Evidence for oligodendrocyte progenitor cell heterogeneity and their role in Alzheimer's disease

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Chapter I: Evidence for the functional diversity of oligodendrocyte progenitor cells

Introduction

Since their discovery as the fourth major glial type in the adult central nervous system, oligodendrocyte progenitor cells (OPCs) have been an important area of study, especially in the remyelination field. Numerous groups are working diligently to elucidate the signaling mechanisms that underlie the differentiation process of OPCs into myelinating oligodendrocytes, and what aspects of this pathway can be harnessed to promote more efficient and complete remyelination following a demyelinating insult. This area of research has the potential to lead to the development of exciting new therapies for debilitating demyelinating disease such as multiple sclerosis (MS), Guillain–Barré syndrome, and other leukodystrophies.

While the large majority of the OPC field has historically focused on the their role as a progenitor pool for mature, myelinating oligodendrocytes, recent literature has begun to explore the idea that the population of OPCs maintained in the adult brain represents multiple diverse subpopulations that fall under the current cellular identification of OPCs[1,2]. The case for OPC heterogeneity is bolstered by emerging evidence that strongly indicates OPCs in the adult brain may have their own role independent of their function as progenitors[3-5]. Understanding the population characteristics of OPCs and investigating their potential diversity may lead to the discovery of novel roles for OPCs in the healthy and diseased brain.

While the field lacks a complete understanding of the heterogeneity of OPCs within the adult brain, the body of literature investigating the function of OPCs since their

discovery in 1983 contains multiple lines of evidence that point to diversity within this population. Here we will review the current evidence that demonstrates the potential for OPC diversity within the adult brain and briefly explore ways to directly address the question of OPC heterogeneity.

Historical background

Oligodendrocyte progenitor cells were initially characterized in 1983, their discovery arising from an effort to characterize glial cells within the optic nerve. Raff and colleagues (1983) observed that cultures of isolated cells from the optic nerve contained a significantly larger percentage of oligodendrocytes when they were maintained in serum-free media than when they were maintained in the presence of serum. When cultured with serum, these same cells produced a large percentage of type-2 astrocytes. Both types of cells largely developed from the same A2B5+ precursor cells. This *in vivo* characterization led to the recognition of a new type of glial cell, originally called O2A progenitor cells because of their ability to generate both type-2 astrocytes and oligodendrocytes in culture [6]. As the body of literature investigating O2A progenitor cells grew, evidence began to suggest that, *in vivo*, O2A progenitors exclusively generated oligodendrocytes and that the production of astrocytes from these cells was a result of culture conditions[7]. While it still remains a topic of debate whether OPCs have the ability to produce astrocytes *in vivo*, fate-labeling studies have led to the general acceptance that the large majority of mature cells produced by OPCs are oligodendrocytes[8,9]. As a result, the term O2A progenitors fell out of favor in most of the literature, being replaced with the most commonly used term 'oligodendrocyte progenitor cells'[10]. However, as the

idea that OPCs may play other roles besides being a progenitor pool gained popularity in recent years, other names for this cell population began to make their way into the literature, including polydendrocyte, after the vast array of processes that each cell has, and NG2-glia, after one of the main markers of OPCs[11,12].

As the variety of names suggests, despite 35 years' worth of literature on this cell type, the precise set of roles they play, especially in the adult brain, remains contested. One confounding factor that makes these cells particularly challenging to study is the limited number of molecular markers used to identify OPCs.

Molecular Identification

One of the challenges that faces the OPC field is the lack of distinct markers that definitively label OPCs and do not overlap with any other cell type. With current known markers, OPCs can only be distinguished from other cells type by the colocalization of two markers. Multiple markers are expressed in all oligo-lineage cells, and can be used to distinguish OPCs and oligodendrocytes from other neural and glial cell types. The transcription factor Sox10 is upregulated early in the development of OPCs from neural precursor cells and expression is maintained in mature oligodendrocytes[13,14]. Olig2 is also a nuclear marker that is known to induce an oligodendroglial identity and is maintained through differentiation[15,16]. While these markers are useful for identifying cells of the oligodendrocyte lineage, they do not distinguish mature myelin-forming oligodendrocytes from OPCs. OPCs are identified using one of the nuclear markers mentioned above, along with either platelet derived growth factor α (PDGFRα) or neural/glial antigen 2 (NG2) to mark the cell body and processes and distinguish them from mature

oligodendrocytes[17]. PDGFRα is a growth factor receptor that marks the cell body and processes of OPCs, but is down regulated as OPCs differentiate, making it an ideal target for distinguishing OPCs from oligodendrocytes[18]. However, endothelial cells also express PDGFRα, so an oligo-lineage nuclear marker must also be used to identify OPCs[19]. NG2, a proteoglycan, is also used to visualize the cell body and processes of OPCs, but is also expressed in pericytes of the brain[20]. Moreover, macrophages have been shown to ectopically express NG2 in the context of injury, making OPC identification and analysis of their behavior even more challenging[21].

Characteristics of OPCs within the Adult Brain

The discovery of OPCs as the fourth main glial type lead to a quickly growing pool of literature that described their origins in the developing brain and spinal cord, their migration to populate the central nervous system (CNS), and the differentiation of a large majority of OPCs into mature myelinating oligodendrocytes[22-24]. But, unlike other progenitor cells that play critical roles in the development of the nervous system, a substantial population of OPCs remain in the adult brain outside of the specific adult neurogenic niches that have been shown to support multipotent stem cells throughout life[25].

OPCs within the adult brain have been shown to populate every region of the CNS. Olig2 and Sox10, pan oligodendroglia markers, are expressed strongly in white matter that is densely populated by mature oligodendrocytes and lowly expressed in the gray matter from less densely packed OPCs[14,15]. As a pool of progenitor cells, OPCs make up a relatively large percentage of cells in the brain, with 2-8% of cells expressing OPC

markers, depending on the brain region. Despite the significant increase in density of oligodendrocytes in the white matter compared to the gray matter, there are only approximately 1.5 times more OPCs in white matter tracts than in gray matter regions. Moreover, in gray matter regions, OPC number matches oligodendrocyte number at approximately a one-to-one ratio[26].

The landscape of OPCs within the adult brain varies vastly from the mature oligodendrocyte “pearls-on-a-string” spacing that is often seen in white matter tracts. OPCs dynamically maintain an evenly tiled spacing, with each OPC occupying its own territory. Cells maintain their tiled nature through contact inhibition with other OPCs, actively retracting their processes upon contact with another OPC[27]. Even tiling is dynamically maintained, with neighboring cells quickly filling a territory left vacant by the death or differentiation of an OPC. Local cells either proliferate or rearrange themselves to repopulate the empty area. While the exact molecular pathway that results in process retraction upon contact between OPCs remains unknown, the mediators of contact inhibition are quickly downregulated as a cell initiates differentiation. Neighboring OPCs quickly overtake the area occupied by a differentiating cell, while a dying OPC maintains it territory until it has been cleared from the area[27]. As a population, OPCs dynamically maintain full coverage of the brain.

Despite clearly delineated territories belonging to each individual OPC, these cells exhibit significant mobility in the mature cortex. OPC mobility in the developing brain has been well documented, with time course analysis of prenatal development indicating widespread migration of OPCs from their birth in the ventral midbrain to populate the entire CNS[24,28]. Earlier studies using time-lapse microscopy showed that even in early

postnatal days, OPCs continue to exhibit substantial mobility in slice-culture[29]. With the emergence of in vivo two photon imaging we can now visualize mobility dynamics of OPCs in living animals over multiple months[27,30]. Using this approach, Hughes and colleagues (2013) demonstrated that adult OPCs maintain their mobility even after establishing their individual territories[27]. Repeated in vivo imaging sessions showed that, within the cortex, OPCs move on average 2 microns per day, with some OPCs moving more than 30 microns in a three day timespan. Moreover, even if the soma remains stationary, OPC processes are actively motile within the cortex, continuously altering their arrangement, branching, and distribution[27]. While new microscopy techniques have allowed for a better understanding of dynamics of OPC behavior within the adult brain, the purpose of the continuous movement of processes and soma translocation remains an open question.

Another defining characteristic of OPCs is their ability to proliferate and maintain a self-renewing capacity throughout adulthood. OPCs maintain a consistent density of cells, at least during early adult life, which requires a pool of proliferative cells to replace dying or differentiating OPCs[31]. Hughes and colleagues (2013) showed that the death of an OPC is followed by proliferation of a neighboring cell 98% of the time and a differentiating cell is replaced through proliferation 76% of the time[27]. They are the most proliferative cells within the CNS, making up 70% of BrdU labeled cells in the mouse brain[26]. This has also been confirmed in human brain tissue, with immunofluorescence showing that Ki67 labeling colocalized with NG2, but not with any markers of neurons, astrocytes, microglia, or oligodendrocytes[32]. While it is generally accepted that OPCs have a high rate of proliferation, there is some disagreement on the percentage of the population that actively

progresses through the cell cycle. Short BrdU pulse-chase experiments of 2 hours shows that, at any time, 1-4% of OPCs are actively mitotic[26]. Multiple studies have looked at long term BrdU incorporation in an attempt to describe what percentage of the OPC population has the ability to actively divide. The exact proportion of the population that actively progresses through the cell cycle remains contested in the literature, as multiple publications report varying levels of BrdU labeling in the OPC population, most likely due to variations in pulse-chase timelines, age of the animals, and which regions of the brain were examined. Simon and colleagues observed 80% of OPCs entering the cell cycle within a month, and saw little increase in that percentage with longer observation time[33]. Others have reported no more than 55% of OPCs showing BrdU labeling after an extended pulse-chase labeling period[31,34,35]. A small subset of other experiments have seen all OPCs labeled with EdU[36]. Regardless of the exact proportion of the population that actively enters the cells cycle, in almost every study a subset of the population does not proliferate, even after 100 days of BrdU administration[34]. It is unclear whether this quiescent population does not proliferate simply because other OPCs replaced dying or differentiating cells more quickly, or if there is an intrinsic quality to this subpopulation of OPCs that results in less proliferation.

Oligodendrocyte Stability

With such as large population of progenitor cells that has the ability, under homeostatic conditions, to replace themselves within 10 days[36], it would be logical to conclude that mature oligodendrocytes require fairly consistent replacement if the main role of OPCs is to serve as a pool of progenitors. There is however, increasing evidence

in both humans and animal models that indicates oligodendrocytes represent a relatively stable population in the healthy brain and that a large number of oligodendrocytes survive the lifetime of the organism.

First indications of the longevity of oligodendrocytes come from studies of what the authors call the "long-lived proteome." Toyama and colleagues (2013) utilized offspring of dams continuously fed a diet rich in ^{15}N. Their pups were then fed a diet rich in ^{14}N, and their brains were subsequently analyzed for proteins that retained significant amounts of ^{15}N. Mass spectrometry of heavy-nitrogen labeled-proteins after a pulse-chase period of 6 months revealed that a number of myelin-specific proteins, including MBP, PLP, MOG, and Cnp1, retained significant amounts of ^{15}N [37,38]. While this does not provide insight into the overall replacement rate of oligodendrocytes, it does indicate that at least some myelin within the brain is maintained for at least 6 months.

While it has been known that oligodendrocytes are produced during adulthood, and their generation is necessary for motor learning tasks, the extent of oligodendrocyte generation and replacement has remained relatively unclear until recently[9,39]. Inducible reporter systems have allowed for a more systematic evaluation of longevity of oligodendrocyte cell bodies themselves, as the turnover of myelin is not necessarily equivalent with the turnover of oligodendrocytes. In 2017, Tripathi and colleagues used an Opalin-GFP reporter system to track the survival of oligodendrocytes. Surprisingly, they found that, of those oligodendrocytes present at P60, between 90-100% of them remained at 6 months, in all of the brain regions examined except for the optic nerve. By 20 months of age, over half of the oligodendrocytes present in all brain regions had differentiated by P60, and this number increased to 90% in the corpus callosum. This lack

of turnover of oligodendrocytes is accompanied by relative population size stability, with the density of oligodendrocytes increasing in the corpus callosum in the first six months of age and then stabilizing for the next year and a half. Based on this lack of turnover, Tripathi and colleagues calculated that by 8 months of age, mice are only generating 0.4 oligodendrocytes/mm^2 each day[40].

The turnover rate of oligodendrocytes in the human brain has also been explored, and the results indicate that a similar oligodendrocyte stability exists within the corpus callosum of humans. Utilizing the incorporation of ^{14}C into cells as a result of the initiation of nuclear bomb testing in 1955, Yeung and colleagues (2014) were able to demonstrate that oligodendrocyte nuclei from the corpus callosum and frontal lobe had levels of ^{14}C that correlated with the first five years of a person's life, regardless of their date of death. This indicates that a significant population of mature oligodendrocytes present at the time of death were born within the first five years of a person's life. While a small population of oligodendrocytes may be added as a result of activity-dependent myelination, mature oligodendrocytes reach the population size seen in adults by 9 years of age and maintain this population size for the majority of a lifetime, showing a small decrease in oligodendrocyte number with age. However, when myelin from the corpus callosum was isolated and carbon dated, these proteins had levels of ^{14}C that more closely correlated with the death of the patient. Calculations of oligodendrocyte production indicates that, on average, in the adult human brain, one out of every 300 oligodendrocytes are replaced each year[41]. This long lifespan and relatively slow production of oligodendrocytes does not seem to necessitate the large dynamic population of progenitor cells that are maintained in the adult brain.

Summary

Within the adult brain, oligodendrocyte progenitor cells maintain a well-coordinated population that covers the entire brain. They maintain their proliferative capacity throughout adulthood and quickly replace a dying or differentiating neighbor. OPCs have the ability to move relatively large distances throughout the brain in a short period of time and maintain active processes within their individual territory. OPCs have been predominately known for their role as a progenitor pool for oligodendrocytes and very few other functions have been ascribed to this population. Based on these characteristics, it would be reasonable to expect that oligodendrocytes require constant replacement and generation of new cells in order to justify such a large and dynamic population. However, multiple lines of evidence indicate that oligodendrocytes are exceptionally stable, with some lasting the entirety of a human lifetime. During adulthood, oligodendrocytes are produced at a surprisingly slow rate and seem to utilize only a fraction of the available progenitor cells. These discrepancies underscore two important questions that remain in the OPC field – what other functional roles might OPCs have apart from their ability to generate oligodendrocytes, and are there subpopulations of OPCs that perform different functions?

Development

3 Waves of OPC development

Evidence for distinct populations of OPCs begins from the time of their birth in the developing CNS. Within the telencephalon, OPCs develop in three different waves from three distinct areas. PDGFRα is first expressed around E12.5, and these OPCs arise from

the medial ganglionic eminence, the most ventral portion of the ventricular zone in the developing mouse [42]. These OPCs are marked by the transcription factor *Nkx2.1* and begin to spread laterally and into the cortex as the mouse develops. Around E16.5 a second set of OPCs begins to migrate from the lateral ganglionic eminence that no longer express *Nkx2.1* but instead express *Gsh2*, a transcription factor that marks the more lateral portions of the neural tube that give rise to interneurons [43]. These OPCs also migrate dorsally to populate the cortex along with the *Nkx2.1* derived OPCs. Around the time of birth, a third wave of OPCs develops from the dorsal portion of the cortex, expressing the transcription factor *Emx1* and lacking expression of both *Gsh2* and *Nkx2.1*. These OPCs don't populate the ventral aspect of the forebrain, but migrate to populate dorsal structures such as the motor cortex and the corpus callosum [24,44]. Interestingly, the first wave of OPCs that are derivatives of *Nkx2.1* expressing cells are not retained in any significant population in the adult brain. By P10, the *Nkx2.1* population has decreased significantly, and by P30 the only region that retains any significant *Nkx2.1* OPC population is the preoptic area, which corresponds to the developmental medial ganglionic eminence that gave rise to this initial wave of OPCs [24].

