfeiffer, R., Skelly, P. J., Loffing, J., Shoemaker, C. B., & Verrey, F. (1998). Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature*, *395*(6699), 288–291. https://doi.org/10.1038/26246

Mayers, J. R., Torrence, M. E., Danai, L. V., Papagiannakopoulos, T., Davidson, S. M., Bauer, M. R., Lau, A. N., Ji, B. W., Dixit, P. D., Hosios, A. M., Muir, A., Chin, C. R., Freinkman, E., Jacks, T., Wolpin, B. M., Vitkup, D., & Vander Heiden, M. G. (2016). Tissue of origin dictates branched-chain amino acid metabolism in mutant Kras-driven cancers. *Science*, *353*(6304), 1161–1165. https://doi.org/10.1126/science.aaf5171

McBrayer, S. K., Mayers, J. R., DiNatale, G. J., Shi, D. D., Khanal, J., Chakraborty, A. A., Sarosiek, K. A., Briggs, K. J., Robbins, A. K., Sewastianik, T., Shareef, S. J., Olenchock, B. A., Parker, S. J., Tateishi, K., Spinelli, J. B., Islam, M., Haigis, M. C., Looper, R. E., Ligon, K. L., … Kaelin, W. G. (2018). Transaminase Inhibition by 2-Hydroxyglutarate Impairs Glutamate Biosynthesis and Redox Homeostasis in Glioma. *Cell*, *175*(1), 101-116.e25. https://doi.org/10.1016/j.cell.2018.08.038

Mccarthy, W. J., Meza, R., Jeon, J., & Moolgavkar, S. H. (2012). Lung cancer in never smokers: Epidemiology and risk prediction models. *Risk Analysis*, *32*(SUPPL.1), S69–S84. https://doi.org/10.1111/j.1539-6924.2012.01768.x

McColl, K., Wildey, G., Sakre, N., Lipka, M. B., Behtaj, M., Kresak, A., Chen, Y., Yang, M., Velcheti, V., Fu, P., & Dowlati, A. (2017). Reciprocal expression of INSM1 and YAP1 defines subgroups in small cell lung cancer. *Oncotarget*, *8*(43), 73745–73756. https://doi.org/10.18632/oncotarget.20572

McDermott, J., & Jimeno, A. (2015). Pembrolizumab: PD-1 inhibition as a therapeutic strategy in cancer. *Drugs of Today*, *51*(1), 7–20. https://doi.org/10.1358/dot.2015.51.1.2250387

Mecca, C., Giambanco, I., Bruscoli, S., Bereshchenko, O., Fioretti, B., Riccardi, C., Donato, R., & Arcuri, C. (2018). PP242 counteracts glioblastoma cell proliferation, migration, invasiveness and stemness properties by inhibiting mTORC2/AKT. *Frontiers in Cellular Neuroscience*, *12*, 99. https://doi.org/10.3389/fncel.2018.00099

Merrick, W. C., & Pavitt, G. D. (2018). Protein synthesis initiation in eukaryotic cells. *Cold Spring Harbor Perspectives in Biology*, *10*(12). https://doi.org/10.1101/cshperspect.a033092

Meuwissen, R., Linn, S. C., Linnoila, R. I., Zevenhoven, J., Mooi, W. J., & Berns, A. (2003). Induction of small cell lung cancer by somatic inactivation of both Trp53 and Rb1 in a conditional mouse model. *Cancer Cell*, *4*(3), 181–189. https://doi.org/10.1016/S1535-6108(03)00220-4

Meyer, C., Cagnon, L., Costa-Nunes, C. M., Baumgaertner, P., Montandon, N., Leyvraz, L., Michielin, O., Romano, E., & Speiser, D. E. (2014). Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunology, Immunotherapy*, *63*(3), 247–257.

https://doi.org/10.1007/s00262-013-1508-5

Milovanovic, I., Stjepanovic, M., & Mitrovic, D. (2017). Distribution patterns of the metastases of the lung carcinoma in relation to histological type of the primary tumor: An autopsy study. *Annals of Thoracic Medicine*, *12*(3), 191–198. https://doi.org/10.4103/atm.ATM_276_16

Miyamoto, H., Shuin, T., Torigoe, S., Iwasaki, Y., & Kubota, Y. (1995). Retinoblastoma gene mutations in primary human bladder cancer. *British Journal of Cancer*, *71*(4), 831–835. https://doi.org/10.1038/bjc.1995.160

Mollaoglu, G., Guthrie, M. R., Böhm, S., Brägelmann, J., Can, I., Ballieu, P. M., Marx, A., George, J., Heinen, C., Chalishazar, M. D., Cheng, H., Ireland, A. S., Denning, K. E., Mukhopadhyay, A., Vahrenkamp, J. M., Berrett, K. C., Mosbruger, T. L., Wang, J., Kohan, J. L., … Oliver, T. G. (2017). MYC Drives Progression of Small Cell Lung Cancer to a Variant Neuroendocrine Subtype with Vulnerability to Aurora Kinase Inhibition. *Cancer Cell*, *31*(2), 270–285. https://doi.org/10.1016/j.ccell.2016.12.005

Mondal, M., Mesmin, B., Mukherjee, S., & Maxfield, F. R. (2009). Sterols are mainly in the cytoplasmic leaflet of the plasma membrane and the endocytic recycling compartment in CHO cells. *Molecular Biology of the Cell*, *20*(2), 581–588. https://doi.org/10.1091/mbc.E08-07-0785

Morita, M., Sato, T., Nomura, M., Sakamoto, Y., Inoue, Y., Tanaka, R., Ito, S., Kurosawa, K., Yamaguchi, K., Sugiura, Y., Takizaki, H., Yamashita, Y., Katakura, R., Sato, I., Kawai, M., Okada, Y., Watanabe, H., Kondoh, G., Matsumoto, S., …

Tanuma, N. (2018). PKM1 Confers Metabolic Advantages and Promotes Cell-Autonomous Tumor Cell Growth. *Cancer Cell*, *33*(3), 355-367.e7. https://doi.org/10.1016/j.ccell.2018.02.004

Motas, N., Motas, C., Davidescu, M., Achim, D., Rus, O., Jianu, E., & Horvat, T. (2018). Neuroendocrine tumors of the lung with surgical resection and lymph node dissection in a tertiary thoracic surgery center. *Acta Endocrinologica*, *14*(2), 219–226. https://doi.org/10.4183/aeb.2018.219

Muthukkumar, S., Ramesh, T. M., & Bondada, S. (1995). Rapamycin, a potent immunosuppressive drug, causes programmed cell death in B lymphoma cells. *Transplantation*, *60*(3), 264–270. https://doi.org/10.1097/00007890-199508000-00010

Naalsund, A., Rostad, H., Strøm, E. H., Lund, M. B., & Strand, T. E. (2011). Carcinoid lung tumors - incidence, treatment and outcomes: A population-based study. *European Journal of Cardio-Thoracic Surgery*, *39*(4), 565–569. https://doi.org/10.1016/j.ejcts.2010.08.036

Nagasaka, M., & Gadgeel, S. M. (2018). Role of chemotherapy and targeted therapy in early-stage non-small cell lung cancer. In *Expert Review of Anticancer Therapy* (Vol. 18, Issue 1, pp. 63–70). Taylor and Francis Ltd. https://doi.org/10.1080/14737140.2018.1409624

Naora, H., Takai, I., Adachi, M., & Naora, H. (1998). Altered cellular responses by varying expression of a ribosomal protein gene: Sequential coordination of enhancement and suppression of ribosomal protein S3a gene expression induces

apoptosis. *Journal of Cell Biology*, *141*(3), 741–753. https://doi.org/10.1083/jcb.141.3.741

Nau, M. M., Brooks, B. J., Battey, J., Sausville, E., Gazdar, A. F., Kirsch, I. R., McBride, O. W., Bertness, V., Hollis, G. F., & Minna, J. D. (1985). L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature*, *318*(6041), 69–73. https://doi.org/10.1038/318069a0

Neinast, M. D., Jang, C., Hui, S., Murashige, D. S., Chu, Q., Morscher, R. J., Li, X., Zhan, L., White, E., Anthony, T. G., Rabinowitz, J. D., & Arany, Z. (2019). Quantitative Analysis of the Whole-Body Metabolic Fate of Branched-Chain Amino Acids. *Cell Metabolism*, *29*(2), 417-429.e4. https://doi.org/10.1016/j.cmet.2018.10.013

Neishabouri, S. H., Hutson, S. M., & Davoodi, J. (2015). Chronic activation of mTOR complex 1 by branched chain amino acids and organ hypertrophy. *Amino Acids*, *47*(6), 1167–1182. https://doi.org/10.1007/s00726-015-1944-y

Neptune, E. R., Podowski, M., Calvi, C., Cho, J. H., Garcia, J. G. N., Tuder, R., Linnoila, R. I., Tsai, M. J., & Dietz, H. C. (2008). Targeted disruption of NeuroD, a proneural basic helix-loop-helix factor, impairs distal lung formation and neuroendocrine morphology in the neonatal lung. *Journal of Biological Chemistry*, *283*(30), 21160–21169. https://doi.org/10.1074/jbc.M708692200