This sequential and distinct generation of three populations of OPCs in the forebrain brings up multiple questions about the role of these OPCs during development, and the potential for each subset to maintain unique roles in the adult brain. The early emergence and postnatal disappearance of the *Nkx2.1* oligolineage population is the most indicative of a non-progenitor role for OPCs, as oligodendrocyte maturation and myelination is just beginning during the first postnatal weeks in rodents [45]. With a significant decrease in the *Nkx2.1* derived population from birth to P10, it is unlikely that

the first wave of OPCs is contributing significantly to the population of myelinating oligodendrocytes. Deletion of any of the three waves of OPCs leads to compensation by the remaining two populations. Deletion does not result in any overt behavioral or myelination abnormalities, although in-depth behavioral analysis has not been performed to assess specific subsets of behavioral changes [24]. It therefore remains an open question whether the three waves of OPCs that populate the forebrain are functionally different or contribute interchangeable populations of OPCs.

Other regions of the developing CNS also exhibit multiple birth places of OPCs, including the spinal cord and hindbrain [46,47]. In both regions, the majority of OPCs come from the ventral aspect of the spinal cord and brain, with a much smaller population developing from the dorsal aspect at a later time point [46,48]. While both the dorsal and ventral born cells express canonical OPC markers, they seem to be regulated by different signaling pathways. *Nkx6.1* and *Nkx6.2*, known regulators of the oligolineage transcription factor *Olig2*, are necessary for the proper production of OPCs from the ventral spinal cord [49,50]. However, in *Nkx6.1/6.2* double knockouts, the dorsal spinal cord retains the ability to produce OPCs, while there is no contribution from the normally dominant ventral population. The two populations also respond to Sonic Hedgehog signaling differently, with inhibition of this pathway preventing ventral OPC development, but having no effect on dorsally derived OPCs [46].

Brain vs. Spinal Cord OPCs

In addition to differences in the development of OPCs that arise from the dorsal and ventral aspects of the developing CNS, OPCs that arise from the spinal cord seem

to be regulated differently from those that arise from the brain. Deletion of *Olig2*, an important regulator of both motor neuron and OPC development, prevents development of OPCs and subsequent oligodendrocytes within the spinal cord [22,51,52]. However, the brains of *Olig2* knockout mice are able to develop OPCs, although the kinetics of their development is slightly delayed. It seems that this development of OPCs despite the lack of *Olig2* is due to compensation by a related transcription factor, *Olig1* [22]. It remains unclear why *Olig1* can compensate for the lack of *Olig2* in OPCs developing within the brain, but cannot compensate for the same deletion in the spinal cord. Deletion of *Nkx6.1* and *Nkx6.2* have even more pronounced differential effects in the brain and the spinal cord. Without *Nkx6.1* or *Nkx6.2*, OPCs that arise from the ventral portion of the spinal cord fail to develop. However, the same deletion results in an expansion of the ventral population of OPCs in the hindbrain [47,53]. It remains unclear how a single signaling pathway can have opposing effects on the same cell type, but this data provides additional evidence for OPC heterogeneity.

Functions as a Progenitor Pool

Canonical Role as Oligodendrocyte Progenitors

The most studied role of OPCs is their ability to generate myelinating oligodendrocytes. This was originally described in culture systems, and, with the development of fate mapping technology, it has been shown that a large number of OPCs in the postnatal brain contribute to the formation of myelinating oligodendrocytes in the first postnatal weeks [54-57]. It was only later that studies were able to unequivocally demonstrate that OPCs present in the adult brain were able to form oligodendrocytes that

had the ability to myelinate axons [9,31,58]. Recent single cell sequencing data has shown that myelinating oligodendrocytes can be divided into six distinct subpopulations, and that these populations are represented at different proportions in varying brain regions [59]. There is currently no data that distinguishes whether these groups of oligodendrocytes arise from distinct subpopulations of OPCs, or if their distinct molecular characteristics are specified after differentiation.

Recent work has shown that the differentiation of OPCs into oligodendrocytes in the adult brain is heavily influenced by neuronal activity, and plays a significant role in motor learning. Gibson and colleagues (2014) demonstrated that stimulation of neurons in the motor cortex lead to a specific increase in the proliferation of OPCs and their differentiation into oligodendrocytes around the projecting axons of these neurons. Oligodendrogenesis resulting from the stimulation of these motor neurons was found to result in enhanced motor performance [60]. Similarly, preventing the formation of new oligodendrocytes in adulthood is sufficient to inhibit the ability to learn a new complex motor task [61,62]. Neuronal activity and myelination in the adult brain seem to be tightly linked, as highly active neurons are much more likely to be subsequently myelinated than those that are less active [63]. Rapid electrical response to neurons has been shown to be a characteristics of a subset of OPCs, at least in the early postnatal brain [64]. It is possible that this subset of OPCs is distinctly responsible for monitoring neuronal activity and the subsequent myelination that results.

Multipotency

Another source of potential heterogeneity within the OPC population arises from studies that show the production of small populations of neurons and astrocytes from OPCs. This idea remains controversial, as there are many contradicting lines of evidence on this topic. Much of the initial speculation about the ability of OPCs to generate neurons and glial cells other than oligodendrocytes developed from *in vitro* studies that demonstrated the ability of cultured OPCs to generate both neurons and astrocytes [23,65-67]. Multiple *in vivo* fate-labeling studies have observed the development of gray matter astrocytes from cells that originally expressed canonical OPC markers [8,68,69]. Zhu and colleagues (2011) found that the development of astrocytes from OPCs was restricted to development and not seen from adult OPCs. They also demonstrated that a single OPC clone either developed OPCs and oligodendrocytes or astrocytes, but they rarely observed both cell types arising from the same clone [69]. Lastly, a small handful of studies have also observed OPCs that develop small populations of neurons within the piriform cortex, which has been replicated in multiple transgenic mouse lines [31,70]. NG2-glia derived neurons have also been observed in the hypothalamus, where they have been shown to produce physiologically-competent neurons that express both NeuN and HuC/D [71].

While it is widely accepted, based current literature, that the vast majority of cells coming from the OPC-lineage in the adult brains are oligodendrocytes, there is still disagreement concerning the ability of OPCs to produce other cells. Multiple studies provide evidence for the generation of both neurons and astrocytes from OPCs. However, other studies fail to find any other cell-types other than OPCs and oligodendrocytes

resulting from OPC lineage tracing [72]. Still others postulate that these populations arise as a result of off-target labeling in OPC-reporter mouse lines [34]. Despite these remaining questions, the potential for OPCs to produce other cell types within the CNS brings up potential evidence for subsets of OPCs that maintain multipotency during adulthood. Both the astrocyte and neuron populations that were shown to develop from OPCs were relatively small and restricted to specific regions of the brain. OPC-derived astrocytes were only found in the gray matter, and labeled neurons were found specifically in the piriform cortex and hypothalamus [8,31,71]. This indicates that the potential production of astrocytes and neurons are restricted to specific populations of OPCs that reside in these areas. These OPCs either represent a specific subpopulation that retains the ability to produce more than just oligodendrocytes, or the environments of these regions permits the production of multiple cell types. Both possibilities indicate that a specific subset of OPCs potentially has the ability to maintain multipotency.

Regional and Molecular Heterogeneity

Gray vs. White Matter

The idea of regional heterogeneity within neurons in the CNS dates as far back as Ramón y Cajal’s drawings in the 1800’s. The same idea of regional heterogeneity in glial subtypes has been a much more recent development, especially in regards to OPCs. Most literature regarding regional specificity of OPCs in the adult brain describes the differences between gray and white matter OPCs.

OPCs are more densely packed in white matter and have distinct morphologies. White matter OPCs have elongated cell bodies, and processes that generally run parallel

to each other and extend from either pole. Gray matter OPCs have rounder cell bodies, with processes that extend from the soma in all directions [26,73]. Clonal analysis of Ascl1+ OPCs has shown that, while Ascl1 is present in OPCs in both the white and gray matter, one clone will only produce cells that reside in a single region, indicating that there is some type of regional fate restriction prior to the proliferation of these clones [74]. Cells in white matter proliferate much more quickly than gray matter OPCs, proliferating more than six times as fast at P21, and almost four times as fast during adulthood [35,36,58]. Cell cycle time not only varies between gray and white matter, but differs between white matter tracts, with OPCs in the corpus callosum proliferating faster than those is the optic nerve [36]. White matter OPCs also produce more oligodendrocytes in a given time period than gray matter OPCs [26,35,36,75]. OPCs in different regions also exhibit different electrophysiological properties, at least during early postnatal development. Gray matter OPCs have a resting membrane potential approximately 15mV lower than those in the white matter, and show different membrane capacitance and input resistance, indicating differences in the array of ion channels present on OPCs in each region. Some gray matter OPCs also exhibit depolarizations reminiscent of action potentials, which are not found in any white matter OPCs [73]. The functional differences that these electrophysiological variations have on gray and white matter OPCs remains unclear.

These distinct groups of OPCs have been shown to respond to mutations and insults in differing ways. The jimpy mouse carries a mutation in the myelin-associated PLP gene and exhibits hypomyelination [76,77]. Analysis of the OPC response to this mutation shows that OPCs in the gray matter of these mice decrease their proliferation, while those in the white matter increase their proliferation. Similar patterns are seen with

oligodendrocyte production, with an overall decrease in the gray matter and a doubling in the white matter [78]. Irradiation insults also cause regionally distinct effects on OPCs, with the gray matter exhibiting a higher percentage of OPCs staining positive for the proliferation marker Ki67 than those in the white matter following radiation treatment [79].

While OPCs in the gray and white matter exhibit a number of differences, the cellular makeup and environment of these regions are quite different, which brings up the question if these differences are intrinsic to distinct subsets of OPCs, or if they are a result of adaptations of homogenous cells to their environment. Transplantation studies have indicated that, while the environment plays a role in the characteristics of OPCs, white and gray matter cells retain some of their differences, despite grafting into the opposite region. When gray and white matter OPCs are transplanted into white matter, they both exhibit an increase in the number of oligodendrocytes derived from transplanted cells over 11 weeks, although the kinetics of the increase in gray matter-derived oligodendrocytes is slower than those originating from the white matter. Despite eventually reaching similar population sizes, gray matter cells overall exhibited many more oligodendrocytes with a pre-myelinating morphology, while the majority of white matter-derived oligodendrocytes had parallel processes typical of myelinating cells. When these cells are grafted into gray matter, white matter-derived cells produce increasing numbers of oligodendrocytes in the weeks following engraftment, but no new oligodendrocytes are produced from gray matter-derived cells. This indicates that, while the white matter environment can encourage gray matter cells to produce more oligodendrocytes, white matter cells harbor intrinsic programming that allows them to maintain the same oligodendrogenesis kinetics despite residing in the gray matter

environment [75]. Slice culture experiments have shown that the addition of PDGF, a growth factor known to be critical for OPC proliferation, causes significantly more proliferation in the white matter than in the gray matter, despite equivalent expression of the receptor PDGFRα in both groups of cells [80,81]. These graded responses to PDGF were maintained when OPCs were transplanted into either gray or white matter [81].

There are a number of differences between white and gray matter OPCs, including characteristics such as morphology, proliferation, differentiation, and membrane properties. While some of these components may be influenced by the environment particular to each region, it is clear that there are some intrinsic differences between gray and white matter OPCs, providing additional evidence for distinct subpopulations that fall under the category of oligodendrocyte progenitor cells.

GPR17+ OPCs

While very little work has been done regarding molecular markers of specific subsets of OPCs, GPR17, a G-protein coupled receptor, has recently been investigated as a potential marker for a subset of OPCs maintained to specifically respond to insult or injury [82-86]. Only a subset of OPCs express GPR17, with some estimating that only one third of OPCs produce GPR17 [87,88]. GPR17 OPCs proliferate less and are less likely to differentiate into oligodendroytes than their GPR17- counterparts [83,84,88]. Even during the peak of murine oligodendrogenesis (P6-P30), only approximately 30% of GRP17+ OPCs differentiate into mature oligodendrocytes [84]. GRP17 seems to act as a repressor of differentiation, as preventing its internalization decreases the number of OPCs that produce oligodendrocytes in culture, and GPR17 knockout mice express the mature

myelin marker MBP in embryonic stages, while wildtype mice don't begin myelin development until postnatal time points [83,89]. The highest expression of GPR17 is seen in OPCs and immature, premyelinating oligodendrocytes, with a significant downregulation as oligodendrocytes fully mature [88,90].

While GRP17+ OPCs seem to be relatively quiescent in the naïve brain, this population reacts strongly to any type of insult or injury. Following stroke, traumatic brain, or experimental autoimmune encephalomyelitis (EAE), GPR17+ OPCs are found surrounding the lesion or injury, and begin to both proliferate and differentiate in response to damage [83,84,91,92]. This same patterning has also been seen in human brains from patients having experienced a traumatic brain injury [93]. This significant expansion and differentiation of GPR17+ OPCs in response to CNS damage has led to the hypothesis that GPR17+ OPCs are maintained in the adult brain in an immature state as a population of cells prepared to sense and quickly respond to damage [94]. The environment of damaged tissue seems to recruit and facilitate the migration of GRP17+ OPCs, as transplanting them into the brain after a middle cerebral artery occlusion results in significantly further migration and longer retention of GPR17+ cells than transplantation into naïve brains [92]. Some literature also suggests that GPR17 signaling in OPCs may result in their differentiation into neurons, potentially contributing to neuronal repopulation in damaged tissue, although this has yet to be fully validated *in vivo* [86,95]. While the functionality of GPR17+ OPCs remains unclear, these cells represent a molecularly distinct subpopulation of OPCs that exhibits a distinct response to CNS damage.

Ascl1+ OPCs

Ascl1, also known as Mash1, is a transcription factor that has also been shown to mark a subset of OPCs. Ascl1 is a transcription factor that is known to play a significant role in neurogenesis and is maintained in OPCs during adulthood [96,97]. OPCs that are born very early in development (E12.5) are much more likely to express Ascl1, while consecutive waves are much less likely to express it [98]. In the adult brain the proportion of OPCs that express Ascl1 varies widely depending on the brain region, from less than a third of OPCs in the striatum expressing Ascl1 to 100% co-expression in the hippocampus and corpus callosum [97,98]. While Ascl1 is downregulated in mature oligodendrocytes, it seems to be positively influencing the differentiation program of OPCs [99]. Deletion of Ascl1 results in significantly fewer myelinating oligodendrocytes in the spinal cord as well as an increase in the number of symmetrical OPC/OPC divisions at the expense of asymmetrical OPC/oligodendrocyte divisions [97,100]. Alterations to Ascl1 seem to have a stronger impact on oligolineage cells in the white matter than on those in the gray matter. White matter OPCs express higher levels of Ascl1, and loss of Ascl1 leads to a loss of oligolineage cells in the white matter only [74,101]. Ascl1+ OPCs differentiate faster in white matter, and are maintained in a progenitor state longer in the gray matter [74,102]. While the exact role of Ascl1 in OPCs remains unclear, current evidence suggests that this transcription factor may identify a subset of OPCs that are more likely to differentiate into oligodendrocytes than Ascl1- OPCs.

Functions beyond Progenitors

OPCs as Electrically Active Cells

With the vast number of neurons and glial cells within the brain, significant crosstalk between neurons and all subtypes of glia is necessary for proper functioning of the brain [103]. OPCs are particularly reactive to neuronal activity, as originally described by Barres and Raff in 1993, when they observed a significant decrease in OPC proliferation after injecting tetrodotoxin into the optic nerve [104]. The extent of the neuron-OPC connection wasn't realized until Bergles and colleagues (2000) performed patch-clamping experiments on OPCs and observed fast, inward currents reminiscent of action potentials. Characterization of these depolarizations indicated that they were caused by multiple glutamatergic synapses converging on a single OPC and activating AMPA receptors [64]. Currently, OPCs are the only known type of glial cell to form canonical synapses with neurons. Further characterization showed that only a subset of OPCs in the neonatal brain exhibited these depolarizations and expressed voltage-gated sodium channels, but that these OPC synapses were found in both gray and white matter [72,105]. However, some argue that all OPCs exhibit synaptic depolarizations and that loss of this response is due to cellular maturation into a late-stage OPC that will eventually differentiate [72,106]. Characterization of glutamate receptors and ion channels on oligolineage cells has shown that, as an OPC differentiates into an oligodendrocyte, it downregulates the number of AMPA and NMDA receptors present on the membrane and completely loses expression of voltage-gated sodium channels, while upregulating glutamate transporters [106]. This indicates that, while oligodendrocytes are still able to

respond to glutamate release, they lose the quick “action-potential like” depolarizations that occur in OPCs as a result of synaptic release of glutamate [107].