Nicholson, S. A., Beasley, M. B., Brambilla, E., Hasleton, P. S., Colby, T. V., Sheppard, M. N., Falk, R., & Travis, W. D. (2002). Small cell lung carcinoma (SCLC): A clinicopathologic study of 100 cases with surgical specimens. *American Journal of*

Surgical Pathology, *26*(9), 1184–1197. https://doi.org/10.1097/00000478-200209000-00009

Nicklin, P., Bergman, P., Zhang, B., Triantafellow, E., Wang, H., Nyfeler, B., Yang, H., Hild, M., Kung, C., Wilson, C., Myer, V. E., MacKeigan, J. P., Porter, J. A., Wang, Y. K., Cantley, L. C., Finan, P. M., & Murphy, L. O. (2009). Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell*, *136*(3), 521–534. https://doi.org/10.1016/j.cell.2008.11.044

Nielsen, S. J., Schneider, R., Bauer, U. M., Bannister, A. J., Morrison, A., O'Carroll, D., Firestein, R., Cleary, M., Jenuwein, T., Herrera, R. E., & Kouzarides, T. (2001). Rb targets histone H3 methylation and HP1 to promoters. *Nature*, *412*(6846), 561–565. https://doi.org/10.1038/35087620

Nishitani, S., Horie, M., Ishizaki, S., & Yano, H. (2013). Branched Chain Amino Acid Suppresses Hepatocellular Cancer Stem Cells through the Activation of Mammalian Target of Rapamycin. *PLoS ONE*, *8*(11), e82346. https://doi.org/10.1371/journal.pone.0082346

Nobukuni, Y., Mitsubuchi, H., Akaboshi, I., Indo, Y., Endo, F., Yoshioka, A., & Matsuda, I. (1991). Maple syrup urine disease: Complete defect of the E1β subunit of the branched chain α-ketoacid dehydrogenase complex due to a deletion of an 11-bp repeat sequence which encodes a mitochondrial targeting leader peptide in a family with the disease. *Journal of Clinical Investigation*, *87*(5), 1862–1866. https://doi.org/10.1172/JCI115209

Noguchi, T., Inoue, H., & Tanaka, T. (1986). The M1- and M2-type isozymes of rat

pyruvate kinase are produced from the same gene by alternative RNA splicing. *Journal of Biological Chemistry*, *261*, 13807–13812.

Noguchi, T., Yamada, K., Inoue, H., Matsuda, T., & Tanaka, T. (1987). The L- and R-type isozymes of rat pyruvate kinase are produced from a single gene by use of different promoters. *The Journal of Biological Chemistry*, *262*, 14366–14371.

O'Reilly, K. E., Rojo, F., She, Q. B., Solit, D., Mills, G. B., Smith, D., Lane, H., Hofmann, F., Hicklin, D. J., Ludwig, D. L., Baselga, J., & Rosen, N. (2006). mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Research*, *66*(3), 1500–1508. https://doi.org/10.1158/0008-5472.CAN-05-2925

Okano, N., Naruge, D., Kawai, K., Kobayashi, T., Nagashima, F., Endou, H., & Furuse, J. (2020). First-in-human phase I study of JPH203, an L-type amino acid transporter 1 inhibitor, in patients with advanced solid tumors. *Investigational New Drugs*, 1–12. https://doi.org/10.1007/s10637-020-00924-3

Owonikoko, T K, Kim, H. R., Govindan, R., Ready, N., Reck, M., Peters, S., Dakhil, S. R., Navarro, A., Rodriguez-Cid, J., Schenker, M., Lee, J. S., Gutierrez, V., Percent, I., Morgensztern, D., Fairchild, J., Baudelet, C., & Park, K. (2019). Nivolumab (nivo) plus ipilimumab (ipi), nivo, or placebo (pbo) as maintenance therapy in patients (pts) with extensive disease small cell lung cancer (ED-SCLC) after first-line (1L) platinum-based chemotherapy (chemo): Results from the double-blind, rando. *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *30*, ii77. https://doi.org/10.1093/annonc/mdz094

Owonikoko, T. K., Niu, H., Nackaerts, K., Csoszi, T., Ostoros, G., Mark, Z., Baik, C.,

Joy, A. A., Chouaid, C., Jaime, J. C., Kolek, V., Majem, M., Roubec, J., Santos, E. S., Chiang, A. C., Speranza, G., Belani, C. P., Chiappori, A., Patel, M. R., … Spigel, D. R. (2020). Randomized Phase II Study of Paclitaxel plus Alisertib versus Paclitaxel plus Placebo as Second-Line Therapy for SCLC: Primary and Correlative Biomarker Analyses. *Journal of Thoracic Oncology*, *15*(2), 274–287. https://doi.org/10.1016/j.jtho.2019.10.013

Oxender, D. L., & Chritensen, H. N. (1963). Distinct Mediating Systems for the Transport of Neutral Amino Acids by the Ehrlich Cell. *The Journal of Biological Chemistry*, *238*(11), 3686–3699.

Ozcan, U., Ozcan, L., Yilmaz, E., Düvel, K., Sahin, M., Manning, B. D., & Hotamisligil, G. S. (2008). Loss of the Tuberous Sclerosis Complex Tumor Suppressors Triggers the Unfolded Protein Response to Regulate Insulin Signaling and Apoptosis. *Molecular Cell*, *29*(5), 541–551. https://doi.org/10.1016/j.molcel.2007.12.023

Pakos‐Zebrucka, K., Koryga, I., Mnich, K., Ljujic, M., Samali, A., & Gorman, A. M. (2016). The integrated stress response. *EMBO Reports*, *17*(10), 1374–1395. https://doi.org/10.15252/embr.201642195

Pan, H., Yin, C., Dyson, N. J., Harlow, E., Yamasaki, L., & Van Dyke, T. (1998). Key roles for E2F1 in signaling p53-dependent apoptosis and in cell division within developing tumors. *Molecular Cell*, *2*(3), 283–292. https://doi.org/10.1016/S1097-2765(00)80273-7

Panda, D. K., Bai, X., Sabbagh, Y., Zhang, Y., Zaun, H.-C., Karellis, A., Koromilas, A. E., Lipman, M. L., & Karaplis, A. C. (2018). Defective interplay between mTORC1

activity and endoplasmic reticulum stress-unfolded protein response in uremic vascular calcification. *American Journal of Physiology-Renal Physiology*, *314*(6), F1046–F1061. https://doi.org/10.1152/ajprenal.00350.2017

Pandya, K. J., Dahlberg, S., Hidalgo, M., Cohen, R. B., Lee, M. W., Schiller, J. H., & Johnson, D. H. (2007). A randomized, phase II trial of two dose levels of temsirolimus (CCI-779) in patients with extensive-stage small-cell lung cancer who have responding or stable disease after induction chemotherapy: A trial of the Eastern Cooperative Oncology Group (E1500). *Journal of Thoracic Oncology*, *2*(11), 1036–1041. https://doi.org/10.1097/JTO.0b013e318155a439

Papadopoulos, K. P., Egile, C., Ruiz-Soto, R., Jiang, J., Shi, W., Bentzien, F., Rasco, D., Abrisqueta, P., Vose, J. M., & Tabernero, J. (2015). Efficacy, safety, pharmacokinetics and pharmacodynamics of SAR245409 (voxtalisib, XL765), an orally administered phosphoinositide 3-kinase/mammalian target of rapamycin inhibitor: A phase 1 expansion cohort in patients with relapsed or refractory lymphoma. *Leukemia and Lymphoma*, *56*(6), 1763–1770. https://doi.org/10.3109/10428194.2014.974040

Papandreou, I., Denko, N. C., Olson, M., Van Melckebeke, H., Lust, S., Tam, A., Solow-Cordero, D. E., Bouley, D. M., Offner, F., Niwa, M., & Koong, A. C. (2011). Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood*, *117*(4), 1311–1314. https://doi.org/10.1182/blood-2010-08-303099

Park, K. S., Liang, M. C., Raiser, D. M., Zamponi, R., Roach, R. R., Curtis, S. J., Walton, Z., Schaffer, B. E., Roake, C. M., Zmoos, A. F., Kriegel, C., Wong, K. K.,

Sage, J., & Kim, C. F. (2011). Characterization of the cell of origin for small cell lung cancer. *Cell Cycle*, *10*(16), 2806–2815. https://doi.org/10.4161/cc.10.16.17012

Passerini, L., Barzaghi, F., Curto, R., Sartirana, C., Barera, G., Tucci, F., Albarello, L., Mariani, A., Testoni, P. A., Bazzigaluppi, E., Bosi, E., Lampasona, V., Neth, O., Zama, D., Hoenig, M., Schulz, A., Seidel, M. G., Rabbone, I., Olek, S., … Bacchetta, R. (2020). Treatment with rapamycin can restore regulatory T-cell function in IPEX patients. *Journal of Allergy and Clinical Immunology*, *145*(4), 1262-1271.e13. https://doi.org/10.1016/j.jaci.2019.11.043

Pavlova, N. N., & Thompson, C. B. (2016). The Emerging Hallmarks of Cancer Metabolism. *Cell Metabolism*, *23*(1), 27–47. https://doi.org/10.1016/j.cmet.2015.12.006

Paz-Ares, L., Dvorkin, M., Chen, Y., Reinmuth, N., Hotta, K., Trukhin, D., Statsenko, G., Hochmair, M. J., Özgüroğlu, M., Ji, J. H., Voitko, O., Poltoratskiy, A., Ponce, S., Verderame, F., Havel, L., Bondarenko, I., Kazarnowicz, A., Losonczy, G., Conev, N. V., … Williamson, M. (2019). Durvalumab plus platinum–etoposide versus platinum–etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. *The Lancet*, *394*(10212), 1929–1939. https://doi.org/10.1016/S0140-6736(19)32222-6

Pedersen, N., Mortensen, S., Sørensen, S. B., Pedersen, M. W., Rieneck, K., Bovin, L. F., & Poulsen, H. S. (2003). Transcriptional gene expression profiling of small cell lung cancer cells. *Cancer Research*, *63*(8).

Pedley, A. M., & Benkovic, S. J. (2017). A New View into the Regulation of Purine

Metabolism: The Purinosome. *Trends in Biochemical Sciences*, *42*(2), 141–154. https://doi.org/10.1016/j.tibs.2016.09.009

Peifer, M., Fernández-Cuesta, L., Sos, M. L., George, J., Seidel, D., Kasper, L. H., Plenker, D., Leenders, F., Sun, R., Zander, T., Menon, R., Koker, M., Dahmen, I., Müller, C., Di Cerbo, V., Schildhaus, H. U., Altmüller, J., Baessmann, I., Becker, C., … Thomas, R. K. (2012). Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nature Genetics*, *44*(10), 1104–1110. https://doi.org/10.1038/ng.2396

Perry, J., & Kleckner, N. (2003). The ATRs, ATMs, and TORs are giant HEAT repeat proteins. In *Cell* (Vol. 112, Issue 2, pp. 151–155). Cell Press. https://doi.org/10.1016/S0092-8674(03)00033-3

Peterson, T. R., Laplante, M., Thoreen, C. C., Sancak, Y., Kang, S. A., Kuehl, W. M., Gray, N. S., & Sabatini, D. M. (2009). DEPTOR Is an mTOR Inhibitor Frequently Overexpressed in Multiple Myeloma Cells and Required for Their Survival. *Cell*, *137*(5), 873–886. https://doi.org/10.1016/j.cell.2009.03.046

Pettengill, O. S., Sorenson, G. D., Wurster‐Hill, D. H., Curphey, T. J., Noll, W. W., Gate, C. C., & Maurer, L. H. (1980). Isolation and growth characteristics of continuous cell lines from small‐cell carcinoma of the lung. *Cancer*, *45*(5), 906–918. https://doi.org/10.1002/1097-0142(19800301)45:5<906::AID-CNCR2820450513>3.0.CO;2-H

Planta, R. J., & Mager, W. H. (1998). The list of cytoplasmic ribosomal proteins of Saccharomyces cerevisiae. *Yeast*, *14*(5), 471–477.

https://doi.org/10.1002/(SICI)1097-0061(19980330)14:5<471::AID-YEA241>3.0.CO;2-U

Polanski, R., Hodgkinson, C. L., Fusi, A., Nonaka, D., Priest, L., Kelly, P., Trapani, F., Bishop, P. W., White, A., Critchlow, S. E., Smith, P. D., Blackhall, F., Dive, C., & Morrow, C. J. (2014). Activity of the monocarboxylate transporter 1 inhibitor azd3965 in small cell lung cancer. *Clinical Cancer Research*, *20*(4), 926–937. https://doi.org/10.1158/1078-0432.CCR-13-2270

Price, D. J., Nemenoff, R. A., & Avruch, J. (1989). Purification of a hepatic S6 kinase from cycloheximide-treated rats. *Journal of Biological Chemistry*, *264*(23), 13825–13833.

Proud, C. G. (2004). mTOR-mediated regulation of translation factors by amino acids. *Biochemical and Biophysical Research Communications*, *313*(2), 429–436. https://doi.org/10.1016/j.bbrc.2003.07.015

Qin, X. Q., Livingston, D. M., Ewen, M., Sellers, W. R., Arany, Z., & Kaelin, W. G. (1995). The transcription factor E2F-1 is a downstream target of RB action. *Molecular and Cellular Biology*, *15*(2), 742–755. https://doi.org/10.1128/mcb.15.2.742

Qu, Y. Y., Zhao, R., Zhang, H. L., Zhou, Q., Xu, F. J., Zhang, X., Xu, W. H., Shao, N., Zhou, S. X., Dai, B., Zhu, Y., Shi, G. H., Shen, Y. J., Zhu, Y. P., Han, C. T., Chang, K., Lin, Y., Zang, W. D., Xu, W., … Zhao, J. Y. (2020). Inactivation of the AMPK–GATA3–ECHS1 pathway induces fatty acid synthesis that promotes clear cell renal cell carcinoma growth. *Cancer Research*, *80*(2), 319–333.

https://doi.org/10.1158/0008-5472.CAN-19-1023

Rabanal-Ruiz, Y., Otten, E. G., & Korolchuk, V. I. (2017). MTORC1 as the main gateway to autophagy. *Essays in Biochemistry*, *61*(6), 565–584. https://doi.org/10.1042/EBC20170027

Rabl, J., Leibundgut, M., Ataide, S. F., Haag, A., & Ban, N. (2011). Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1. *Science*, *331*(6018), 730–736. https://doi.org/10.1126/science.1198308

Raffel, S., Falcone, M., Kneisel, N., Hansson, J., Wang, W., Lutz, C., Bullinger, L., Poschet, G., Nonnenmacher, Y., Barnert, A., Bahr, C., Zeisberger, P., Przybylla, A., Sohn, M., Tönjes, M., Erez, A., Adler, L., Jensen, P., Scholl, C., … Trumpp, A. (2017). BCAT1 restricts αkG levels in AML stem cells leading to IDHmut-like DNA hypermethylation. *Nature*, *551*(7680), 384–388. https://doi.org/10.1038/nature24294

Rajeswaran, A., Trojan, A., Burnand, B., & Giannelli, M. (2008). Efficacy and side effects of cisplatin- and carboplatin-based doublet chemotherapeutic regimens versus non-platinum-based doublet chemotherapeutic regimens as first line treatment of metastatic non-small cell lung carcinoma: A systematic review of randomi. *Lung Cancer*, *59*(1), 1–11. https://doi.org/10.1016/j.lungcan.2007.07.012

Ramanathan, A., Robb, G. B., & Chan, S. H. (2016). mRNA capping: Biological functions and applications. *Nucleic Acids Research*, *44*(16), 7511–7526. https://doi.org/10.1093/nar/gkw551

Ready, N., Farago, A. F., de Braud, F., Atmaca, A., Hellmann, M. D., Schneider, J. G.,

Spigel, D. R., Moreno, V., Chau, I., Hann, C. L., Eder, J. P., Steele, N. L., Pieters, A., Fairchild, J., & Antonia, S. J. (2019). Third-Line Nivolumab Monotherapy in Recurrent SCLC: CheckMate 032. *Journal of Thoracic Oncology*, *14*(2), 237–244. https://doi.org/10.1016/j.jtho.2018.10.003

Rebsamen, M., Pochini, L., Stasyk, T., De Araújo, M. E. G., Galluccio, M., Kandasamy, R. K., Snijder, B., Fauster, A., Rudashevskaya, E. L., Bruckner, M., Scorzoni, S., Filipek, P. A., Huber, K. V. M., Bigenzahn, J. W., Heinz, L. X., Kraft, C., Bennett, K. L., Indiveri, C., Huber, L. A., & Superti-Furga, G. (2015). SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. *Nature*, *519*(7544), 477–481. https://doi.org/10.1038/nature14107

Reichard, P., Baldesten, A., & Rutberg, L. (1961). Formation of deoxycytidine phosphates from cytidine phosphates in extracts from Escherichia coli. *The Journal of Biological Chemistry*, *236*, 1150–1157.