Despite almost two decades of work on the OPC-neuron synapse, its functional purpose remains unclear. AMPA signaling has been shown to significantly increase the motility of OPCs, and blocking both NMDA and AMPA receptors has been shown to increase OPC proliferation and significantly change OPC morphology [108-110]. Preventing vesicular release in general results in a reduced number of oligodendrocytes as well as a reduction in the number of myelin sheaths that each oligodendrocyte is able to produce [111]. OPC-neuron synapses also seem to play a role in demyelination repair. Following a demyelinating lesion in the cerebellar peduncle, many OPCs exhibited depolarizations in response to an electrical stimulus, while OPCs in this tract in a healthy adult mouse did not produce any depolarizations. Inhibiting AMPA/kainate receptors within the lesion inhibited remyelination, while blocking all neuronal activity resulted in a significant increase in OPC proliferation [107]. Based on this data, many hypothesize that the OPC-neuron synapse temporally controls OPC proliferation and differentiation as a mechanism to regulate myelination. While this certainly may be the case, especially with the high concentration of AMPA receptors found on immature OPCs, the nano-second time scale of OPC depolarizations seems to indicate a role for these synapses that must occur very quickly. Since OPC differentiation takes at least two days in the perinatal brain, and 4 days at later time points, it is unlikely that the only purpose of the OPC-neuron synapse is to regulate OPC differentiation and myelination [30].

Glial Scar Formation

The glial scar is a region of reactive gliosis that surrounds a lesion or injury within the CNS. Reactive astrocytes are most often seen as the main component of the glial scar, but OPCs also seem to be contributing to its formation [112]. Immediately following a laser injury to the brain, OPCs begin extending their processes to surround the lesion, reaching the injury sight slightly after microglia, followed later by the migration of the cell soma. These cells remain in the area of the lesion for much of the recovery, and disappear as the glial scar is removed [27]. In both mouse models of traumatic brain injury and stroke, as well as human instances of spinal cord injury, OPCs can be found surrounding the area of focal injury with a concomitant increase in their proliferation [113-117]. The glial scar contains significant amounts of chondroitin sulphate proteoglycans (CSPGs), which includes the OPC marker NG2 [118,119]. The increase in NG2 within the glial scar is, at least in part, contributed by the compact ring of OPCs that forms around the lesion [116,118]. OPCs within the glial scar have also been shown to express the CSPG neurocan [118]. Whether the increase in OPCs within the glial scar is beneficial or detrimental to repair and recovery following injury is up for debate. CSPGs within the glial scar, including NG2, have been shown to inhibit axon outgrowth into the lesioned area [119,120]. Specific ablation of NG2+ cells, or all proliferative cells within the lesion, have been shown to at least partially rescue the lack of axonal entrance into the lesion [116,117,121]. However, deletion of NG2 cells from the glial scar increases edema and hemorrhaging within the lesion and proliferating OPCs at the sight of injury have been shown to contribute to later oligodendrogenesis [116,122].

The functional purpose of the significant increase in OPCs within the glial scar has yet to be fully described, but recent evidence indicates that they may play a significant role in orchestrating other cell types in the formation of the glial scar. Ablation of all NG2+ expressing cells within and surrounding a lesion prevents not only the accumulation of OPCs within the scar, but also prevents the clear astrocytic border that normally encloses the area of focal damage [116]. The deletion of β-catenin, and therefore canonical Wnt-signaling, in OPCs also reduces the number of OPCs that surround the lesion as well as the number of microglia/macrophages and reactive astrocytes that surround the damage. Within this model, more axons cross into the area of damage, but it remains to be seen if this abrogation of normal glial scar formation results in functional improvement following CNS injury [121]. Some evidence has also suggested that OPCs may be a small source of astrocytes specifically within the glial scar [123,124]. Whether the OPCs that contribute to the glial scar represent a specific subpopulation of OPCs that are particularly prepared to respond to injury is unclear, but it is known that those cells participating in glial scar formation change fundamental characteristics usually found in OPCs. Specifically, they lose the contact inhibition that usually maintains organized tiling of these cells in the brain and begin forming clusters [27,116,121].

Maintaining Homeostasis

With the emergence of transgenic lines that allow for the specific and effective ablation of OPCs, literature is currently emerging that more conclusively indicates non-myelinating roles for OPCs [125]. Some of these studies provide evidence that OPCs play a significant role in maintaining homeostatic conditions in the CNS that allow for proper

neuronal functioning. In 2016, Djogo and colleagues showed that specific ablation of OPCs from the median eminence (ME) of the hypothalamus resulted in significant and sustained weight gain and food intake increase. Without ME OPCs, neurons expressing the receptor for leptin lose the ability to respond to leptin, despite no loss in the number of leptin-sensing neurons. Further analysis showed that OPC processes seem to be contacting leptin receptor positive dendrites and that their ablation leads to the subsequent degeneration of these neurites. The mechanism through which OPCs maintain the health of these dendrites is still unclear, but the authors hypothesize that the loss of ME OPCs may be the cause of weight-gain side effects resulting from cancer radiation treatment [5]. OPCs also seem to be maintaining homeostatic conditions in the hippocampus. Nakano and colleagues (2017) ablated OPCs in the hippocampus and saw a subsequent loss of hippocampal neurons. The loss of hippocampal OPCs resulted in microglial activation and IL-1β release, which in turn lead to local neuronal death. The addition of hepatocyte growth factor (HGF), a growth factor known to be made by OPCs, was able to rescue neuronal death despite the loss of OPCs [4,126]. This study provides some evidence that OPCs may play a part in regulating microglial activation in the healthy brain to maintain an environment conducive to proper neuronal functioning.

Growth factors produced by OPCs may also play a role in maintaining blood-brain barrier (BBB) integrity. *In vitro*, conditioned media from OPCs is sufficient to decrease the permeability of a monolayer of endothelial cells, an effect that is abrogated by blocking TGF-β signaling. Deletion of TGF-β1 from OPCs *in vivo* results in brain hemorrhages by the day of birth, which is accompanied by leakage of the blood-brain barrier as well as alterations to tight junctions [127]. This production of growth factors that seems to be

maintaining homeostasis in the healthy brain may also serve to insulate the brain from potential injury. Culturing OPCs in a chamber connected to hippocampal slice culture exposed to oxygen and glucose deprivation results in less cell death than slices cultured alone. This effect is at least partially mediated by IL-10 and BDNF produced by OPCs [128]. Evidence supporting the functional role of OPCs in maintaining homeostasis in the healthy brain is still recent, and the full range of effects that OPCs might have on other CNS resident cells remains to be described.

Diseases

Multiple Sclerosis

The known progenitor role of OPCs has fostered a large amount of research into how the environment of a demyelinating lesion, and the concomitant inflammatory cascade, may affect the viability of OPCs and their ability to differentiate into oligodendrocytes [129-131]. While this line of inquiry is extremely important, especially for the generation of novel remyelination therapeutics, recent data suggests that OPCs may be playing an instrumental role in transducing key signals in demyelinating diseases, most notably experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. Kang and colleagues (2013) demonstrated that EAE severity was significantly reduced when Act1, an integral protein in the IL-17 signaling pathway, was deleted from OPCs. Robust clinical score reduction was not observed when Act1 was deleted from any other resident CNS cell population. This deletion not only improved clinical scores, but also reduced demyelination and parenchymal infiltration by immune cells. Cultured OPCs

respond to IL-17 by producing cytokines and proteins that degrade the extracellular matrix including IL6, Cxcl1, Csf2, Mmp3, and Mmp9 [132,133].

A handful of other studies have also indicated a role for OPCs in modulating the local immune environment. OPCs extracted from mice undergoing cuprizone-induced demyelination upregulate the pro-inflammatory cytokine IL1-β as well as the monocyte chemoattractant CCL2, a phenotype that is also seen in active human MS lesions [134,135]. Following cerebral hypoperfusion, OPCs have been shown to quickly induce production of Mmp9, an enzyme that has the ability to degrade the extracellular matrix [136,137]. This induction of Mmp9 results in blood brain barrier breakdown and ultimately plays a role in the pathological demyelination that results from this type of injury [136] Within the context of EAE, OPCs may also be playing a role in blood brain barrier maintenance, as deletion of NG2 increases permeability of the BBB and alters the morphology of tight junctions [138]. A significant amount of work remains in order to outline the exact role that OPCs have in the pathological progression of EAE, as well as other inflammatory or demyelinating diseases. However, it is clear from this evidence that OPCs have the potential to significantly alter the immune environment within the CNS and may contribute to pathological processes that result in demyelination or parenchymal inflammation.

Depression

Interest in the potential role of glia in psychiatric disorders, and depression in particular, stems from early observational studies that described changes to cellular composition in the brains of patients diagnosed with mental health disorders [139,140]. Twenty years ago, Ongur and colleagues (1998) described a significant loss of glial cells

in the frontal cortex of patients who had been diagnosed with bipolar disorder or depression [140]. Further analysis of proliferating cells in the brain demonstrated a significant loss of cells that incorporated BrdU, and many of these cells co-stained with OPC markers such as Olig2 and NG2 [141-143]. Some evidence indicates that this loss can be partially rescued with antidepressant treatment, indicating that the recovery of actively cycling cells within the brain at least has the potential to be involved in recovery from depression [142]. Loss of proliferative cells, including OPCs, the most proliferative cells in the CNS, during depression seems to be potentially tied to areas of the brain known to be involved in this disorder. Following 4 weeks of unpredictable chronic mild stress, Liu and colleagues (2018) saw a decrease in the transcript abundance of common OPC markers such as PDGFRα and NG2 in the prefrontal cortex and nucleus accumbens, with a concomitant increase in these same markers in the corpus callosum [144]. However, the exact dynamics of regionally specific changes in cycling cells remains unclear, as some studies show contradictory changes in specific brain regions [142,143]. With the observance of a loss of OPCs during depression, multiple groups began to investigate how the mature oligodendrocyte population and myelination changed in this context. Myelin genes, including MOG, MAG, and CNP are downregulated in the prefrontal cortex after animal models of social defeat and unpredictable chronic mild stress, as well as in the temporal cortex and the amygdala of patients who had been diagnosed with Major Depressive Disorder [145-148]. This decrease in myelin genes is not always accompanied by a decrease in oligodendrocyte cell number. Both Lehmann (2017) and Liu (2012) demonstrated stability in oligodendrocyte number despite a downregulation in myelin genes, indicating

an effect of depression on myelin stability, but not necessarily the health of oligodendrocytes themselves [146,149].

Until recently, the effects of depression on oligolineage cells, and OPCs in particular, remained purely observational. In 2015, Birey and colleagues outlined a potential role for OPCs in the pathophysiology of depression, independent of their role as progenitors. Using a diphtheria toxin ablation system, they demonstrated that global ablation of OPCs resulted in both anxiety and depression-like behavior. This ablation modulated electrical activity of glutamate neurons in the prefrontal cortex, although notably this change was not seen in the striatum or sensory cortex, indicating varying roles for OPCs in different brain regions. This change in post-synaptic potentials in glutamatergic neuron after OPC ablation was accompanied by a significant decrease in astrocytic uptake of glutamate, driven by the downregulation of membrane-bound GLAST and GLT-1. Interestingly, local ablation of OPCs in the PFC alone was sufficient to drive a subset of depressive behaviors, indicating that the function of OPCs in specific brain regions may be particularly critical for regulating behavior. Allowing OPCs to repopulate following ablation rescued the behavioral, neuronal, and astrocytic phenotype observed after ablation. OPC secretion of the growth factor FGF2 is lost in the PFC of mice following social defeat. Knockdown of FGF2 in PFC OPCs phenocopies behavioral deficits observed following OPC ablation [3]. This data strongly suggests that OPCs play an integral role in the progression of a depressive phenotype, independent of their ability to generate myelinating oligodendrocytes. However, many important questions remain to be answered, including the upstream cause of OPC loss, as well as clarification on whether

the loss of OPCs in depression is a cause or an effect of the behavioral symptoms exhibited in depression.

Conclusions

Historically, OPCs have been viewed simply as a pool of progenitors available to produce myelinating oligodendrocytes as needed in the adult brain. However, their dynamic characteristics, including constant proliferation, rapid migration, and maintenance of even tiling throughout the brain, along with the relative stability of adult oligodendrocytes, indicates that OPCs within the mature brain potentially have multiple, diverse roles. Characteristics indicating this potential diversity begin as early as development, with OPCs arising from multiple areas within the CNS. Cells arising from different regions seem to respond differently to the same signaling pathway. Regional heterogeneity is maintained in the adult brain, with white and gray matter OPCs exhibiting diverse characteristics. Some molecular heterogeneity within OPCs has been described, with GPR17 and Ascl1 marking subsets of OPCs that seem to have specific roles in the brain. Some roles of OPCs that have been explored are their ability to participate in glial scar formation, as well as maintain homeostasis within the healthy brain. These diverse roles of OPCs also have implications for their involvement in disease, with recent evidence implicating integral roles for these cells in disorders such as multiple sclerosis and depression.

Until recently, much of what was known about OPCs outside of their progenitor context was largely observational. Recent studies have begun to explore their functional role in both the healthy and diseased brain, but more systematic study of their diversity

and its functional significance is integral to our understanding of how OPCs contribute to the normal functioning of the CNS. The goal of my project is to outline molecular heterogeneity and identify potential subpopulations of OPCs in the healthy brain and to describe how these populations change in depression. Modulating specific markers of these subpopulations in the context of depression will allow us to describe how OPC may be contributing to a depressive phenotype, and what changes may be leading to their depletion in depression.

Chapter II: Materials and Methods

Animals

PDGFRα-CreER mice (Jackson #018280) were crossed to *R26-EYFP* (Jackson, #006148) animals to generate *PDGFRα-CreER*; *R26-EYFP* mice, a previously described model[150]. C57BL/6J (Jackson, #000664) were purchased from Jackson or bred at the University of Virginia. 5xFAD mice (Jackson #34848) were bred at the University of Virginia and were used at 5-6 months old[151]. Mice were maintained on a 12 hour light/dark cycle with lights on at 7am. Behavior was performed on mice used in single-cell sequencing run 1. Testing consisted of sucrose preference, elevated plus maze, open field, and forced swim test. All animal experiments were approved and complied with regulations of the Institutional Animal Care and Use Committee at the University of Virginia (protocol #3918).

Human Tissue Samples

Human post-mortem brain samples were provided by Dr. Stefan Prokop at the University of Florida's Neuromedicine Human Brain and Tissue bank. All tissue collection and preparation was approved by the Institutional Review Board at the University of Florida. Clinical details of all human specimens analyzed are details in Table 1.

Tamoxifen injections

Tamoxifen (C8267, Sigma-Aldrich) was dissolved in corn oil at 37°C overnight at 20 mg/mL. Tamoxifen was administered *i.p*. at 200 mg/kg with a maximum dose of 4 mg per injection. For single-cell sequencing experiments, six-week old mice were given two

injections of tamoxifen, three days apart. For validation of Cre recombination in *PDGFRα-CreER*; *R26-EYFP* brains, five to six week old mice were injected with 0, 1, 2, or 3 doses of tamoxifen, each given three days apart. For those mice receiving three doses of tamoxifen, the final dose was given at 150 mg/kg.