Reinacher, M., & Eigenbrodt, E. (1981). Immunohistological demonstration of the same type of pyruvate kinase isoenzyme (M2-Pk) in tumors of chicken and rat. *Virchows Archiv B Cell Pathology Including Molecular Pathology*, *37*(1), 79–88. https://doi.org/10.1007/BF02892557

Ricciardi, M., Zanotto, M., Malpeli, G., Bassi, G., Perbellini, O., Chilosi, M., Bifari, F., & Krampera, M. (2015). Epithelial-to-mesenchymal transition (EMT) induced by inflammatory priming elicits mesenchymal stromal cell-like immune-modulatory properties in cancer cells. *British Journal of Cancer*, *112*(6), 1067–1075. https://doi.org/10.1038/bjc.2015.29

Rodrigues, G., Videtic, G. M. M., Sur, R., Bezjak, A., Bradley, J., Hahn, C. A., Langer, C., Miller, K. L., Moeller, B. J., Rosenzweig, K., & Movsas, B. (2011). Palliative thoracic radiotherapy in lung cancer: An American Society for Radiation Oncology evidence-based clinical practice guideline. *Practical Radiation Oncology*, *1*(2), 60–71. https://doi.org/10.1016/j.prro.2011.01.005

Rodríguez-Enríquez, S., Torres-Márquez, M. E., & Moreno-Sánchez, R. (2000). Substrate oxidation and ATP supply in AS-30D hepatoma cells. *Archives of Biochemistry and Biophysics*, *375*(1), 21–30. https://doi.org/10.1006/abbi.1999.1582

Ross, J. F., Liu, X., & Dynlacht, B. D. (1999). Mechanism of transcriptional repression of E2F by the retinoblastoma tumor suppressor protein. *Molecular Cell*, *3*(2), 195–205. https://doi.org/10.1016/S1097-2765(00)80310-X

Rudin, C. M., Durinck, S., Stawiski, E. W., Poirier, J. T., Modrusan, Z., Shames, D. S., Bergbower, E. A., Guan, Y., Shin, J., Guillory, J., Rivers, C. S., Foo, C. K., Bhatt, D., Stinson, J., Gnad, F., Haverty, P. M., Gentleman, R., Chaudhuri, S., Janakiraman, V., … Seshagiri, S. (2012). Comprehensive genomic analysis identifies SOX2 as a frequently amplified gene in small-cell lung cancer. *Nature Genetics*, *44*(10), 1111–1116. https://doi.org/10.1038/ng.2405

Rudin, C. M., Poirier, J. T., Byers, L. A., Dive, C., Dowlati, A., George, J., Heymach, J. V, Johnson, J. E., Lehman, J. M., MacPherson, D., Massion, P. P., Minna, J. D., Oliver, T. G., Quaranta, V., Sage, J., Thomas, R. K., Vakoc, C. R., & Gazdar, A. F. (2019). Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nature Reviews Cancer*, *19*(5), 289–297.

https://doi.org/10.1038/s41568-019-0133-9

Ruel, L., Stambolic, V., Ali, A., Manoukian, A. S., & Woodgett, J. R. (1999). Regulation of the protein kinase activity of Shaggy(zeste-white3) by components of the Wingless pathway in Drosophila cells and embryos. *Journal of Biological Chemistry*, *274*(31), 21790–21796. https://doi.org/10.1074/jbc.274.31.21790

Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P., & Snyder, S. H. (1994). RAFT1: A mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*, *78*(1), 35–43. https://doi.org/10.1016/0092-8674(94)90570-3

Sabers, C. J., Martin, M. M., Brunn, G. J., Williams, J. M., Dumont, F. J., Wiederrecht, G., & Abraham, R. T. (1995). Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *Journal of Biological Chemistry*, *270*(2), 815–822. https://doi.org/10.1074/jbc.270.2.815

Sancak, Y., Bar-Peled, L., Zoncu, R., Markhard, A. L., Nada, S., & Sabatini, D. M. (2010). Ragulator-rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell*, *141*(2), 290–303. https://doi.org/10.1016/j.cell.2010.02.024

Sancak, Y., Peterson, T. R., Shaul, Y. D., Lindquist, R. A., Thoreen, C. C., Bar-Peled, L., & Sabatini, D. M. (2008). The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science (New York, N.Y.)*, *320*(5882), 1496–1501. https://doi.org/10.1126/science.1157535

Sancak, Y., Thoreen, C. C., Peterson, T. R., Lindquist, R. A., Kang, S. A., Spooner, E.,

Carr, S. A., & Sabatini, D. M. (2007). PRAS40 Is an Insulin-Regulated Inhibitor of the mTORC1 Protein Kinase. *Molecular Cell*, *25*(6), 903–915. https://doi.org/10.1016/j.molcel.2007.03.003

Sarbassov, D. D., Ali, S. M., Sengupta, S., Sheen, J. H., Hsu, P. P., Bagley, A. F., Markhard, A. L., & Sabatini, D. M. (2006). Prolonged Rapamycin Treatment Inhibits mTORC2 Assembly and Akt/PKB. *Molecular Cell*, *22*(2), 159–168. https://doi.org/10.1016/j.molcel.2006.03.029

Sato, N., Ohtake, Y., Kato, H., Abe, S., Kohno, H., & Ohkubo, Y. (2003). Effects of polyamines on histone polymerization. *Journal of Protein Chemistry*, *22*(3), 303–307. https://doi.org/10.1023/A:1025032906494

Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell*, *168*(6), 960–976. https://doi.org/10.1016/j.cell.2017.02.004

Schaffer, B. E., Park, K. S., Yiu, G., Conklin, J. F., Lin, C., Burkhart, D. L., Karnezis, A. N., Sweet-Cordero, E. A., & Sage, J. (2010). Loss of p130 accelerates tumor development in a mouse model for human small-cell lung carcinoma. *Cancer Research*, *70*(10), 3877–3883. https://doi.org/10.1158/0008-5472.CAN-09-4228

Schneider, B. J., Saxena, A., & Downey, R. J. (2011). Surgery for early-stage small cell lung cancer. In *JNCCN Journal of the National Comprehensive Cancer Network* (Vol. 9, Issue 10, pp. 1132–1139). Harborside Press. https://doi.org/10.6004/jnccn.2011.0094

Schuler, W., Sedrani, R., Cottens, S., Häberlin, B., Schulz, M., Schuurman, H. J., Zenke, G., Zerwes, H. G., & Schreier, M. H. (1997). SDZ RAD, a new rapamycin

derivative: Pharmacological properties in vitro and in vivo. *Transplantation*, *64*(1), 36–42. https://doi.org/10.1097/00007890-199707150-00008

Schumacher, T., Brink, I., Mix, M., Reinhardt, M., Herget, G., Digel, W., Henke, M., Moser, E., & Nitzsche, E. (2001). FDG-PET imaging for the staging and follow-up of small cell lung cancer. *European Journal of Nuclear Medicine*, *28*(4), 483–488. https://doi.org/10.1007/s002590100474

Schwarze, P. E., & Seglen, P. O. (1985). Reduced autophagic activity, improved protein balance and enhanced in vitro survival of hepatocytes isolated from carcinogen-treated rats. *Experimental Cell Research*, *157*(1), 15–28. https://doi.org/10.1016/0014-4827(85)90148-X

Schwarzer, R., Tondera, D., Arnold, W., Giese, K., Klippel, A., & Kaufmann, J. (2005). REDD1 integrates hypoxia-mediated survival signaling downstream of phosphatidylinositol 3-kinase. *Oncogene*, *24*, 1138–1149. https://doi.org/10.1038/sj.onc.1208236

Sehgal, S. N., Baker, H., & Vezina, C. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *The Journal of Antibiotics*, *28*(10), 727–732. https://doi.org/10.7164/antibiotics.28.727

Sekiguchi, T., Hirose, E., Nakashima, N., Ii, M., & Nishimoto, T. (2001). Novel G Proteins, Rag C and Rag D, Interact with GTP-binding Proteins, Rag A and Rag B. *Journal of Biological Chemistry*, *276*(10), 7246–7257. https://doi.org/10.1074/jbc.M004389200

Selwan, E. M., & Edinger, A. L. (2017). Branched chain amino acid metabolism and

cancer: The importance of keeping things in context. In *Translational Cancer Research* (Vol. 6, Issue 3, pp. S578–S584). https://doi.org/10.21037/tcr.2017.05.05

Serrano M, Lin AW, McCurrach ME, Beach D, L. S. (1997). Oncogenic ras provokes premature cell senescence associciated with accumulation of p53 and p16INK4a. *Cell*, *88*(5), 593–602.

Seufferlein, T., & Rozengurt, E. (1996). Rapamycin inhibits constitutive p70(s6k) phosphorylation, cell proliferation, and colony formation in small cell lung cancer cells. *Cancer Research*, *56*(17), 3895–3897.

Shaffer, A. L., Shapiro-Shelef, M., Iwakoshi, N. N., Lee, A. H., Qian, S. B., Zhao, H., Yu, X., Yang, L., Tan, B. K., Rosenwald, A., Hurt, E. M., Petroulakis, E., Sonenberg, N., Yewdell, J. W., Calame, K., Glimcher, L. H., & Staudt, L. M. (2004). XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity*, *21*(1), 81–93. https://doi.org/10.1016/j.immuni.2004.06.010

Shang, L., Chen, S., Du, F., Li, S., Zhao, L., & Wang, X. (2011). Nutrient starvation elicits an acute autophagic response mediated by Ulk1 dephosphorylation and its subsequent dissociation from AMPK. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(12), 4788–4793. https://doi.org/10.1073/pnas.1100844108

Shen, K., Huang, R. K., Brignole, E. J., Condon, K. J., Valenstein, M. L., Chantranupong, L., Bomaliyamu, A., Choe, A., Hong, C., Yu, Z., & Sabatini, D. M. (2018). Architecture of the human GATOR1 and GATOR1-Rag GTPases

complexes. *Nature*, *556*(7699), 64–69. https://doi.org/10.1038/nature26158

Shi, Y., Frankel, A., Radvanyi, L. G., Penn, L. Z., Miller, R. G., & Mills, G. B. (1995). Rapamycin Enhances Apoptosis and Increases Sensitivity to Cisplatin in Vitro. *Cancer Research*, *55*(9).

Showkat, M., Beigh, M. A., & Andrabi, K. I. (2014). mTOR Signaling in Protein Translation Regulation: Implications in Cancer Genesis and Therapeutic Interventions. *Molecular Biology International*, *2014*, 1–14. https://doi.org/10.1155/2014/686984

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(1), 7–34. https://doi.org/10.3322/caac.21551

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, *70*(1), 7–30. https://doi.org/10.3322/caac.21590

Sigoillot, F. D., Berkowski, J. A., Sigoillot, S. M., Kotsis, D. H., & Guy, H. I. (2003). Cell Cycle-dependent Regulation of Pyrimidine Biosynthesis. *Journal of Biological Chemistry*, *278*(5), 3403–3409. https://doi.org/10.1074/JBC.M211078200

Silva, L. S., Poschet, G., Nonnenmacher, Y., Becker, H. M., Sapcariu, S., Gaupel, A., Schlotter, M., Wu, Y., Kneisel, N., Seiffert, M., Hell, R., Hiller, K., Lichter, P., & Radlwimmer, B. (2017). Branched‐chain ketoacids secreted by glioblastoma cells via MCT 1 modulate macrophage phenotype. *EMBO Reports*, *18*(12), 2172–2185. https://doi.org/10.15252/embr.201744154

Silvera, D., Arju, R., Darvishian, F., Levine, P. H., Zolfaghari, L., Goldberg, J., Hochman, T., Formenti, S. C., & Schneider, R. J. (2009). Essential role for eIF4GI

overexpression in the pathogenesis of inflammatory breast cancer. *Nature Cell Biology*, *11*(7), 903–908. https://doi.org/10.1038/ncb1900

Singer, S. J., & Nicolson, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science*, *175*(4023), 720–731. https://doi.org/10.1126/science.175.4023.720

Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A. M., & Czaja, M. J. (2009). Autophagy regulates lipid metabolism. *Nature*, *458*(7242), 1131–1135. https://doi.org/10.1038/nature07976

Smith, S. M., Van Besien, K., Karrison, T., Dancey, J., McLaughlin, P., Younes, A., Smith, S., Stiff, P., Lester, E., Modi, S., Doyle, L. A., Vokes, E. E., & Pro, B. (2010). Temsirolimus has activity in non-mantle cell non-Hodgkin's lymphoma subtypes: The University of Chicago phase II consortium. *Journal of Clinical Oncology*, *28*(31), 4740–4746. https://doi.org/10.1200/JCO.2010.29.2813

Sofer, A., Lei, K., Johannessen, C. M., & Ellisen, L. W. (2005). Regulation of mTOR and Cell Growth in Response to Energy Stress by REDD1. *Molecular and Cellular Biology*, *25*(14), 5834–5845. https://doi.org/10.1128/mcb.25.14.5834-5845.2005

Sonenberg, N., & Hinnebusch, A. G. (2009). Regulation of Translation Initiation in Eukaryotes: Mechanisms and Biological Targets. In *Cell* (Vol. 136, Issue 4, pp. 731–745). https://doi.org/10.1016/j.cell.2009.01.042

Song, Y., Sun, Y., Lei, Y., Yang, K., & Tang, R. (2020). YAP1 promotes multidrug resistance of small cell lung cancer by CD74-related signaling pathways. *Cancer Medicine*, *9*(1), 259–268. https://doi.org/10.1002/cam4.2668

Sonkin, D., Vural, S., Thomas, A., & Teicher, B. A. (2019). Neuroendocrine negative SCLC is mostly RB1 WT and may be sensitive to CDK4/6 inhibition. *BioRxiv*, 516351. https://doi.org/10.1101/516351

Sorrells, D. L., Black, D. R., Meschonat, C., Rhoads, R., De Benedetti, A., Gao, M., Williams, B. J., & Li, B. D. L. (1998). Detection of eIF4E gene amplification in breast cancer by competitive PCR. *Annals of Surgical Oncology*, *5*(3), 232–237. https://doi.org/10.1007/BF02303778

Steuer, C. E., Behera, M., Kim, S., Chen, Z., Saba, N. F., Pillai, R. N., Owonikoko, T. K., Khuri, F. R., & Ramalingam, S. S. (2015). Atypical carcinoid tumor of the lung: A surveillance, epidemiology, and end results database analysis. *Journal of Thoracic Oncology*, *10*(3), 479–485. https://doi.org/10.1097/JTO.0000000000000419

Strosberg, J. R. (2012). A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Phase III Trial (RADIANT-3) of Everolimus in Patients With Advanced Pancreatic Neuroendocrine Tumors: Updated Safety and Overall Survival Results. *Gastrointestinal Cancer Research : GCR*, *5*(4 Suppl 2), S13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434535/

Suh, E. H., Hackett, E. P., Wynn, R. M., Chuang, D. T., Zhang, B., Luo, W., Sherry, A. D., & Park, J. M. (2019). In vivo assessment of increased oxidation of branched-chain amino acids in glioblastoma. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-018-37390-0

Sullivan, L. B., Gui, D. Y., Hosios, A. M., Bush, L. N., Freinkman, E., & Vander Heiden, M. G. (2015). Supporting Aspartate Biosynthesis Is an Essential Function of

Respiration in Proliferating Cells. *Cell*, *162*(3), 552–563. https://doi.org/10.1016/j.cell.2015.07.017

Sullivan, L. B., Luengo, A., Danai, L. V., Bush, L. N., Diehl, F. F., Hosios, A. M., Lau, A. N., Elmiligy, S., Malstrom, S., Lewis, C. A., & Vander Heiden, M. G. (2018). Aspartate is an endogenous metabolic limitation for tumour growth. *Nature Cell Biology*, *20*(7), 782–788. https://doi.org/10.1038/s41556-018-0125-0

Sun, S. Y., Rosenberg, L. M., Wang, X., Zhou, Z., Yue, P., Fu, H., & Khuri, F. R. (2005). Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Research*, *65*(16), 7052–7058. https://doi.org/10.1158/0008-5472.CAN-05-0917

Suryawan, a, Hawes, J. W., Harris, R. a, Shimomura, Y., Jenkins, a E., & Hutson, S. M. (1998). A molecular model of human branched-chain amino acid metabolism. *Am J Clin Nutr*, *68*(1), 72–81. http://www.ncbi.nlm.nih.gov/pubmed/9665099

Sweatt, A. J., Wood, M., Suryawan, A., Wallin, R., Willingham, M. C., & Hutson, S. M. (2004). Branched-chain amino acid catabolism: unique segregation of pathway enzymes in organ systems and peripheral nerves. *American Journal of Physiology. Endocrinology and Metabolism*, *286*(1), E64–E76. https://doi.org/10.1152/ajpendo.00276.2003

Symonds, H., Krall, L., Remington, L., Saenz-Robles, M., Lowe, S., Jacks, T., & Van Dyke, T. (1994). p53-Dependent apoptosis suppresses tumor growth and progression in vivo. *Cell*, *78*(4), 703–711. https://doi.org/10.1016/0092-8674(94)90534-7

T'Ang, A., Varley, J. M., Chakraborty, S., Murphree, A. L., & Fung, Y. K. T. (1988). Structural rerrangement of the retinoblastoma gene in human breast carcinoma. *Science*, *242*(4876), 263–266. https://doi.org/10.1126/science.3175651

Tada, T., Kumada, T., Toyoda, H., Yasuda, S., Koyabu, T., & Nakashima, M. (2019). Impact of Branched-Chain Amino Acid Granule Therapy in Patients with Hepatocellular Carcinoma Who Have Normal Albumin Levels and Low Branched-Chain Amino Acid to Tyrosine Ratios. *Nutrition and Cancer*, *71*(7), 1132–1141. https://doi.org/10.1080/01635581.2019.1597905

Takano, A., Usui, I., Haruta, T., Kawahara, J., Uno, T., Iwata, M., & Kobayashi, M. (2001). Mammalian Target of Rapamycin Pathway Regulates Insulin Signaling via Subcellular Redistribution of Insulin Receptor Substrate 1 and Integrates Nutritional Signals and Metabolic Signals of Insulin. *Molecular and Cellular Biology*, *21*(15), 5050–5062. https://doi.org/10.1128/mcb.21.15.5050-5062.2001

Takegoshi, K., Honda, M., Okada, H., Takabatake, R., Matsuzawa-Nagata, N., Campbell, J. S., Nishikawa, M., Shimakami, T., Shirasaki, T., Sakai, Y., Yamashita, T., Takamura, T., Tanaka, T., & Kaneko, S. (2017). Branched-chain amino acids prevent hepatic fibrosis and development of hepatocellular carcinoma in a non-alcoholic steatohepatitis mouse model. *Oncotarget*, *8*(11), 18191–18205. https://doi.org/10.18632/oncotarget.15304

Taniwaki, M., Daigo, Y., Ishikawa, N., Takano, A., Tsunoda, T., Yasui, W., Inai, K., Kohno, N., & Nakamura, Y. (2006). Gene expression profiles of small-cell lung cancers: Molecular signatures of lung cancer. *International Journal of Oncology*, *29*(3), 567–575. https://doi.org/10.3892/ijo.29.3.567

Tannapfel, A., John, K., Miše, N., Schmidt, A., Buhlmann, S., Ibrahim, S. M., & Pü Tzer, B. M. (2008). Autonomous growth and hepatocarcinogenesis in transgenic mice expressing the p53 family inhibitor DNp73. *Carcinogenesis*, *29*(1), 211–218. https://doi.org/10.1093/carcin/bgm236

Tarhini, A., Kotsakis, A., Gooding, W., Shuai, Y., Petro, D., Friedland, D., Belani, C. P., Dacic, S., & Argiris, A. (2010). Phase II study of everolimus (RAD001) in previously treated small cell lung cancer. *Clinical Cancer Research*, *16*(23), 5900–5907. https://doi.org/10.1158/1078-0432.CCR-10-0802

Taylor, C. P. (1997). Mechanisms of action of gabapentin. In *Revue neurologique*.