Case number	Main neuropathologic diagnosis	Secondary neuropathologic diagnoses	Thal	Braak	CERAD	Gender	Age
control 1	PART, definite, Braak II		0	II	none	f	72
control 2	PART, Braak I	CAA widespread, mild	0	I	none	m	71
control 3	No significant pathological findings		0	0	none	f	55
control 4	CVD	ADNC low; CAA focal, mild	1	II	none	f	90
control 5	No significant pathological findings	Atherosclerosis (moderate)	0	0	none	m	71
control 6	No significant pathological findings	Atherosclerosis (moderate)	0	0	none	m	88
control 7	PART, definite, Braak I	ARTAG, subependymal	0	I	none	m	77
control 8	CVD		0	0	none	m	72
control 9	PART, definite, Braak I		0	I	none	f	73
control 10	PART, definite, Braak II		0	II	none	f	82
control 11	PART, definite, Braak II	subacute microinfarct corpus callosum	0	II	none	f	90
control 12	PART, definite, Braak I		0	I	none	f	78
control 13	PART, definite, Braak II	CAA focal, moderate	0	II	none	m	77
control 14	PART, definite, Braak I	ARTAG, subependymal	0	I	none	m	51
control 15	PART, definite, Braak I		0	I	none	m	52
AD 1	ADNC high	CAA widespread, moderate	4	V	frequent	m	84
AD 2	ADNC high	CAA widespread, moderate ; LATE NC stage 1	5	VI	frequent	m	81
AD 3	ADNC high	CAA focal, moderate	4	V	frequent	f	85
AD 4	ADNC high	CAA widespread, moderate to severe	5	V	frequent	m	74
AD 5	ADNC high	CAA widespread, moderate	5	VI	frequent	m	83
AD 6	ADNC high	CAA widespread, moderate	4	VI	frequent	m	75
AD 7	ADNC high	CAA widespread, moderate	5	VI	frequent	m	70
AD 8	ADNC high	CAA, moderate, widespread	5	V	frequent	f	86
AD 9	ADNC high	CAA focal, moderate; LATE NC stage 1	5	VI	frequent	m	74
AD 10	ADNC high	CAA, widespread, moderate	5	V	frequent	f	64
AD 11	ADNC high	CAA widespread, moderate	5	VI	frequent	m	59
AD 12	ADNC high	CAA widespread, moderate	5	VI	frequent	m	83
AD 13	ADNC high	CAA focal, mild	5	V	frequent	m	78
AD 14	ADNC high	CAA focal, moderate	5	V	frequent	m	95
AD 15	ADNC high	CAA widespread, mild to moderate; LATE NC stage 2	4	V	frequent	f	78
AD 16	ADNC high	CAA widespread, mild to moderate	5	V	frequent	f	97
AD 17	ADNC high	CAA widespread, moderate	5	V	frequent	f	78
AD 18	ADNC high	CAA widespread, mild	4	V	frequent	m	77
AD 19	ADNC high	CAA widespread, mild to moderate	5	VI	frequent	m	66
AD 20	ADNC high	CAA widespread, moderate	5	VI	frequent	m	63
AD 21	ADNC high	CAA focal, moderate	5	VI	frequent	f	86
AD 22	ADNC high	CAA widespread, mild; LATE NC stage 1	5	V	frequent	f	82
AD 23	ADNC high	CAA focal, mild; LATE NC stage 1	5	V	frequent	f	90
AD 24	ADNC high	LATE NC stage 2	5	V	frequent	m	87
AD 25	ADNC high	CAA focal, mild to moderate	5	V	frequent	m	73
AD 26	ADNC high	CAA focal, moderate; LATE NC stage 1	5	VI	frequent	f	84

Table 1: Human Samples. Clinical details for all the human brain specimens used in this manuscript

OPC Culture

OPCs were cultured as previously described, with a few modifications[152]. Briefly, postnatal cortices (P0-P4) were rapidly dissected and meninges removed. The tissue was digested in 2ml of Accutase (Gibco, A1110501) supplemented with 50 units/mL DNase

(Worthington Biochemical, LS002139). Cells were then passed through a 70μm filter and grown in suspension in neurosphere media consisting of DMEM/F12 (Gibco, 11320082), B27 (Gibco, 17504044), Pen-Strep (Gibco, 15140122), and 10ng/mL EGF (Peprotech, 315-09). Following expansion as neurospheres, cells were switched to oligosphere media consisting of DMEM/F12 (Gibco, 11320082), B27 (Gibco, 17504044), Pen-Strep (Gibco, 15140122), 10ng/mL FGF (Peprotech, 450-33), and 10ng/mL PDGF-AA (Peprotech, 315-17). Cells were allowed to grow in suspension for at least 2 days. Cells were then plated as attached OPCs on 0.01% Poly-L-Lysine coated plates (Electron Microscopy Sciences, 19320-B) in the same media. Cells were allowed to attach for at least 12 hours and then subsequent assays were performed.

OPC Proliferation

OPCs were plated in proliferation media (described above) supplemented with 8μg/mL clusterin (Sino Biological 50485-M08H) or an equivalent volume of vehicle (H_20 or PBS) for the control samples. OPCs were allowed to proliferate for 40-72 hours. Cell number was assessed using the Cell Counting Kit-8 (Dojindo, CK04) according to manufacturer's instructions. Optical density (OD) was measured at 450nm. For data analysis, the OD of a media only control was subtracted from the OD of all experimental samples. The OD of wells treated with clusterin were then normalized to the biologically identical control well.

OPC Differentiation

OPCs were plated in proliferation media (described above) or differentiation media consisting of DMEM/F12 (Gibco, 11320082), B27 (Gibco, 17504044), Pen-Strep (Gibco,

15140122), 10ng/mL FGF (Peprotech, 450-33), 10ng/mL CNTF (Peprotech, 450-13), and 40ng/mL T3 (Sigma, T6397). For clusterin conditions, 8μg/mL clusterin (Sino Biological 50485-M08H) was added to the differentiation media and an equivalent volume of vehicle (H20 or PBS) was added to the differentiation and proliferation control samples. For IL-9 rescue experiments, 100ng/ml IL-9 (Peprotech 219-19) was added to the differentiation media and an equivalent volume of vehicle (0.1% BSA) was added to the differentiation and proliferation control samples. For VEGF inhibition experiments, 10μg/ml of a VEGF function-blocking antibody (R&D Systems MAB9947-SP) was added to differentiation media and 10μg/ml of control IgG (Biolegend 403501) was added to the differentiation and proliferation control samples. OPCs were allowed to differentiate for 48-72 hours and were then subsequently processed for RNA extraction and qPCR or for immunofluorescence.

Aβ Preparation and *in vitro* treatment

Aβ oligomers were prepared as previously described[153]. Briefly, human Amyloid Beta$_{1-42}$ (Echelon Biosciences, 641-15) was dissolved in HFIP (Sigma, 52517) to make a 1mM solution and was allowed to desiccate overnight. The resulting peptide film was diluted to a 5mM solution in DMSO and subsequently diluted to a 100μM solution in phenol-free F-12 cell culture media (Gibco, 11039-021) and allowed to incubate overnight at 4°C. For the analysis of clusterin expression following Aβ-treatment, OPCs were treated with 3μM Aβ or a vehicle control for 4 hours. For experiments that included samples treated with CytoD, cells were pretreated for 30 minutes with 1μM CytoD (Millipore-Sigma, C8273) or DMSO and subsequently treated with 3μM Aβ or vehicle control (also containing CytoD

or DMSO) for 4 hours. For CypHer-labeled Aβ experiments, 400μM Aβ oligomers in DMSO and phenol-free F-12 cell culture media was incubated with an equivalent volume of 0.1M sodium bicarbonate (Fisher Scientific, S233-500) and 400μM CypHer5e (Cytiva, PA15401) for 30 minutes at room temperature. Following incubation, the solution was spun through a buffer exchange column (Thermo Scientific, 89882) to remove any excess dye.

Myelin Preparation and *in vitro* treatment

Myelin was prepared from mouse brains as previously described[154]. Purified myelin was passed through an insulin syringe prior to use to ensure cells were treated with a homogenous solution. Cells were treated with 100μg/ml myelin or vehicle control for 4 hours. For two replicates that included samples treated with CytoD, cells were pretreated for 30 minutes with 1μM CytoD (Millipore-Sigma, C8273) or DMSO and subsequently treated with 100μg/ml myelin or vehicle control (also containing CytoD or DMSO) for 4 hours. For the remaining two replicates that included samples treated with CytoD, no pretreatment was performed. Pretreatment with CytoD did not alter clusterin expression when compared to no pretreatment with CytoD, so all experiments were combined and plotted together. Cells treated with myelin were washed once with PBS prior to RNA preparation.

Cytokine and H_20_2 *in vitro* treatment

OPCs were treated with 10ng/ml TNFα, 10ng/ml IFNγ, 10μM H_20_2 or the relevant control for 3 hours and then processed for qPCR.

Apoptotic cell preparation and *in vitro* treatment

To create apoptotic cells, Jurkats were treated with 150mJ of UV energy and incubated for 2-4 hours at 37°C in complete media consisting of RPMI 1640 Media (Gibco, 11875101), 10% FBS (R&D Systems, S12450H), and Pen-Strep (Gibco, 15140122). Apoptotic cells were washed and added to cultured OPCs at approximately a 1:1 ratio for 6 hours. OPCs were washed once prior to RNA isolation.

ELISA

OPCs were grown as described above. A 10cm dish of approximately 2 million OPCs was treated with 3μM Aβ or an equivalent volume of vehicle for 72 hours. Cells were lysed for 15 minutes in RIPA buffer (PBS, 1% Triton X-100, 0.5% deoxycholic acid, 1% sodium dodecyl sulfate) supplemented with 1x protease inhibitor (MedChem Express HY-K0010). The insoluble material was removed by spinning the lysate at 15,000g for 15 minutes. The resulting lysate was used to quantify the amount of clusterin present in each sample using the Mouse Clusterin ELISA Kit (Thermo Fisher EM18RB) according to manufacturer's instructions.

Luminex Assay

OPCs were grown as described above. Cells were treated with 8μg/ml clusterin for 24 hours (1 replicate) or 72 hours (1 replicate). Media was then collected and spun to remove all cellular debris. Media was concentrated using a 3KD cutoff concentrator column (Thermo Fisher 88526). The resulting concentrate was analyzed using the Milliplex 32-

plex Mouse Cytokine/Chemokine Panel (Millipore Sigma MCYTMAG-70K-Px32) according to manufacturer's instructions.

Aβ Injections

C57BL/6J mice (8-14 weeks) were injected with CypHer-Aβ (ipsilateral) and NHS-Fluorescein (contralateral, Thermo Fisher 46410) as previously described[155]. Briefly, mice were anesthetized with a mixture of ketamine and xylazine and a small burr hole was drilled in the skull. 1μL of 100μM Aβ was injected at a speed of 200nL/minute into the right hemisphere. 1μL of 200μM NHS-Fluorescein diluted in PBS was injected into the left hemisphere at the same speed. Injections were targeted for 2mM lateral, 0mM anterior, and -1.5mM deep relative to bregma. Mice were given ketoprofen following surgery and were euthanized 12 hours post-injection.

Immunofluorescence

Mice were deeply anesthetized with pentobarbitol and subsequently perfused with 5 units/mL heparin in saline followed by 10% buffered formalin, each for approximately one minute. For brain tissue, brains were rapidly dissected and post-fixed in 10% buffered formalin overnight at 4°C. Tissue was then transferred into 30% sucrose in PBS and allowed to sink for at least 24 hours. Brains were frozen in OCT, sectioned, and stored in PBS plus 0.02% NaAz until further staining.

Tissue or cultured cells were blocked with PBS, 1% BSA, 0.5% Triton-X 100, 2% normal donkey serum, and 1:200 CD16/CD32 (14-0161-82, 1:200, eBioscience) for at least one

hour at room temperature. For stains utilizing a mouse primary antibody, tissue was blocked in Mouse on Mouse Blocking Reagent (MKB-2213, Vector Laboratories) according to manufacturer's instructions for at least 1 hour at room temperature. Samples were incubated in primary antibodies overnight at 4°C with gentle agitation. Samples were then washed three times in TBS containing 0.3% Triton-X 100 and incubated in secondary antibodies overnight at 4°C with gentle agitation. Following secondary incubation, samples were stained with Hoechst (1:700, ThermoFisher Scientific, H3570) for 10 minutes at room temperature, washed three times in TBS containing 0.3% Triton-X 100, and mounted on slides using Aqua Mount Slide Mounting Media (Lerner Laboratories). Images were collected on a Leica TCS SP8 confocal microscope and processed using Fiji.

Antibodies for Immunofluorescence

Primary antibodies used for immunofluorescence were PDGFRα (1:200, R&D Systems, AF1062), Olig2 (1:200, Millipore, MABN50), GFP-488 (1:400, Fisher Scientific, A21311), GFP (1:1000, Invitrogen, A10262), Clusterin (1:250, Abcam, AB184100), and Aβ (1:300, Cell Signaling Technology, 8243S). Secondary antibodies used were Donkey anti-Goat Cy3 (2μg/mL, Jackson ImmunoResearch, 705-165-147), Donkey anti-Mouse 647 (2μg/mL, Jackson ImmunoResearch, 715-605-150), Donkey anti-Mouse 546 (2μg/mL, Life Technologies, A10036), Donkey anti-Chicken 488 (2μg/mL, Jackson ImmunoResearch, 703-545-155), Donkey anti-Goat 488 (2μg/ml, Jackson ImmunoResearch, 705-545-147), Donkey anti-Rabbit Cy3 (2μg/ml, Jackson ImmunoResearch, 711-165-152), Donkey anti-Rabbit 647 (2μg/ml, Jackson

ImmunoResearch, 711-605-152), and Donkey anti-Goat 647 (2μg/mL, Invitrogen, A21447).

Isolation of CNS cells

To prepare cells for single-cell sequencing, adult mice (8-20 weeks) were anesthetized with pentobarbitol and subsequently perfused with 5 units/mL heparin in saline for approximately one minute. Brains were rapidly dissected and finely minced. For single-cell sequencing experiments, tissue was digested in HBSS with calcium and magnesium (Gibco, 14025-092) supplemented with 20 units per mL papain (Worthington Biochemical LS003126) and 50 units per mL DNase (Worthington Biochemical, LS002139). Tissue was digested at 37°C with gentle shaking for 45 minutes, with trituration after every 15-minute interval to dissociate the tissue. Following digestion, a 40% Percoll gradient (GE Healthcare, 17-0891-01) was used to remove myelin and other debris from the samples. Resulting single-cell suspensions from 4-5 mice were pooled for each sequencing sample and subsequently stained for FACS sorting.

FACS sorting

For single-cell sequencing experiments, single-cell suspensions were stained for 30 minutes at room temperature with the following antibodies: O4-APC (O4, 10μL/test, Miltenyi, 130-095-891), CD11b-e450 (M1/70, 0.5 μL/test, eBioscience, 48-0112-82), TER119-APC/Cy7 (TER-119, 1.25 μL/test, Biolegend, 116223), PDGFRα-PE/Cy7 (APA5, 0.625 μL/test, Invitrogen, 25-1401-82), CD45-PerCP/Cy5.5 (30-F11, 0.5 μL/test, eBioscience, 45-0451-82), and CD16/31 (93, 0.5 μL/test, Invitrogen, 14-0161-82).

Viability was determined using Ghost Dye Violet 510 (0.5 µL/test, Tonbo biosciences, 13-0870). Cells were sorted using a 16-color BD influx cell sorter. Cells used for sequencing were gated on live/singlets/TER119-/CD45-/CD11b-/YFP+. Following sorting, cells were washed three times with 0.04% BSA and then processed for sequencing according to the 10x Genomics protocol.

Flow Cytometry

OPCs were incubated with 3µM CypHer-Aβ with 8µg/ml clusterin, 1µM CytoD, or a PBS vehicle control for 90 minutes. Cells were removed from the plate with 0.25% Trypsin-EDTA (Gibco 25200056), washed, and stained with Ghost Dye Violet 510 (0.5 µL/test, Tonbo biosciences, 13-0870). Cells were analyzed using a 3 laser, 10-color Gallios flow cytometer (Beckman-Coulter).

Single-Cell Sequencing and Analysis

Library Preparation and Sequencing: Samples were processed for single-cell sequencing according to manufacturer's instructions using the Chromium Next GEM Single-cell 3' Reagent Kit (10xGenomics) and Chromium Controller (10xGenomics). Single-cell libraries were sequenced using the NextSeq 500 Sequencing System (Illumina). Library preparation and sequencing was completed by the Genome Analysis and Technology Core at the University of Virginia.

Quantification: All steps of the quantification process were performed with Cellranger. The fastq files for the samples were quantified using the mkfastq utility and were quantified against the mm10 mouse genome with the count utility.