Tee, A. R., Manning, B. D., Roux, P. P., Cantley, L. C., & Blenis, J. (2003). Tuberous Sclerosis Complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Current Biology*, *13*(15), 1259–1268. https://doi.org/10.1016/S0960-9822(03)00506-2

Tenkerian, C., Krishnamoorthy, J., Mounir, Z., Kazimierczak, U., Khoutorsky, A., Staschke, K. A., Kristof, A. S., Wang, S., Hatzoglou, M., & Koromilas, A. E. (2015). MTORC2 balances AKT activation and eIF2α serine 51 phosphorylation to promote survival under stress. *Molecular Cancer Research*, *13*(10), 1377–1388. https://doi.org/10.1158/1541-7786.MCR-15-0184-T

Terada, N., Patel, H. R., Takase, K., Kohno, K., Nairn, A. C., & Gelfand, E. W. (1994). Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(24), 11477–11481.

https://doi.org/10.1073/pnas.91.24.11477

Terao, K., & Ogata, K. (1979). Proteins of small subunits of rat liver ribosomes that interact with poly(U): I. effects of preincubation of poly(u) with 40 S subunits on the interactions of 40 S subunit proteins with aurintricarboxylic acid and with n,n'-p-phenylenedimaleimide. *Journal of Biochemistry*, *86*(3), 597–603. https://doi.org/10.1093/oxfordjournals.jbchem.a132563

Thedieck, K., Polak, P., Kim, M. L., Molle, K. D., Cohen, A., Jenö, P., Arrieumerlou, C., & Hall, M. N. (2007). PRAS40 and PRR5-like protein are new mTOR interactors that regulate apoptosis. *PLoS ONE*, *2*(11), e1217. https://doi.org/10.1371/journal.pone.0001217

Thelander, L., & Reichard, P. (1979). Reduction of Ribonucleotides. *Annual Review of Biochemistry*, *48*(1), 133–158. https://doi.org/10.1146/annurev.bi.48.070179.001025

Thewes, V., Simon, R., Hlevnjak, M., Schlotter, M., Schroeter, P., Schmidt, K., Wu, Y., Anzeneder, T., Wang, W., Windisch, P., Kirchgäßner, M., Melling, N., Kneisel, N., Büttner, R., Deuschle, U., Sinn, H. P., Schneeweiss, A., Heck, S., Kaulfuss, S., … Tönjes, M. (2017). The branched-chain amino acid transaminase 1 sustains growth of antiestrogen-resistant and ERα-negative breast cancer. *Oncogene*, *36*(29), 4124–4134. https://doi.org/10.1038/onc.2017.32

Thoreen, C. C., Kang, S. A., Chang, J. W., Liu, Q., Zhang, J., Gao, Y., Reichling, L. J., Sim, T., Sabatini, D. M., & Gray, N. S. (2009). An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1.

Journal of Biological Chemistry, *284*(12), 8023–8032. https://doi.org/10.1074/jbc.M900301200

Tolan, D. R., & Traut, R. R. (1981). Protein topography of the 40 S ribosomal subunit from rabbit reticulocytes shown by cross-linking with 2-iminothiolane. *Journal of Biological Chemistry*, *256*(19), 10129–10136.

Tomita, Y., Marchenko, N., Erster, S., Nemajerova, A., Dehner, A., Klein, C., Pan, H., Kessler, H., Pancoska, P., & Moll, U. M. (2006). WT p53, but not tumor-derived mutants, bind to Bcl2 via the DNA binding domain and induce mitochondrial permeabilization. *Journal of Biological Chemistry*, *281*(13), 8600–8606. https://doi.org/10.1074/jbc.M507611200

Tönjes, M., Barbus, S., Park, Y. J., Wang, W., Schlotter, M., Lindroth, A. M., Pleier, S. V, Bai, A. H. C., Karra, D., Piro, R. M., Felsberg, J., Addington, A., Lemke, D., Weibrecht, I., Hovestadt, V., Rolli, C. G., Campos, B., Turcan, S., Sturm, D., … Radlwimmer, B. (2013). BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. *Nature Medicine*, *19*(7), 901–908. https://doi.org/10.1038/nm.3217

Travis, W. D., Brambilla, E., Nicholson, A. G., Yatabe, Y., Austin, J. H. M., Beasley, M. B., Chirieac, L. R., Dacic, S., Duhig, E., Flieder, D. B., Geisinger, K., Hirsch, F. R., Ishikawa, Y., Kerr, K. M., Noguchi, M., Pelosi, G., Powell, C. A., Tsao, M. S., & Wistuba, I. (2015). The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances since the 2004 Classification. *Journal of Thoracic Oncology*, *10*(9), 1243–1260. https://doi.org/10.1097/JTO.0000000000000630

Tremblay, F., Marette, A., Feedback, A. N., Leading, M., Insulin, T. O., In, R., & Muscle, S. (2001). Amino Acid and Insulin Signaling via the mTOR/p70 S6 Kinase Pathway. *Journal of Biological Chemistry*, *276*(41), 38052–38060. https://doi.org/10.1074/jbc.M106703200

Tripathi, S. C., Fahrmann, J. F., Celiktas, M., Aguilar, M., Marini, K. D., Jolly, M. K., Katayama, H., Wang, H., Murage, E. N., Dennison, J. B., Watkins, D. N., Levine, H., Ostrin, E. J., Taguchi, A., & Hanash, S. M. (2017). MCAM mediates chemoresistance in small-cell lung cancer via the PI3K/AKT/ SOX2 signaling pathway. *Cancer Research*, *77*(16), 4414–4425. https://doi.org/10.1158/0008-5472.CAN-16-2874

Tsai, K. Y., Hu, Y., Macleod, K. F., Crowley, D., Yamasaki, L., & Jacks, T. (1998). Mutation of E2f-1 suppresses apoptosis and inappropriate S phase entry and extends survival of Rb-deficient mouse embryos. *Molecular Cell*, *2*(3), 293–304. https://doi.org/10.1016/S1097-2765(00)80274-9

Tsun, Z. Y., Bar-Peled, L., Chantranupong, L., Zoncu, R., Wang, T., Kim, C., Spooner, E., & Sabatini, D. M. (2013). The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. *Molecular Cell*, *52*(4), 495–505. https://doi.org/10.1016/j.molcel.2013.09.016

Umemura, S., Mimaki, S., Makinoshima, H., Tada, S., Ishii, G., Ohmatsu, H., Niho, S., Yoh, K., Matsumoto, S., Takahashi, A., Morise, M., Nakamura, Y., Ochiai, A., Nagai, K., Iwakawa, R., Kohno, T., Yokota, J., Ohe, Y., Esumi, H., … Goto, K. (2014). Therapeutic priority of the PI3K/AKT/mTOR pathway in small cell lung cancers as revealed by a comprehensive genomic analysis. *Journal of Thoracic*

Oncology, *9*(9), 1324–1331. https://doi.org/10.1097/JTO.0000000000000250

Van Slegtenhorst, M., Nellist, M., Nagelkerken, B., Cheadle, J., Snell, R., Van Den Ouweland, A., Reuser, A., Sampson, J., Halley, D., & Van Der Sluijs, P. (1998). Interaction between hamartin and tuberin, the TSC1 and TSC2 gene products. *Human Molecular Genetics*, *7*(6), 1053–1057. https://doi.org/10.1093/hmg/7.6.1053

Vembar, S. S., & Brodsky, J. L. (2008). One step at a time: Endoplasmic reticulum-associated degradation. In *Nature Reviews Molecular Cell Biology* (Vol. 9, Issue 12, pp. 944–957). Nat Rev Mol Cell Biol. https://doi.org/10.1038/nrm2546

Venkatanarayan, A., Raulji, P., Norton, W., Chakravarti, D., Coarfa, C., Su, X., Sandur, S. K., Ramirez, M. S., Lee, J., Kingsley, C. V., Sananikone, E. F., Rajapakshe, K., Naff, K., Parker-Thornburg, J., Bankson, J. A., Tsai, K. Y., Gunaratne, P. H., & Flores, E. R. (2015). IAPP-driven metabolic reprogramming induces regression of p53-deficient tumours in vivo. *Nature*, *517*(7536), 626–630. https://doi.org/10.1038/nature13910

Verrey, F. (2003). System L: Heteromeric exchangers of large, neutral amino acids involved in directional transport. In *Pflugers Archiv European Journal of Physiology* (Vol. 445, Issue 5, pp. 529–533). Springer Verlag. https://doi.org/10.1007/s00424-002-0973-z

Vezina, C., Kudelski, A., & Sehgal, S. N. (1975). Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *The Journal of Antibiotics*, *28*(10), 721–726. https://doi.org/10.7164/antibiotics.28.721

Voortman, J., Lee, J. H., Killian, J. K., Suuriniemi, M., Wang, Y., Lucchi, M., Smith, W. I., Meltzer, P., Wang, Y., & Giaccone, G. (2010). Array comparative genomic hybridization-based characterization of genetic alterations in pulmonary neuroendocrine tumors. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(29), 13040–13045. https://doi.org/10.1073/pnas.1008132107

Wallin, R., Hall, T. R., & Hutson, S. M. (1990). Purification of branched chain aminotransferase from rat heart mitochondria. *Journal of Biological Chemistry*, *265*(11), 6019–6024.

Walter, P., & Ron, D. (2011). The unfolded protein response: From stress pathway to homeostatic regulation. In *Science* (Vol. 334, Issue 6059, pp. 1081–1086). American Association for the Advancement of Science. https://doi.org/10.1126/science.1209038

Wang, M., Hu, Y., & Stearns, M. E. (2009). RPS2: A novel therapeutic target in prostate cancer. *Journal of Experimental and Clinical Cancer Research*, *28*(1). https://doi.org/10.1186/1756-9966-28-6

Wang, P., Wu, S., Zeng, X., Zhang, Y., Zhou, Y., Su, L., & Lin, W. (2018). BCAT1 promotes proliferation of endometrial cancer cells through reprogrammed BCAA metabolism. *International Journal of Clinical and Experimental Pathology*, *11*(12), 5536–5546. http://www.ncbi.nlm.nih.gov/pubmed/31949641

Wang, S., Tang, J., Sun, T., Zheng, X., Li, J., Sun, H., Zhou, X., Zhou, C., Zhang, H., Cheng, Z., Ma, H., & Sun, H. (2017). Survival changes in patients with small cell

lung cancer and disparities between different sexes, socioeconomic statuses and ages. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-01571-0

Wang, Y., Nartiss, Y., Steipe, B., McQuibban, G. A., & Kim, P. K. (2012). ROS-induced mitochondrial depolarization initiates PARK2/PARKIN-dependent mitochondrial degradation by autophagy. *Autophagy*, *8*(10), 1462–1476. https://doi.org/10.4161/auto.21211

Wang, Z. Q., Faddaoui, A., Bachvarova, M., Plante, M., Gregoire, J., Renaud, M. C., Sebastianelli, A., Guillemette, C., Gobeil, S., Macdonald, E., Vanderhyden, B., & Bachvarov, D. (2015). BCAT1 expression associates with ovarian cancer progression: Possible implications in altered disease metabolism. *Oncotarget*, *6*(31), 31522–31543. https://doi.org/10.18632/oncotarget.5159

Warburg, O. (1956). On the origin of cancer cells. *Science*, *123*(3191), 309–314. https://doi.org/10.1126/science.123.3191.309

Warburg, O., Wind, F., & Negelein, E. (1927). The metabolism of tumors in the body. *Journal of General Physiology*, *8*(6), 519–530. https://doi.org/10.1085/jgp.8.6.519

Warner, J. R. (1999). The economics of ribosome biosynthesis in yeast. In *Trends in Biochemical Sciences* (Vol. 24, Issue 11, pp. 437–440). Trends Biochem Sci. https://doi.org/10.1016/S0968-0004(99)01460-7

Watkins, J. C. (2000). L-glutamate as a central neurotransmitter: Looking back. *Biochemical Society Transactions*, *28*(4), 297–310. https://doi.org/10.1042/bst0280297

Weinmann, R., & Roeder, R. G. (1974). Role of DNA dependent RNA polymerase III in

the transcription of the tRNA and 5S RNA genes. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(5), 1790–1794. https://doi.org/10.1073/pnas.71.5.1790

Weintraub, S. J., Chow, K. N. B., Luo, R. X., Zhang, S. H., He, S., & Dean, D. C. (1995). Mechanism of active transcriptional repression by the retinoblastoma protein. In *Nature* (Vol. 375, Issue 6534, pp. 812–816). Nature. https://doi.org/10.1038/375812a0

Wessner, B., Strasser, E. M., Spittler, A., & Roth, E. (2003). Effect of single and combined supply of glutamine, glycine, N-acetylcysteine, and R, S-α-lipoic acid on glutathione content of myelomonocytic cells. *Clinical Nutrition*, *22*(6), 515–522. https://doi.org/10.1016/S0261-5614(03)00053-0

Wiest, D. L., Burkhardt, J. K., Hester, S., Hortsch, M., Meyer, D. I., & Argon, Y. (1990). Membrane biogenesis during B cell differentiation: Most endoplasmic reticulum proteins are expressed coordinately. *Journal of Cell Biology*, *110*(5), 1501–1511. https://doi.org/10.1083/jcb.110.5.1501

Wildner, H., Gierl, M. S., Strehle, M., Pla, P., & Birchmeier, C. (2008). Insm1 (IA-1) is a crucial component of the transcriptional network that controls differentiation of the sympatho-adrenal lineage. *Development*, *135*(3), 473–481. https://doi.org/10.1242/dev.011783

Witzig, T. E., Reeder, C. B., Laplant, B. R., Gupta, M., Johnston, P. B., Micallef, I. N., Porrata, L. F., Ansell, S. M., Colgan, J. P., Jacobsen, E. D., Ghobrial, I. M., & Habermann, T. M. (2011). A phase II trial of the oral mTOR inhibitor everolimus in

relapsed aggressive lymphoma. *Leukemia*, *25*(2), 341–347. https://doi.org/10.1038/leu.2010.226

Wolfson, R. L., Chantranupong, L., Saxton, R. A., Shen, K., Scaria, S. M., Cantor, J. R., & Sabatini, D. M. (2015). Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science*, *351*(6268), 43–48. https://doi.org/10.1126/science.aab2674

Wolfson, R. L., Chantranupong, L., Wyant, G. A., Gu, X., Orozco, J. M., Shen, K., Condon, K. J., Petri, S., Kedir, J., Scaria, S. M., Abu-Remaileh, M., Frankel, W. N., & Sabatini, D. M. (2017). KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. *Nature*, *543*, 438–442. https://doi.org/10.1038/nature21423

Wong, Q. W. L., Li, J., Ng, S. R., Lim, S. G., Yang, H., & Vardy, L. A. (2014). RPL39L is an example of a recently evolved ribosomal protein paralog that shows highly specific tissue expression patterns and is upregulated in ESCs and HCC tumors. *RNA Biology*, *11*(1), 33–41. https://doi.org/10.4161/rna.27427

Woo, S. Y., Kim, D. H., Jun, C. B., Kim, Y. M., Haar, E. Vander, Lee, S. Il, Hegg, J. W., Bandhakavi, S., Griffin, T. J., & Kim, D. H. (2007). PRR5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor β expression and signaling. *Journal of Biological Chemistry*, *282*(35), 25604–25612. https://doi.org/10.1074/jbc.M704343200

Xing, X., Zhang, L., Wen, X., Wang, X., Cheng, X., Du, H., Hu, Y., Li, L., Dong, B., Li, Z., & Ji, J. (2014). PP242 suppresses cell proliferation, metastasis, and angiogenesis of gastric cancer through inhibition of the PI3K/AKT/mTOR pathway.

Anti-Cancer Drugs, *25*(10), 1129–1140. https://doi.org/10.1097/CAD.0000000000000148

Xu, Y., Yu, W., Yang, T., Zhang, M., Liang Phd, C., Cai, X., & Shao, Q. (2018). *Overexpression of BCAT1 is a prognostic marker in gastric cancer ☆*. https://doi.org/10.1016/j.humpath.2018.02.003

Xue, L., Fletcher, G. C., & Tolkovsky, A. M. (2001). Mitochondria are selectively eliminated from eukaryotic cells after blockade of caspases during apoptosis. *Current Biology*, *11*(5), 361–365. https://doi.org/10.1016/S0960-9822(01)00100-2

Y-H Lee, E. P., Chang, C.-Y., Hu, N., Wang, Y.-C. J., Lai, C.-C., Herrup, K., Lee, W.-H., & Bradleyll, A. (1992). *Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis*.