Pre-processing: Seurat was used for the single-cell analysis[156,157], and for each of the sequencing run datasets, we followed the same procedure. First, a QC step was performed to identify and remove cells that were potential outliers. This included removing potential multiplets (i.e., cells that had clear outlier gene expression) and cells that had approximately ten percent or more of mitochondrial gene expression (i.e., cells that were likely to have high technical variation). After filtering out these suspect cells, the data was normalized and log-transformed (using the 'LogNormalize' method), unwanted sources of technical variation were regressed out (i.e., the number of detected molecules and mitochondrial contribution to gene expression) [158], and the counts were scaled.

Integration: To make comparative analyses possible between multiple sequencing run datasets, the datasets were integrated with Seurat using the alignment strategy described previously[156]. The first step was to select the genes to be used as the basis for the alignment. The union of the 1000 genes with highest variability in each of the datasets was taken and then filtered this down to only those genes found in each of the datasets, resulting in 2,285 genes for the alignment. Next, the common sources of variation between the six datasets (3 sequencing runs with 2 samples each) was identified by running a canonical correlation analysis (CCA) with the highly variable genes as features. By examining the strength of the thirty calculated canonical correlations (CCs), the first

twelve CCs were identified to be driving the variation between the datasets. The subspaces [156] (i.e., the first twelve CCs) were then aligned, resulting in an integrated dataset with features on a common scale.

Analysis: Seurat was used on the aligned dataset to identify eight clusters of cells, and then t-SNE was used to visualize the similarity between cells. Next, cell types were assigned to these clusters based upon the expression of pre-defined marker genes, and then identified cluster markers by finding the differentially expressed genes in one cluster compared to all other clusters (one-vs-all). All analyzed single-cell sequencing data has been uploaded in a searchable database located at http://165.22.7.10:3838/seurat_viewer/seurat_viewer_4.Rmd. The sequencing data has been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GSE147075).

Subclustering

Reclustering was performed in Seurat (version 3). Briefly, cells from each original cluster of interest were used to create new Seurat objects consisting of only cells from the clusters of interest. Next, the count data was split by sex and re-normalized with the command: "NormalizeData(object, verbose = FALSE, normalization.method = "LogNormalize", scale.factor = 10000, assay = "RNA")". The Seurat function "FindVariableFeatures" with the "vst" method was used to identify the 2,000 most variable genes in each sex. Next, FindIntegrationAnchors was applied with dims = 1:30 to integrate the data with CCA using the first thirty CC vectors. "IntegrateData" was called

to integrate the two sexes. Next, the call: “ScaleData(object, scale.max=10, model.use="linear", use.umi=FALSE, do.scale=TRUE, do.center=TRUE, block.size=1000, min.cells.to.block=3000,verbose = FALSE)” was used to re-center and scale the counts matrix for each new Seurat object representing a cluster. “RunPCA(object, npcs = 30, verbose = FALSE)” was used to perform PCA, and “RunTSNE(object,dims.use = 1:14, max_iter=2000)”, “FindNeighbors(object, reduction = "pca", dims = 1:14)”, and “FindClusters(object, resolution = 0.6)” were called to identify new sub-clusters. Visualizations were performed using the Seurat function “VlnPlot”.

Multiplex RNAscope (Mouse)

C57B/6J mice (8-10 weeks) from Jackson were anesthetized with pentobarbitol and subsequently perfused with ice-cold 5 units/mL heparin in saline for approximately 1 minute. Brains were rapidly dissected, flash frozen in OCT (Fisher Healthcare, 4585), and stored at -80°C until further processing. Frozen tissue was cut sagittally (15µm), immediately slide-mounted, allowed to dry for approximately one hour at -20°C and then stored at -80°C. All tissue was used within three months of dissection.

Tissue was processed using the V1 RNAscope Fluorescent Multiplex Reagent Kit (Advanced Cell Diagnostics, 320850) according to manufacturer’s instructions. Briefly, tissue was fixed for 15 minutes in 10% buffered formalin (Fisher Scientific, 23-245685) at 4°C, dehydrated, and then incubated in Protease IV (Advanced Cell Diagnostics, 320850) at room temperature for 30 minutes. Target probes were hybridized to the tissue for two hours at 40°C, followed by hybridization of AMP1-FL (30 minutes, 40°C), AMP2-FL (15

minutes, 40°C), AMP3-FL (30 minutes, 40°C), and AMP4-FL (15 minutes, 40°C). Samples were counterstained with supplied DAPI or Hoechst 33342 (1:700, ThermoFisher Scientific, H3570) and mounted on slides using ProLong Glass Antifade Mountant (ThermoFisher, P36980). The following target probes were used: *Olig1* (Advanced Cell Diagnostics, 480651-C2), *Olig2* (Advanced Cell Diagnostics, 447091-C2), *Pdgfrα* (Advanced Cell Diagnostics, 480661), Clusterin (Advanced Cell Diagnostics, 427891-C3), *Gpr17* (Advanced Cell Diagnostics,318131-C3), RNAscope 3-plex Positive Control Probes (Advanced Cell Diagnostics, 320881), and RNAscope 3-plex Negative Control Probes (Advanced Cell Diagnostics, 320871). Sections were imaged using a Leica TCS SP8 confocal microscope.

RNAscope Quantification

Following imaging, max projected confocal images were analyzed using CellProfiler Software. RNA expression per cell was quantified using a modified version of a previously published pipeline[159]. Briefly, automated steps were used to draw nuclear masks and subsequently quantify the number of RNA puncta from each channel that colocalized with each nuclear mask. Threshold values for each channel were set based on negative control images. Automatic nuclear identification was reviewed and any nuclear mask that clearly contained a large group of nuclei or was located on the edge of an image such that part of the nuclei was not visible was excluded from further analysis. Cells were considered positive for an OPC marker (*Pdgfra*, *Olig1*, or *Olig2*) if four or more puncta colocalized with a particular nucleus to account for background in the assay [160]. OPCs were defined by the co-expression of two canonical OPC transcripts encoding for cell

surface markers (*Pdgfra* or *Cspg4*) and oligolineage transcription factors (*Olig1* or *Olig2*). The number of transcripts of cluster markers clusterin or *Gpr17* were recorded for each identified OPC. OPCs were considered *Clu*- or *GPR17*- if they contained 10 or fewer puncta.

Multiplex RNAscope (Human)

Human tissue was embedded in paraffin and cut into 15µM sections. Slices were heated for 48 hours to allow attachment. Tissue was subsequently processed using the V2 RNAscope Fluorescent Multiplex Reagent Kit (Advanced Cell Diagnostics, 323100) according to manufacturer's instructions. Briefly, tissue was dehydrated using xylene and ethanol wash, treated with H_20_2, and then incubated in Target Retrieval Buffer at 95°C for 15 minutes. Tissue was incubated with the supplied Protese Plus regent for 30 minutes. Target probes were hybridized to the tissue for two hours at 40°C, followed by hybridization of AMP1-FL (30 minutes, 40°C), AMP2-FL (15 minutes, 40°C), AMP3-FL (30 minutes, 40°C), and AMP4-FL (15 minutes, 40°C). Tissue was then treated with an HRP reagent for a single probe, followed by a unique secondary, and an HRP blocker. This process was repeated for each probe used. Samples were counterstained with supplied DAPI or Hoechst 33342 (1:700, ThermoFisher Scientific, H3570) and mounted on slides using ProLong Glass Antifade Mountant (ThermoFisher, P36980). The following target probes were used: Human *OLIG2* (Advanced Cell Diagnostics, 424191-C2), Human *PDGFRA* (Advanced Cell Diagnostics, 604481), Human *CLU* (Advanced Cell Diagnostics, 584771-C3), and the RNAscope 4-plex Negative Control Probes (Advanced Cell Diagnostics, 321831). The following dyes were used: Opal 520 (Akoya Biosciences,

FP1487001), Opal 690 (Akoya Biosciences, FP1497001), and Opal 620 (Akoya Biosciences, FP1495001). Sections were imaged using a Leica TCS SP8 confocal microscope.

Immunohistochemistry (Human)

5 μm thick tissue sections of formalin fixed, paraffin embedded (FFPE) brain tissue specimens were rehydrated in Xylene and descending alcohol series and heat-induced epitope retrieval (HIER) was performed in a pressure cooker (Tintoretriever, Bio SB) for 15 min at high pressure in a 0.05% Tween-20 solution. Endogenous peroxidase was quenched by incubation of sections in 1.5% hydrogen peroxide/0.005% Triton-X-100 diluted in pH 7.4 sterile phosphate buffered saline (PBS) (Invitrogen) for 20 min, following multiple washes in tap water and subsequently, 0.1 M Tris, pH 7.6. Non-specific antibody binding was minimized with sections incubated in 2% FBS/0.1 M Tris, pH 7.6. Clusterin (Proteintech) primary antibody was diluted in 2% FBS/0.1 M Tris, pH 7.6 at a dilution of 1:500. Sections were incubated with primary antibody over night at 4°C, washed one time in 0.1 M Tris, pH 7.6, followed by 2% FBS/0.1 M Tris, pH 7.6 for 5 min, incubated in goat ant-rabbit IgG HRP Conjugated secondary antibody (Millipore Sigma) for 1 hour, additionally washed one time in 0.1 M Tris, pH 7.6, followed by 2% FBS/0.1 M Tris, pH 7.6 for 5 min, and incubated in VectaStain ABC Peroxidase HRP Kit (diluted in 2% FBS/0.1 M Tris, pH 7.6 at 1:1000) for 1 hour. After a final wash in 0.1 M Tris, pH 7.6 for 5 min, immunocomplexes were visualized using the Vector Laboratories ImmPACT DAB Peroxidase (HRP) 3,3′-diaminobenzidine. Tissue sections were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO) for 2 minutes, dehydrated in ascending

alcohol series and Xylene and cover slipped using Cytoseal 60 mounting medium (Thermo Scientific). For analysis of stains, slides of frontal cortex specimens stained with Clusterin antibodies were scanned on an Aperio AT2 scanner (Leica biosystems) at 20x magnification and digital slides analyzed using the QuPath platform (version 0.3.1, https://QuPath.github.io/ , PMID: 29203879) on a Dell PC (Intel® Xeon® W-1270 CPU @ 3.40GHz/ 64 GB RAM/ 1 TB SSD Hard Drive) running Windows 10. Cortex and white matter were annotated for regional analysis. After exclusion of tissue and staining artifacts we used the 'Positive Pixel Detection' tool (Downsample factor 2, Gaussian sigma 1 µm, Hematoxylin threshold ('Negative') 1.5 OD units, DAB threshold ('Positive') 0.5 OD units) to determine the percentage of area covered by Clusterin staining.

Combined in situ Hybridization and Immunohistochemistry (Human)

For in-situ hybridization, 5µm thick paraffin-embedded tissue sections on slides were rehydrated in xylene and series of ethanol solutions (100%, 90%, and 70%). Following air drying for 5 min at RT, slides were incubated with RNAscope® Hydrogen peroxide for 10 min at RT, followed by 3 washing steps in distilled water. Antigen retrieval was performed in a steam bath for 15 min using RNAscope® 1x target retrieval reagent. After a rinse in distilled water and incubation in 10% ethanol for 3 min slides were air dried at 60°C. Subsequently, slides were incubated with RNAscope® Protease plus reagent for 30 min at 40°C in a HybEZ™ oven, followed by 3 washes in distilled water. Slides were then incubated with the following RNAscope® probe for 2 hours at 40°C in a HybEZ™ oven: Hs-Clusterin (cat. number 606241). Following washes with 1X Wash buffer, slides were incubated with RNAscope®AMP1 solution for 30 min at 40°C. Subsequent

incubations with other RNAscope® AMP solutions, followed each by two washes with 1x RNAscope® Wash buffer were completed as follows: AMP2 – 15 min at 40°C; AMP3 – 30 min at 40°C; AMP4 – 15 min at 40°C; AMP5 – 30 min at RT and AMP6 – 15 min at RT. After two washes in 1x RNAscope® wash buffer slides were incubated in RNAscope® Fast RED-B and RED-A mixture (1:60 ratio) for 10 min at RT, followed by two washes in tap water. For in situ hybridization/immunohistochemistry double labeling, sections were incubated in 2% FBS/0.1 M Tris, pH 7.6 for 5 min following RNAscope® Fast RED incubation and two washes in tap water. Primary antibodies were diluted in 2% FBS/0.1 M Tris, pH 7.6 at the following dilutions: Ab5 (PMID: 16341263), 1:1000. Sections were incubated with primary antibody over night at 4°C, washed two times in 0.1 M Tris, pH 7.6 for 5 min each and incubated with biotinylated secondary antibody (Vector Laboratories; Burlingame, CA) diluted in 2% FBS/0.1 M Tris, pH 7.6 for 1 hour at room temperature. An avidin-biotin complex (ABC) system (Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA) was used to enhance detection of the immunocomplexes, which were visualized using the chromogen 3,3′-diaminobenzidine (DAB kit; KPL, Gaithersburg, MD). Tissue sections were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO), air dried at 60°C for 30 min and cover slipped using EcoMount™ mounting medium (Biocare Medical).

RT-qPCR

RNA was extracted from samples using the ISOLATE II RNA Micro Kit (Bioline, BIO-52075) or the ISOLATE II RNA Mini Kit (Bioline, BIO-52073). Isolated RNA was reverse transcribed using the SensiFAST cDNA Synthesis Kit (Bioline, BIO-65054) or the iScript

cDNA Synthesis Kit (Bio-Rad, 1708891). RT-qPCR was performed using the SensiFAST Probe No-ROX Kit (Bioline, BIO-86005) and TaqMan probes for *Gapdh* (ThermoFisher, Mm99999915_g1), *Mbp* (ThermoFisher, Mm01266402_m1 and Mm01266403_m1), *Plp1* (ThermoFisher, Mm00456892_m1), *Cnp* (ThermoFisher, Mm01306641_m1), *Myrf* (ThermoFisher, Mm01194959_m1), and *Clu* (ThermoFisher, Mm00442773_m1). Additionally, the SensiFAST SYBR No-ROX Kit (Bioline, BIO-98005) was used with primers for *Plp1* (Forward: GCCCCTACCAGACATCTAGC, Reverse: AGTCAGCCGCAAAACAGACT) and *Myrf* (CGGCGTCTCGACAGCCTCAA, Reverse: GACACGGCAAGAGAGCCGTCA). Data was collected using the CFX384 Real-Time System (Bio-rad).

RNA Flow Cytometry

Isolated cells were stained according to the PrimeFlow RNA Assay kit (ThermoFisher Scientific, 88-18005-204). Probes used include *Olig2* (Affymetrix, VPFVKKV-210), *Gpr17* (Affymetrix, VPGZE6T-210), *Clu* (Affymetrix, VB10-3283998-210) and *Pdgfra* (Affymetrix, VB6-3197712-210). A probe targeting *Actb* (Affymetrix, VB1-10350-210) was used as a positive control to ensure good RNA quality. Samples were run using a 16-color Life Technologies Attune Nxt flow cytometer and data was analyzed using FlowJo software.

Statistical Analysis

Statistical analysis of all data (except single-cell sequencing data) was done using Prism 9 (Graphpad software). Significance was set at $p<0.05$.

Chapter III: Oligodendrocyte progenitor cells in the adult brain are transcriptionally diverse

Abstract

Oligodendrocyte progenitor cells (OPCs) account for approximately 5% of the adult brain and have been historically studied for their role in myelination. In the adult brain, OPCs maintain their proliferative capacity and ability to differentiate into oligodendrocytes throughout adulthood, despite the fact that relatively few mature oligodendrocytes are produced post-developmental myelination. Recent work has begun to demonstrate that OPCs likely perform multiple functions in both homeostasis and disease and can significantly impact behavioral phenotypes such as food intake and depressive symptoms. However, the exact mechanisms through which OPCs might influence brain function remains unclear. In this work, we demonstrate that adult OPCs are transcriptionally diverse and separate into two distinct populations in the homeostatic brain. These two groups show distinct transcriptional signatures and enrichment of biological processes unique to individual OPC populations. We have validated these OPC populations using multiple methods, including multiplex RNA in situ hybridization and RNA flow cytometry. This study provides an important resource that profiles the transcriptome of adult OPCs and will provide a significant foundation for further investigation into novel OPC functions.