Yamashita, J., Ohmoto, M., Yamaguchi, T., Matsumoto, I., & Hirota, J. (2017). Skn-1a/Pou2f3 functions as a master regulator to generate Trpm5-expressing chemosensory cells in mice. *PLOS ONE*, *12*(12), e0189340. https://doi.org/10.1371/journal.pone.0189340

Yan, R., Zhao, X., Lei, J., & Zhou, Q. (2019). Structure of the human LAT1–4F2hc heteromeric amino acid transporter complex. *Nature*, *568*(7750), 127–130. https://doi.org/10.1038/s41586-019-1011-z

Yan, Z., Degregori, J., Shohet, R., Leone, G., Stillman, B., Nevins, J. R., & Williams, R. S. (1998). Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(7), 3603–3608. https://doi.org/10.1073/pnas.95.7.3603

Yang, C., Ko, B., Hensley, C. T., Jiang, L., Wasti, A. T., Kim, J., Sudderth, J., Calvaruso, M. A., Lumata, L., Mitsche, M., Rutter, J., Merritt, M. E., & DeBerardinis, R. J. (2014). Glutamine oxidation maintains the TCA cycle and cell survival during impaired mitochondrial pyruvate transport. *Molecular Cell*, *56*(3), 414–424. https://doi.org/10.1016/j.molcel.2014.09.025

Yang, G. F., Xie, D., Liu, J. H., Luo, J. H., Li, L. J., Hua, W. F., Wu, H. M., Kung, H. F., Zeng, Y. X., & Guan, X. Y. (2009). Expression and amplification of eIF-5A2 in human epithelial ovarian tumors and overexpression of EIF-5A2 is a new independent predictor of outcome in patients with ovarian carcinoma. *Gynecologic Oncology*, *112*(2), 314–318. https://doi.org/10.1016/j.ygyno.2008.10.024

Yang, J. J., Kang, J. S., & Krauss, R. S. (1998). Ras Signals to the Cell Cycle Machinery via Multiple Pathways To Induce Anchorage-Independent Growth. *Molecular and Cellular Biology*, *18*(5), 2586–2595. https://doi.org/10.1128/mcb.18.5.2586

Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T. I., Jones, D. P., & Wang, X. (1997). Prevention of apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. *Science*, *275*(5303), 1129–1132. https://doi.org/10.1126/science.275.5303.1129

Yao, J. C., Fazio, N., Singh, S., Buzzoni, R., Carnaghi, C., Wolin, E., Tomasek, J., Raderer, M., Lahner, H., Voi, M., Pacaud, L. B., Rouyrre, N., Sachs, C., Valle, J. W., Fave, G. D., Van Cutsem, E., Tesselaar, M., Shimada, Y., Oh, D. Y., … Pavel, M. E. (2016). Everolimus for the treatment of advanced, non-functional neuroendocrine tumours of the lung or gastrointestinal tract (RADIANT-4): A

randomised, placebo-controlled, phase 3 study. *The Lancet*, *387*(10022), 968–977. https://doi.org/10.1016/S0140-6736(15)00817-X

Ye, F., Xu, R., Ge, Y., Zheng, Y., Liu, X., Deng, P., & Xu, X. (2020). LINC00963 Confers Oncogenic Properties in Glioma by Regulating the miR-506/BCAT1 Axis. *Cancer Management and Research*, *Volume 12*, 2339–2351. https://doi.org/10.2147/cmar.s246332

Yeo, C. Q. X., Alexander, I., Lin, Z., Lim, S., Aning, O. A., Kumar, R., Sangthongpitag, K., Pendharkar, V., Ho, V. H. B., & Cheok, C. F. (2016). P53 Maintains Genomic Stability by Preventing Interference between Transcription and Replication. *Cell Reports*, *15*(1), 132–146. https://doi.org/10.1016/j.celrep.2016.03.011

Yip, C. K., Murata, K., Walz, T., Sabatini, D. M., & Kang, S. A. (2010). Structure of the Human mTOR Complex I and Its Implications for Rapamycin Inhibition. *Molecular Cell*, *38*(5), 768–774. https://doi.org/10.1016/j.molcel.2010.05.017

Yoshida, T., Mett, I., Bhunia, A. K., Bowman, J., Perez, M., Zhang, L., Gandjeva, A., Zhen, L., Chukwueke, U., Mao, T., Richter, A., Brown, E., Ashush, H., Notkin, N., Gelfand, A., Thimmulappa, R. K., Rangasamy, T., Sussan, T., Cosgrove, G., … Tuder, R. M. (2010). Rtp801, a suppressor of mTOR signaling, is an essential mediator of cigarette smoke-induced pulmonary injury and emphysema. *Nature Medicine*, *16*(7), 767–773. https://doi.org/10.1038/nm.2157

Yu, C., Niu, X., Jin, F., Liu, Z., Jin, C., & Lai, L. (2016). Structure-based Inhibitor Design for the Intrinsically Disordered Protein c-Myc. *Scientific Reports*, *6*. https://doi.org/10.1038/srep22298

Zarai, Y., Margaliot, M., & Tuller, T. (2016). On the ribosomal density that maximizes protein translation rate. *PLoS ONE*, *11*(11), e0166481. https://doi.org/10.1371/journal.pone.0166481

Zdzisińska, B., Żurek, A., & Kandefer-Szerszeń, M. (2017). Alpha-Ketoglutarate as a Molecule with Pleiotropic Activity: Well-Known and Novel Possibilities of Therapeutic Use. In *Archivum Immunologiae et Therapiae Experimentalis* (Vol. 65, Issue 1, pp. 21–36). Birkhauser Verlag AG. https://doi.org/10.1007/s00005-016-0406-x

Zeng, Y., Tian, X., Wang, Q., He, W., Fan, J., & Gou, X. (2018). Attenuation of everolimus-induced cytotoxicity by a protective autophagic pathway involving ERK activation in renal cell carcinoma cells. *Drug Design, Development and Therapy*, *12*, 911–920. https://doi.org/10.2147/DDDT.S160557

Zhai, W., & Comai, L. (2000). Repression of RNA Polymerase I Transcription by the Tumor Suppressor p53. *Molecular and Cellular Biology*, *20*(16), 5930–5938. https://doi.org/10.1128/mcb.20.16.5930-5938.2000

Zhang, H. S., Gavin, M., Dahiya, A., Postigo, A. A., Ma, D., Luo, R. X., Harbour, J. W., & Dean, D. C. (2000). Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and RB-hSWI/SNF. *Cell*, *101*(1), 79–89. https://doi.org/10.1016/S0092-8674(00)80625-X

Zhang, L., Pan, X., & Hershey, J. W. B. (2007). Individual overexpression of five subunits of human translation initiation factor eIF3 promotes malignant transformation of immortal fibroblast cells. *Journal of Biological Chemistry*, *282*(8),

5790–5800. https://doi.org/10.1074/jbc.M606284200

Zhang, L., & Han, J. (2017). Branched-chain amino acid transaminase 1 (BCAT1) promotes the growth of breast cancer cells through improving mTOR-mediated mitochondrial biogenesis and function. *Biochemical and Biophysical Research Communications*, *486*(2), 224–231. https://doi.org/10.1016/j.bbrc.2017.02.101

Zhang, X., Guo, M., Fan, J., Lv, Z., Huang, Q., Han, J., Wu, F., Hu, G., Xu, J., & Jin, Y. (2016). Prognostic significance of serum LDH in small cell lung cancer: A systematic review with meta-analysis. *Cancer Biomarkers*, *16*(3), 415–423. https://doi.org/10.3233/CBM-160580

Zhang, Y., Gao, X., Saucedo, L. J., Ru, B., Edgar, B. A., & Pan, D. (2003). Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nature Cell Biology*, *5*, 578–581. https://doi.org/10.1038/ncb999

Zhang, Z. Y., Monleon, D., Verhamme, P., & Staessen, J. A. (2018). Branched-chain amino acids as critical switches in health and disease. *Hypertension*, *72*(5), 1012–1022. https://doi.org/10.1161/HYPERTENSIONAHA.118.10919

Zheng, Y. H., Hu, W. J., Chen, B. C., Grahn, T. H. M., Zhao, Y. R., Bao, H. L., Zhu, Y. F., & Zhang, Q. Y. (2016). BCAT1, a key prognostic predictor of hepatocellular carcinoma, promotes cell proliferation and induces chemoresistance to cisplatin. *Liver International*, *36*(12), 1836–1847. https://doi.org/10.1111/liv.13178

Zhou, L., Xie, Z., Shao, Z., Chen, W., Xie, H., Cui, X., Qin, G., & Zhao, N. (2018). Modeling the relationship between baseline lactate dehydrogenase and prognosis in patients with extensive-disease small cell lung cancer: A retrospective cohort

study. *Journal of Thoracic Disease*, *10*(2), 1043–1049. https://doi.org/10.21037/jtd.2018.02.16

Zhou, W., Feng, X., Ren, C., Jiang, X., Liu, W., Huang, W., Liu, Z., Li, Z., Zeng, L., Wang, L., Zhu, B., Shi, J., Liu, J., Zhang, C., Liu, Y., & Yao, K. (2013). Over-expression of BCAT1, a c-Myc target gene, induces cell proliferation, migration and invasion in nasopharyngeal carcinoma. *Molecular Cancer*, *12*, 53. https://doi.org/10.1186/1476-4598-12-53

Zhu, W., Shao, Y., & Peng, Y. (2017). MicroRNA-218 inhibits tumor growth and increases chemosensitivity to CDDP treatment by targeting BCAT1 in prostate cancer. *Molecular Carcinogenesis*, *56*(6), 1570–1577. https://doi.org/10.1002/mc.22612

Zindy, F., Eischen, C. M., Randle, D. H., Kamijo, T., Cleveland, J. L., Sherr, C. J., & Roussel, M. F. (1998). Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes and Development*, *12*(15), 2424–2433. https://doi.org/10.1101/gad.12.15.2424

Zinszner, H., Kuroda, M., Wang, X. Z., Batchvarova, N., Lightfoot, R. T., Remotti, H., Stevens, J. L., & Ron, D. (1998). CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes and Development*, *12*(7), 982–995. https://doi.org/10.1101/gad.12.7.982

Zoncu, R., Bar-Peled, L., Efeyan, A., Wang, S., Sancak, Y., & Sabatini, D. M. (2011). mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. *Science (New York, N.Y.)*, *334*(6056), 678–

683. https://doi.org/10.1126/science.1207056