Introduction

First described in the early 1980's, oligodendrocyte progenitor cells (OPCs) are the fourth major glial subtype present in the brain. Depending on the region examined, OPCs makeup anywhere from 2-8% of the cells in the adult central nervous system (CNS)[6,26]. Adult OPCs belong to the same population of progenitors that give rise to oligodendrocytes during CNS development. However, a large fraction of OPCs do not differentiate, but instead remain in a progenitor state throughout adulthood, a property not consistent with the relatively small need to generate new oligodendrocytes[9,26,27,32,33]. While it has been demonstrated that the differentiation of OPCs into myelinating oligodendrocytes is critical for processes such as motor learning during adulthood, recent evidence indicates that mature oligodendrocytes are a relatively stable population in the adult brain[40,41,60]. The slow rate at which oligodendrocytes are replaced throughout life does not correlate with the maintenance of a highly dynamic and energetically costly population of OPCs[39-41]. With this discordance between the dynamics of the OPC population and the relatively small need for newly differentiated oligodendrocytes in adulthood, the field has begun to explore alternate functions of adult OPCs[1,161].

Under homeostatic conditions, OPCs express distinct ion channel profiles that vary with both the brain region and developmental stage of the organism, indicating that subpopulations of OPCs maintain unique electrical properties and therefore may be performing multiple functions within the brai[162]. Furthermore, loss of OPCs, either globally or regionally, has been shown to result in significant depressive-like behavior, persistent weight-gain and leptin-insensitivity, as well as microglial activation and

subsequent neuronal death[3-5]. In pathological conditions, OPCs can upregulate cytokine production in response to IL-17 signaling and greatly contribute to CNS pathogenesis[132]. Surprisingly, OPCs also upregulate antigen presentation machinery in the demyelinating CNS and can regulate T cell function[163-165]. Taken together, these studies illustrate the dynamic role OPCs can play in the adult CNS and build a strong case in support of exploring adult OPCs diversity at the transcriptional level. A profiling of the overall transcriptional profiles of adult OPCs will provide an important resource for further functional investigation of OPCs in the CNS.

Here, we have developed an inducible OPC reporter mouse strain, which expresses YFP in PDGFRα-expressing cells after tamoxifen administration. After validation of the model, we used this tool to isolate OPCs from the adult brain by fluorescent activated cell sorting (FACS) and perform single-cell sequencing. We demonstrate the presence of two novel populations of transcriptionally distinct OPCs in the adult brain. Gene Ontology (GO) term analysis and gene expression analysis of identified OPC subtypes support specialization of OPCs, encompassing potential functions such as extra-cellular matrix modulation and neuronal regulation. Sequencing results were validated in vivo by measuring co-expression of canonical OPC markers along with cluster-specific genes identified from our sequencing dataset using RNAscope and qPCR. Taken together our results present a unique toolbox to support functional exploration of OPCs under homeostatic and pathological conditions.

Results

Validation of Inducible OPC Reporter Mouse Line

In order to label oligodendrocyte progenitor cells (OPCs) in the adult mouse brain with as little off-target labeling as possible, we utilized a PDGFRα-Cre^{ER}; R26-EYFP mouse line [9]. Animals were injected with tamoxifen at six weeks of age to avoid labeling the pool of OPCs that differentiate into oligodendrocytes during developmental myelination [45]. Two tamoxifen injections were sufficient to label the majority (93.56% ± 1.98) of OPCs in the brain (Fig. 1A, C). Immunohistochemistry revealed that yellow fluorescent protein positive

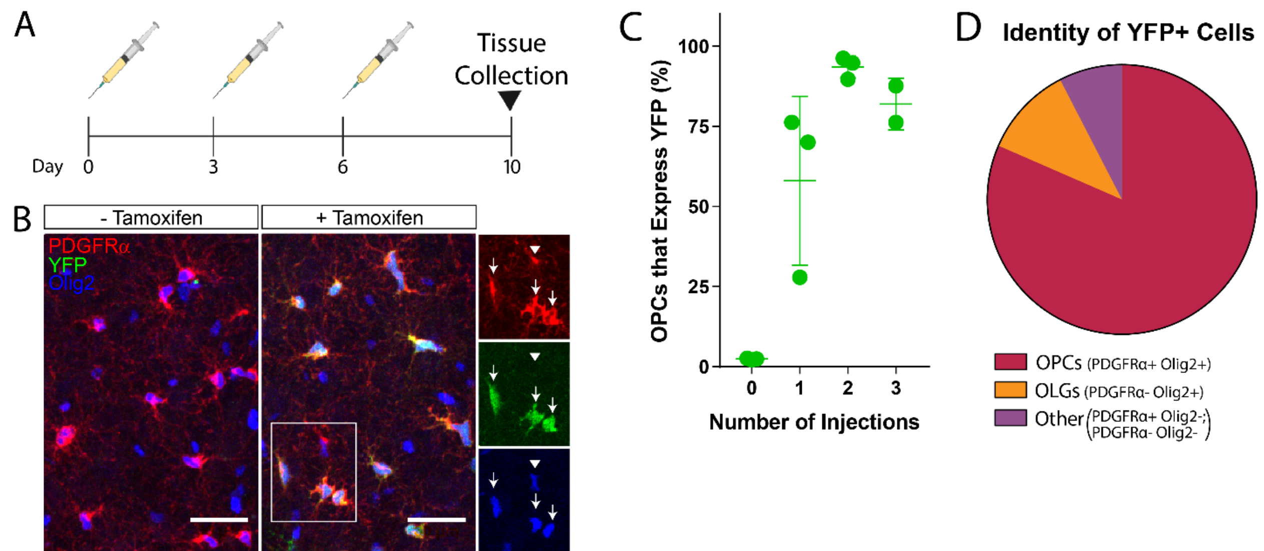

Figure 1: Validation of PDGFRα-Cre^{ER}; R26-EYFP reporter mouse. (A) Timeline of tamoxifen injections and tissue harvest used to validate YFP expression in OPCs and titrate optimal tamoxifen dosing paradigm. (B) Immunofluorescence of PDGFRα (red), Olig2 (blue), and YFP (green) in PDGFRα-Cre^{ER}; R26-eYFP mice receiving no tamoxifen injections (- Tamoxifen) or 2 tamoxifen injections (+Tamoxifen). Arrows represent OPCs expressing YFP and arrowheads represent OPCs lacking YFP expression. Scale bar = 30μM. (C) Percentage of OPCs (PDGFRα+/Olig2+) that also express YFP following 0, 1, 2, or 3 tamoxifen injections. N=3 independent experiments, n=2-3 per group. Error bars represent SEM. (D) Proportion of YFP+ cells identified as OPCs (PDGFRα+/Olig2+), Oligodendrocytes (OLG, PDGFRα-/Olig2+), or neither of these cell types (Other, PDGFRα+/Olig2- or PDGFRα-/Olig2-) following 2 tamoxifen injections. N=2 independent experiments, n=3 samples. Data quantified in (C) and (D) include images from the prefrontal cortex, hippocampus, and corpus callosum.

cells (YFP+) following two tamoxifen injections were composed of OPCs (81.5%; PDGFRα+/Olig2+), mature oligodendrocytes (10.9%, PDGFRα-/Olig2+) or other cell types not belonging to the canonical oligolineage (7.6%, PDGFRα-/Olig2- or PDGFRα+/Olig2-), presumably endothelial cells, as they are also known to express PDGFRα (Fig. 1B,D) [166].

Isolation and Sequencing of YFP+ Cells

Whole brains, including the cerebellum but excluding the spinal cord, were collected from adult PDGFRα-Cre^{ER}; R26-EYFP mice and processed into a single-cell suspension for FACS. Four to five brains were pooled for each sample, and male and female brains were processed separately to allow for analysis of potential sex differences in YFP+ cells (Fig. 2A). YFP+ cells were selected by gating on live cells while excluding immune (CD45+) and red blood cells (TER119+), thus ensuring that the population of YFP+ cells collected were viable and highly enriched (Fig. 2B). YFP+ cells were barcoded and prepared for

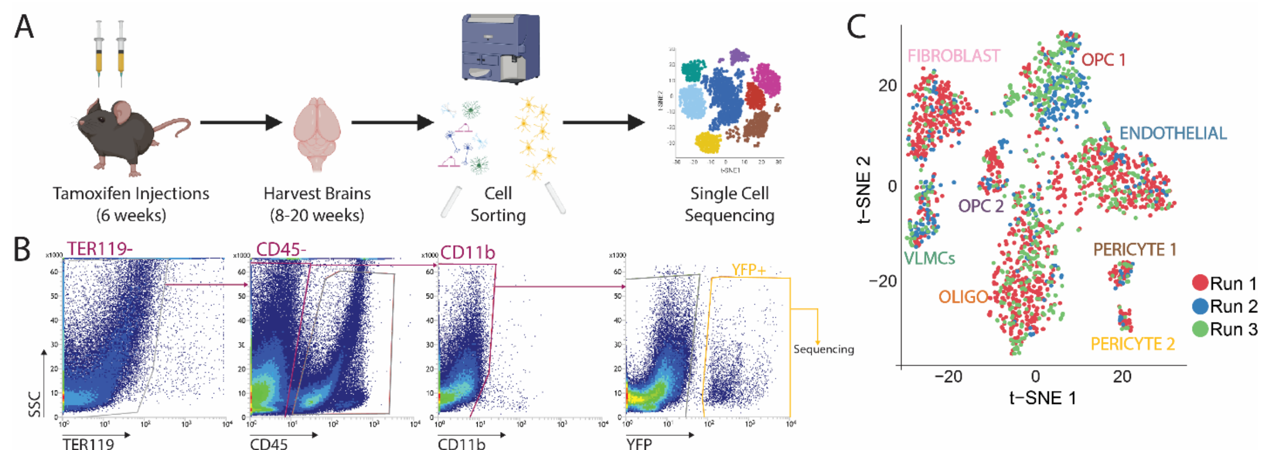

Figure 2: Isolation of YFP+ cells from PDGFRα-creERT2 x YFP brains for sequencing. (A) Experimental strategy used for the isolation and single-cell sequencing of cells analyzed in Figure 1. (B) Gating strategy for YFP+ cell sorting following Live/Scatter/Singlet gating. (C) tSNE map depicting cell clusters colored by sequencing run.

single-cell sequencing using Next GEM reagents and Chromium microfluidics supplied by 10x Genomics. Cell sorting and sequencing was performed three independent times for a total of 6 independently sequenced samples. Unbiased clustering of each independent run revealed overlap between distinct sequencing runs and no clustering of cells driven by sequencing run alone (Fig. 2C). For all future analysis, all sequencing runs were combined to form one dataset.

Profiling the Molecular Signature of OPCs in the Adult Brain

Unbiased clustering of sequenced cells using the Seurat package [156,157] revealed that cells sorted from PDGFRα-Cre^{ER}; R26-EYFP brains clustered into 8 distinct populations (Fig. 3A). Mature oligodendrocytes comprised one cluster, having potentially differentiated following initial tamoxifen labeling of PDGFRα expressing progenitor cells. Also captured in the sequencing were four cell types outside the oligolineage that are known to either express PDGFRα or come from PDGFRα expressing precursors, including one population of fibroblasts, one population of endothelial cells, one population of vascular and leptomeningeal cells (VLMCs), and two populations of pericytes [59,166-169]. These clusters were identified by expression of known cell type markers such as *Igfbp6* and *Fn1* (fibroblasts), *Tek*, *Pecam1*, and *Kdr* (endothelial cells), *Lum*, *Col1a2*, and *Col3a1* (VLMCs), as well as *Rgs5*, *Pdgfrb*, and *Des* (pericytes) (data not shown) [59,170-173]. The remaining 2 clusters of cells (OPC1 and OPC2) expressed at least 2 of the 5 canonical OPC markers *Ptprz*, *PDGFRα*, *Olig1*, *Olig2*, and *Cspg4* (Fig. 3B) [15-18,174]. Importantly, each OPC population expressed a unique transcriptional signature distinct from the gene expression in every other cluster (Fig. 3C).

In order to further investigate how these clusters of OPCs are distinct from one another, we identified a significantly upregulated gene from each cluster that offered potential indications of distinct functions of these subpopulations (Fig. 3D). OPC1 expressed high levels of *Clusterin*, a multifunctional protein that has been identified as a risk factor for late-onset Alzheimer Disease (LOAD) [175]. OPC2 were positive for G-protein coupled

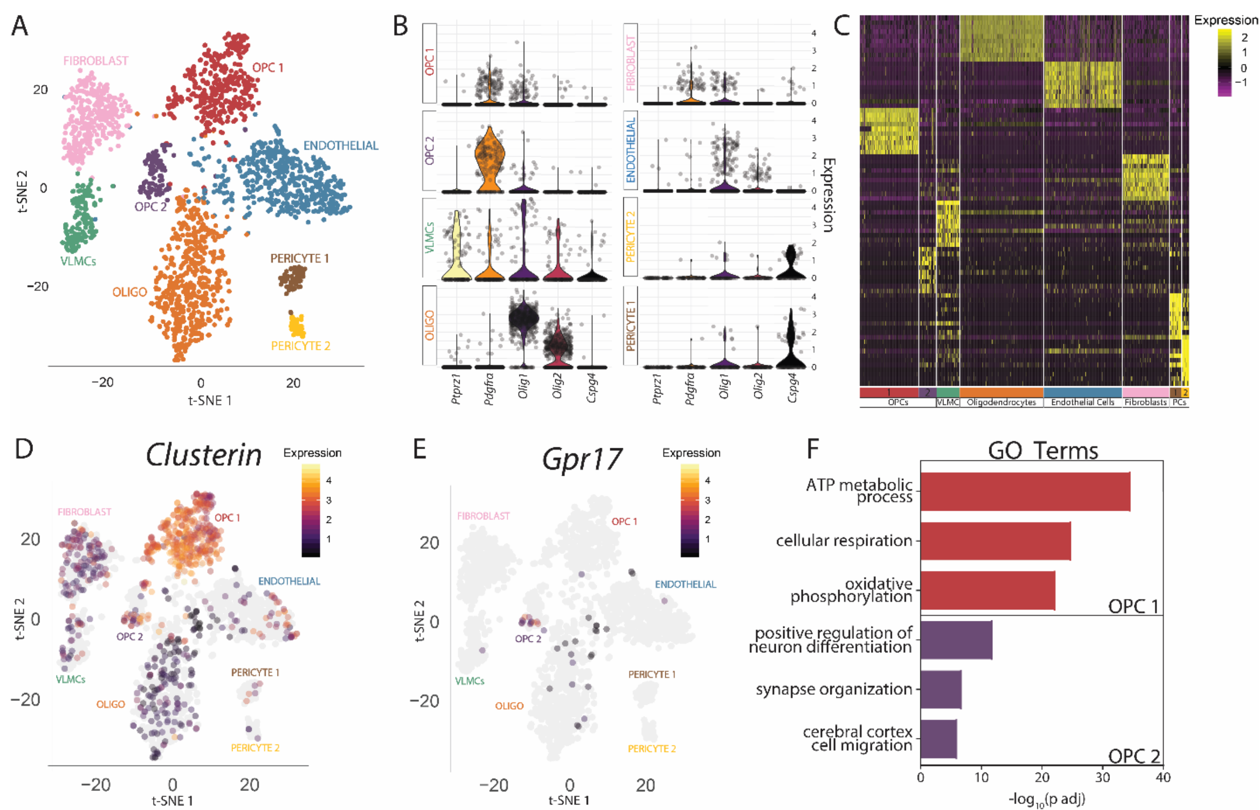

Figure 3: Adult OPCs cluster in two distinct subpopulations. (A) tSNE plot of all sequenced cells isolated from PDGFRα-Cre^{ER} reporter brains. Clusters were labeled with cell-type classifications based on expression of common cell-type markers. N=3 independent experiments, n=6 samples. (B) Violin plots depicting expression of common OPC markers in each cluster. Each dot represents a cell. Expression value is plotted on the y-axis. (C) Heatmap depicting the scaled and log-normalized expression values of the top 10 most highly enriched genes in each cluster. (D) Cell-specific expression of markers used for cluster validation including *clusterin* (OPC1) and *Gpr17* (OPC2) overlaid on tSNE map. (E) Significant GO terms uniquely upregulated in one OPC cluster compared to each OPC cluster and VLMCs. PC=Pericytes.

receptor *Gpr17*, the only currently known marker of molecular diversity in OPCs, when compared to all other cell types sequenced (Fig. 3D) [84,88].

Lastly, in order to gain insight into how these subpopulations of OPCs potentially differ at the functional level, we analyzed GO terms that were unique to each OPC cluster (Fig. 3E). OPC1 showed upregulation in genes involved in ATP metabolic processes, cellular respiration, and oxidative phosphorylation, including *Chchd10*, *Mdh1*, and *Uqcrq*. OPC2 was characterized by the upregulation in genes involved in the positive regulation of neuron differentiation, synapse organization, and cerebral cortex cell migration, including *Stmn2*, *Pfn2*, and *Dcx*. Searchable gene expression data for these clusters is available on our website (http://165.22.7.10:3838/seurat_viewer/seurat_viewer_4.Rmd). In sum, our single sequencing data reveal two unique subpopulations of OPCs that reside in the adult brain under homeostatic conditions and support functional diversity based on unique gene expression profile.

Subclustering Further Elucidates Potential Functions of OPC Clusters

Since the inclusion of multiple cell types in a single-cell sequencing dataset has the potential to mask transcriptional differences between subsets of one cell type, we decided to reanalyzed each cluster of OPCs individually on order to bypass this problem. This additional analysis identified 3 subclusters in OPC1 (OPC1a, OPC1b, OPC1c; Fig 4A). However, OPC1a and OPC1b were very transcriptionally similar, and we believe that together they represent one functional subcluster within OPC1. This analysis also identified 2 subclusters in OPC2 (OPC2a, OPC2b; Fig 4C). Analysis of genes significantly upregulated in each cluster further highlighted potential alternative functions for each

subset of OPCs. For example, OPC1a and OPC1b highly express genes related to metabolic activity (*Cox5b*, *Ndufb3*, *Atp5g2*; Fig 4B) and OPC1c shows significant upregulation of genes related to the regulation of the extracellular matrix (*Adamts1*,

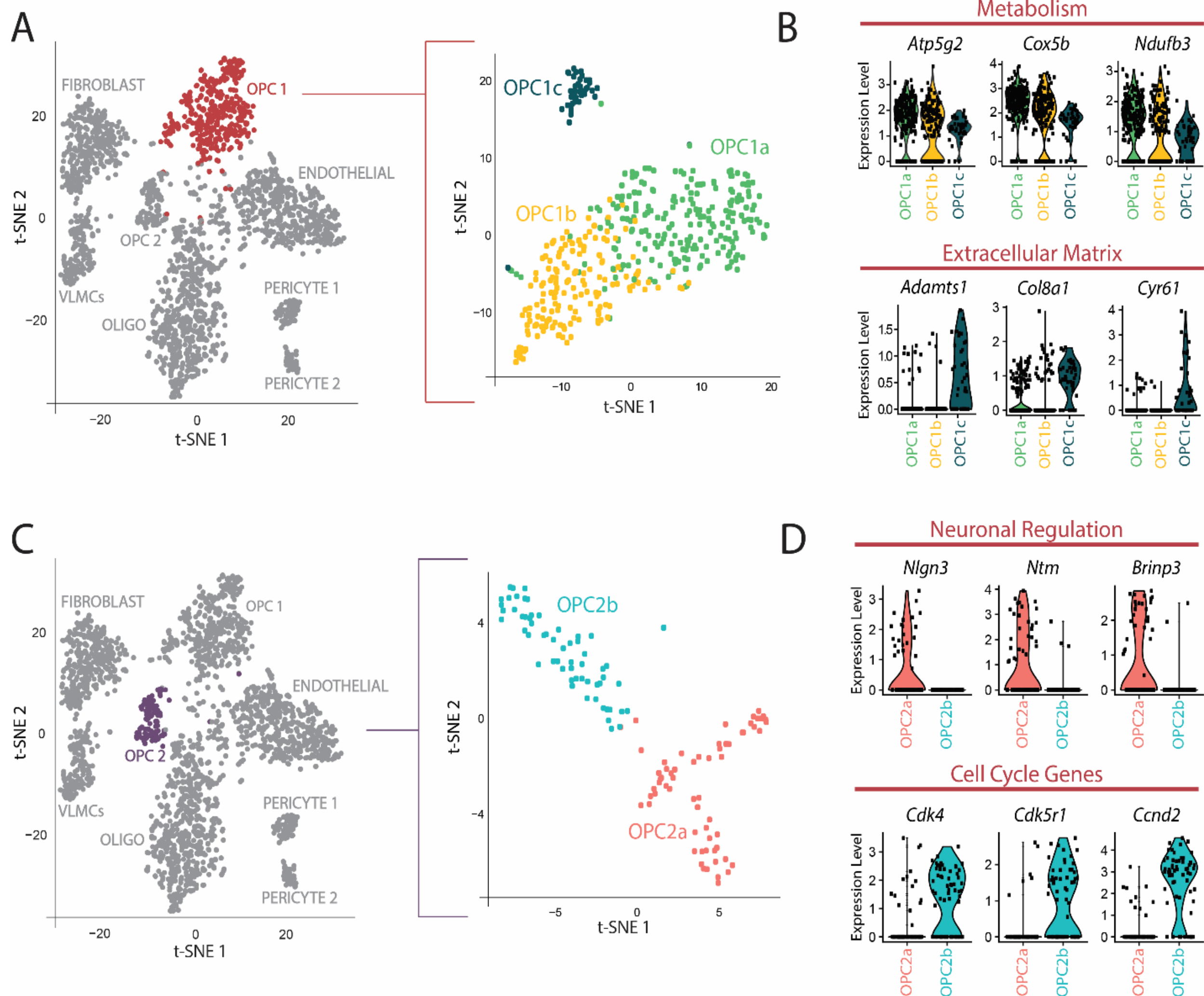

Figure 4: Subclustering reveals potential functions of OPCs. (A) Subclustering of OPC1 into 3 subclusters (OPC1a, OPC1b, and OPC1c). (B) Violin plots depicting expression of genes related to metabolism that are significantly upregulated in OPC1a and genes related to the extracelluar matrix that are significantly upregulated in OPC1c. Each dot represents a cell. (C) Subclustering of OPC2 into 2 subclusters (OPC2a and OPC2b). (D) Violin plots depicting expression of genes related to neuronal regulation that are significantly upregulated in OPC2a and genes related to the cell cycle that are significantly upregulated in OPC2b. Each dot represents a cell.

Col8a1, *Cyr61*; Fig 4B). Similarly, OPC2a expresses genes related to neuronal regulation (*Nlgn3*, *Ntm*, *Brinp3*; Fig 2D) and OPC2b expresses genes that are known to regulate the cell cycle (*Cdk4*, *Cdk5r1*, *Ccnd2*; Fig 4D) and are likely the actively proliferating subset of OPCs that that have been previously described [169]. Further subsetting our clusters of OPCs into transcriptionally distinct subclusters could lay the foundation for a more targeted exploration of novel functions of OPCs.

Sex Differences in OPCs

Since sex differences have been reported in multiple types of glia including microglia and astrocytes, we investigated whether OPCs isolated from male and female mice were different [176,177]. Cells from male and female animals were present in all 8 clusters (Fig. 5A). However, while not statistically significant due to the limited replications inherent in single-cell sequencing experiments, cells from each sex were not equally distributed into each cluster. Indeed, males exhibited a higher frequency of cells in OPC1 while cells from

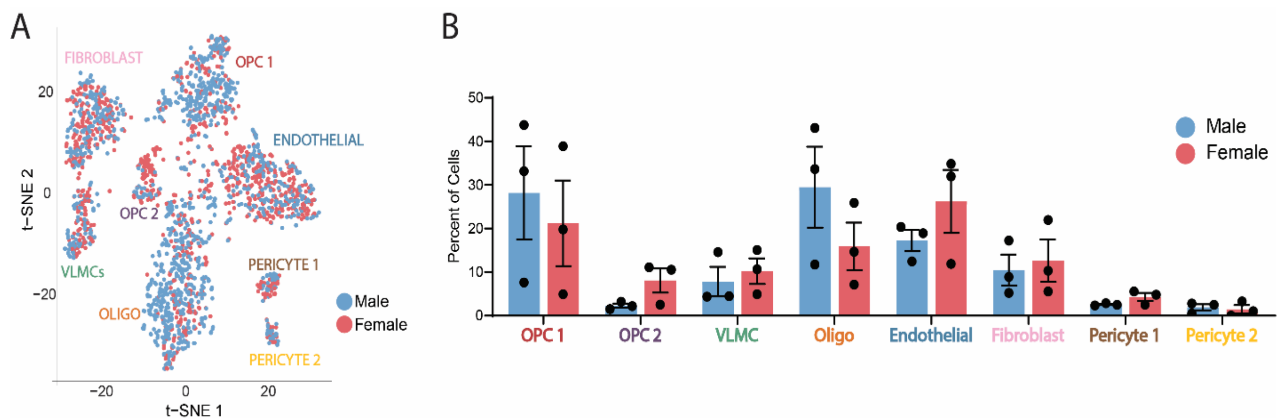

Figure 5: The transcriptional profile of OPCs does not seem to be different between sexes. (A) tSNE map depicting cell clusters colored by sequencing sex. (B) Percent of male and female cells that fall within each cluster. Each bar represents the percentage of all male (blue) or female (red) cells sequenced.

females were present at higher frequency of cells in OPC2 (Fig. 5B). Cells from males were also more abundant in the mature oligodendrocyte cluster. While the distribution of cells across OPC clusters may have sex-specific differences, the number of significantly different genes between male and female cells in each cluster fell within the range of statistical noise (data not shown). While this data indicates no sex-specific OPC signature in the adult brain, the proportions of OPC subpopulations may be sexually dimorphic and further investigation into these potential differences is warranted.

In Vivo Validation of OPC Subpopulations

Since gene expression can be altered by tissue processing before sequencing, we validated the expression of each OPC cluster marker using RNAscope in the adult mouse brain [178]. OPCs were defined by the co-expression of two canonical OPC transcripts encoding for cell surface markers (*Pdgfra* or *Cspg4*) and oligolineage transcription factors (*Olig1* or *Olig2*). Using a Cell Profiler pipeline to unbiasedly quantify RNA puncta expression per cell [159], we subsequently quantified expression of each OPC cluster gene. OPCs in both gray and white matter express a range of both *Clusterin* and *Gpr17* transcripts, with a population of cells expressing little to no RNA for these markers, a population of cells showing medium expression, and a smaller population of cells expressing very high levels of these transcripts (Fig 6A-D). We were therefore able to detect and validate, using the novel selected cluster markers and canonical OPC genes, each cluster of OPCs *in vivo*.

Expression of Canonical Markers in OPC Clusters

While single-cell sequencing provides gene expression data on an individual cell basis, its relatively shallow depth of sequencing can result in little to no detected expression of genes with known low expression, such as transcription factors [179,180]. In order to confirm that the 2 identified clusters of OPCs express canonical OPC markers using a more

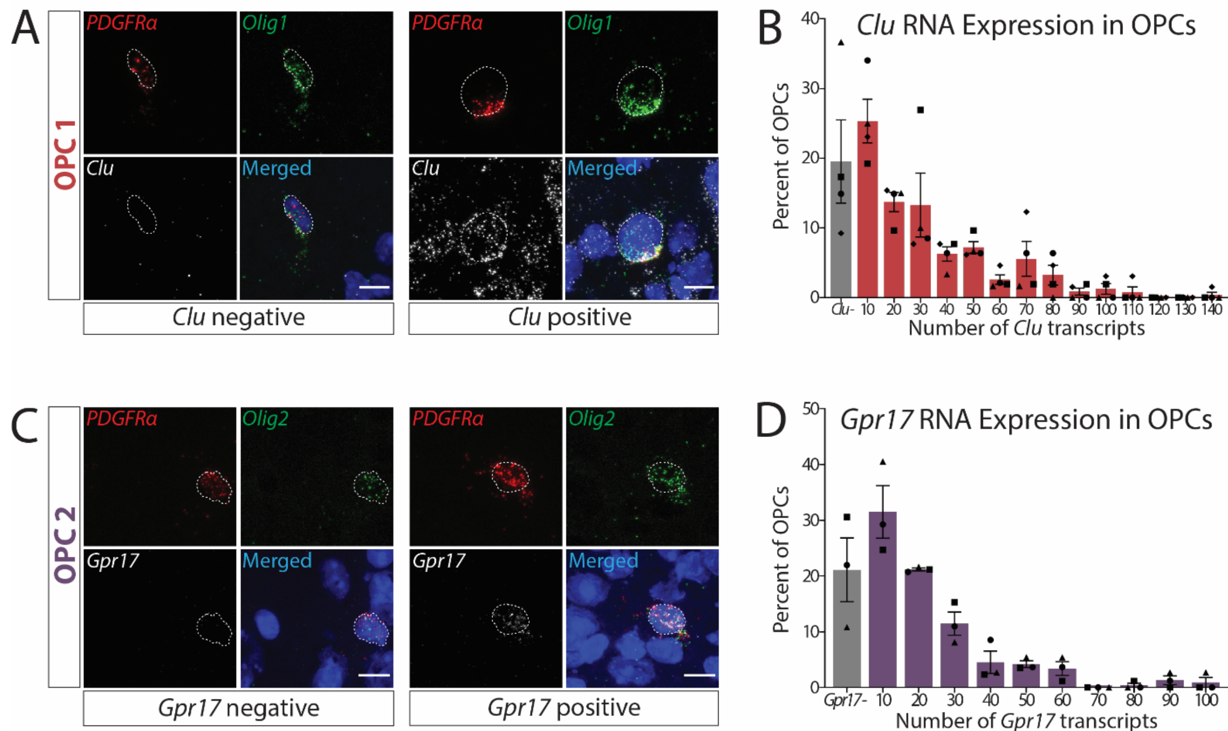

Figure 6: In vivo OPC expression of cluster markers *clusterin* and *Gpr17*. (A) RNAscope expression of *Pdgfra* (red), *Olig1* (green), *Clu* (white), and Hoechst (blue). (B) Quantification of the number of *Clu* transcripts in individual OPCs. Data plotted as a histogram of *Clu* expression in OPCs with a bin width of 10 transcripts. N=2 individual experiments, n=4 samples, with a total of 205 OPCs quantified. Each shape correlates to an individual mouse. (C) RNAscope expression of *Pdgfra* (red), *Olig2* (green), *Gpr17* (white), and Hoechst (blue). (D) Quantification of the *Gpr17* expression in OPCs with a bin width of 5 transcripts. N=3 individual experiments, n=4 samples, with a total of 247 OPCs quantified. Each shape correlates to an individual mouse. In all RNAscope experiments, OPCs were identified as cells co-expressing an OPC surface marker (*Pdgfra* or *Cspg4*) and an oligolineage transcription factor (*Olig1* or *Olig2*). Each sample includes quantification of marker expression from the cortex, hippocampus, corpus callosum, and cerebellum. Scale bar = 10μM.

sensitive method of gene detection, we individually sorted each cluster of OPCs based on expression of cell surface proteins. OPC1 was identified by expression of Aquaporin1 (Fig. 7A) and OPC2 was identified by expression of Neurexin1 (Fig. 7B). Each cluster of OPCs was sorted by FACS based on their co-expression of YFP and Aquaporin1 (for OPC1), or Neurexin1 (for OPC2) (Fig. 7C). Expression of *Olig1*, *Olig2, Pdgfra*, and *Cspg4* was then determined by qPCR. While each cluster demonstrated expression of at least 2 canonical OPC markers, each cluster demonstrated a unique combination of each of these genes (Fig. 8A). Specifically, OPC1 expresses *Olig1*, *Olig2*, and *Pdgfra* with relatively low expression of *Cspg4*, and OPC2 expresses both *Pdgfra* and *Cspg4* with relatively low expression of *Olig1* and *Olig2*. Our results offer additional validation of canonical OPC marker expression in the 2 clusters of adult OPCs identified by single-cell sequencing.

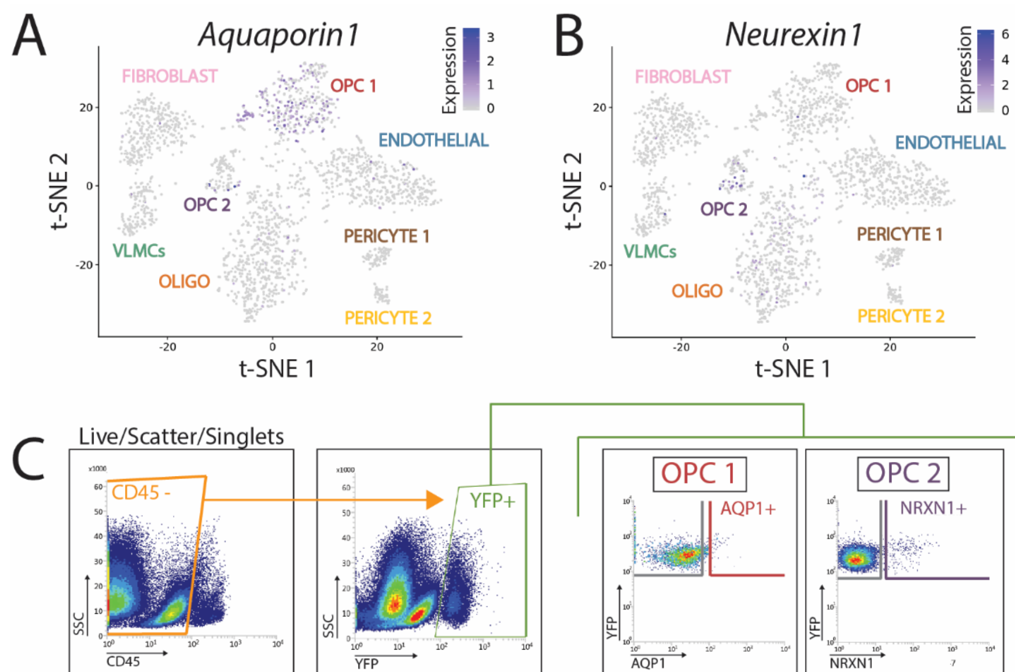

Figure 7: Isolation of Specific OPC clusters. Expression of genes used to sort out individual OPC clusters including *Aquaporin1* (A) and *Neurexin 1* (B) overlaid on tSNE map. (C) Gating strategy used to isolate OPC1 (YFP+/AQP1+) and OPC2 (YFP+/NRXN1+) clusters from brains of PDGFRα-Cre^{ER}; EYFP mice.

Clusterin and Gpr17 are exclusively expressed in OPC1 and OPC2 subsets respectively

While both RNAscope and RT-qPCR of canonical OPC markers within each cluster have demonstrated that these clusters of OPCs are expressed within the brain and belong to the oligolineage, neither of these techniques have demonstrated that these markers

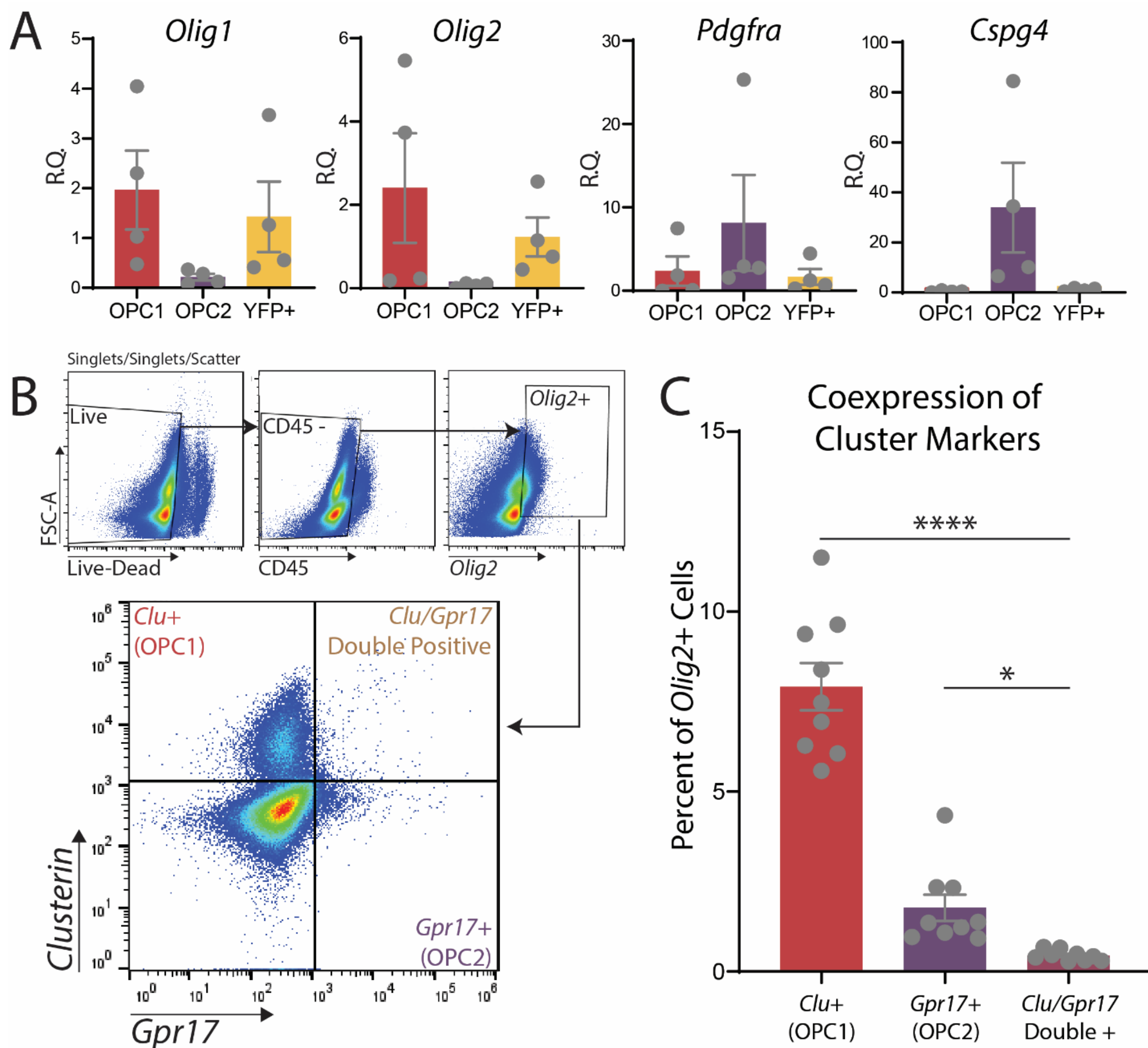

Figure 8: OPC clusters express multiple canonical OPC markers, but do not express markers of multiple clusters. (A) qPCR relative quantification of canonical OPC markers *Olig1*, *Olig2*, *Pdgfrα*, and *Cspg4* in OPC1 (YFP+/AQP1+), OPC2 (YFP+/NRXN1+), and all YFP+ cells. Values plotted are the $2^{-\Delta\Delta Cq}$ relative to the average ΔCq in all YFP+ cells. N=4 independent experiments, n=4 samples per group. (B) Representative PrimeFlow gating of brain cells stained for CD45 protein and *Clu*, *Gpr17*, *Pdgfrα*, and *Olig2* RNA. (C) Quantification of Live/CD45-/Olig2+ cells that express *Clu* alone, *Gpr17* alone, or both *Clusterin* and *Gpr17*. N=2 individual experiments, n=9 samples. Analyzed using one-way repeated measures ANOVA. ****$p<0.0001$, *$p<0.05$. Error bars represent SEM.

characterize clusters that are unique from one another. Using PrimeFlow, a technique that allows for the combination of cellular-resolution RNA detection with the multiplexing capabilities of flow cytometry, we demonstrate that a subset of *Olig2* expressing cells express *Clusterin* (OPC1), and a mutually exclusive population expresses *Gpr17* (OPC2), with very few OPCs expressing detectable levels of both cluster markers (Fig. 8B,C). While this does not rule out the possibility that an individual OPC might express genes enriched in different clusters at different times, it does demonstrate that, at any given point, genetic markers of these two clusters of OPCs largely do not overlap.

Parenchymal Fibroblasts

While our dataset contains the typical brain-resident PDGFRα-expressing cells (OPCs and endothelial cells) as well as cell types known to arise from PDGFRα-expressing progenitors (oligodendrocytes and pericytes), it also contained a cluster of fibroblasts, likely arising from the meninges as these cells expressed markers recently attributed to the dural meninges, as well as a cluster of cells transcriptionally similar to those previously defined as VLMCs [18,59,166,169,181]. VLMCs are actively expressing significant amounts PDGFRα, but do not express other markers of OPCs or endothelial cells, the most significant expressers of PDGFRα in the CNS (Fig 3B). Based on this expression and the transcriptional similarity of this VLMC cluster to a previously described cluster of “fibroblast-like” CNS-resident cells, we postulated that these cells may represent parenchymal fibroblasts [182]. To validate that this cluster of cells resides in the parenchyma and not the meninges, we used RNAscope to stain the brain for *Lumican*, one of the top genes upregulated in the VLMC cluster, as well as for *Pdgfra* and the oligolineage marker

Olig1 (Fig 9A). Indeed, we observed cells within the brain parenchyma that expressed *Lumican* and *Pdgfra*, but lacked expression of *Olig1* and are therefore not OPCs (Fig 9B). While we observed some OPCs (*PDGFRα+ Olig1+*) that expressed *Lumican*, approximately half (48.73%±10.58%) of all *Lumican+* cells expressed *Pdgfra*, but no *Olig1* (Fig 9B, C). In sum, we have demonstrated that a population of VLMCs, as identified by their co-expression of *Lumican* and *Pdgfra* but lack of *Olig1* expression, resides within the brain parenchyma and likely represents a novel population of brain-resident fibroblasts.

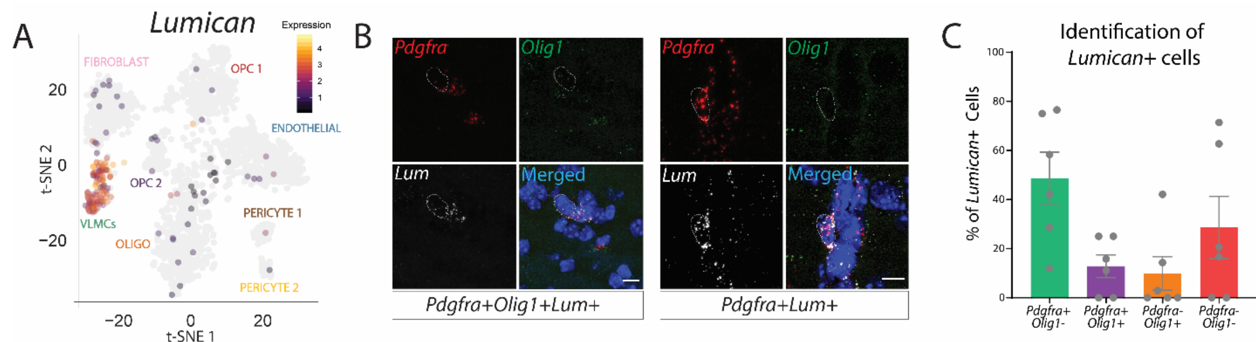

Figure 9: *Lumican+* fibroblast-like cells are found in the brain parenchyma. (A) Expression of *Lumican*, a marker of the VLMC cluster overlaid on the tSNE map. (B) Representative RNAscope images of an OPC (*Pdgfra+Olig1+)* and a fibroblast-like cell *(Pdgfra+Olig1-)* expressing *Lumican* in the brain parenchyma. (C) Quantification of the percentage of *Lumican+* cells in each mouse that also expressed *Pdgfra*, *Olig1*, both, or neither. . N=3 individual experiments, n=4 samples.

Discussion

With the development of novel tools that allow for the analysis of tissue at single-cell resolution, interest has surged in outlining how cell types that express the same canonical cell type markers may represent more diverse subpopulations than previously thought [59,183-186]. Here, we demonstrate that OPCs from the adult brain cluster into two distinct subpopulations, characterized by unique transcriptional signatures and Gene Ontology

profiles. Furthermore, we demonstrate that a population of fibroblast-like cells reside within the parenchyma.

A small number of studies have investigated the transcriptional profiles of OPCs present during development and have described relatively little transcriptional diversity. Marques and colleagues transcriptionally profiled oligolineage cells from both juvenile and adult brains and, while mature oligodendrocytes clustered into seven subpopulations, their data indicated one population of progenitor cells [59]. However, their population of sequenced OPCs was relatively small (approximately 300 cells) and the majority of their OPCs came from juvenile animals [59]. A more recent study from the same group characterized the transcriptional profiles of OPCs from E13.5, E17.5, and P7 mice and found three clusters of OPCs that shared similar transcriptional signatures, but largely clustered by the age of the cells, with one cluster of cycling OPCs [169]. From these data, they concluded that, during development, the three known waves of developmental OPCs converge into a transcriptionally homogenous group of OPCs by P7. Importantly, this sequencing dataset only profiles prenatal and early postnatal OPCs, a time window in which OPCs are preparing to generate a large population of mature oligodendrocytes to support the developmental myelination that occurs during early postnatal time points [45]. Therefore, it is likely that OPCs during this early stage of development may represent a relatively homogenous population of progenitors destined to give rise to myelinating glia [45]. However, following developmental myelination, oligodendrocytes represent a relatively stable population that require minimal replacement, yet OPCs continue to represent approximately 5% of cells in the adult brain and tile every brain region [26,187]. It is therefore

reasonable to hypothesize that as the CNS matures, and no longer requires the production of large numbers of mature oligodendrocytes, OPCs may develop diverse transcriptional repertoires, as demonstrated here, to perform alternative functions throughout adulthood. Indeed, recent data from Spitzer and colleagues demonstrated that OPCs throughout the brain express a diverse array of electrophysiological properties and ion channels which become more diverse with age [162]. Additionally, data obtained from zebrafish has demonstrated that OPCs can be categorized into two functionally distinct subpopulations that demonstrate different calcium dynamics [188]. Interestingly, one population of OPCs was found to rarely differentiate *in vivo*, although these cells maintained their differentiation capacity, indicating that the main functions of this population of OPCs is likely something other than serving as a progenitor pool for mature oligodendrocytes [188].

Additionally, recent studies have begun to investigate OPC diversity within the human brain. Single-cell sequencing data from human patients at fetal, adolescent, and adult timepoints reveal multiple transcriptionally distinct populations of oligo-lineage cells that largely clustered based on the age of the patient[189]. Another study profiled the transcriptional diversity of OPCs produced from human embryonic stem cells and found multiple distinct OPC subsets[190]. The addition of our study to the previously published datasets, detailing the transcriptional profile of developmental OPCs as well as the transcriptional state of human OPCs will provide the field with a better understanding of how OPCs might change as the brain matures. Indeed, our work highlights how the function of OPCs might shift as development ends and the brain enters adulthood.

Our in-depth analysis of canonical OPC marker expression in each OPC cluster surprisingly indicated that both OPC1 and OPC2 expressed a unique subset of common OPC markers. While PDGFRα, CSPG4, Olig1, and Olig2 have historically all been thought to be expressed in the vast majority of OPCs [191], recent data from zebrafish indicates that only approximately 80% of gray-matter OPCs and 30% of white matter OPCs express *Cspg4* transcripts [188]. This differential expression of canonical OPC markers may indicate that OPCs can express a unique array of OPC genes based on their function, and canonical OPC markers may not be as critical to OPC functioning as previously thought. This idea is supported by the recent description of the development and differentiation of a PDGFRα-independent subpopulation of OPCs [192].

One of the most prominent genes distinguishing OPC1 from OPC2 is *clusterin*. Importantly, clusterin has been shown to be upregulated in the brains of patients with Alzheimer's Disease (AD) as well as patients with Multiple Sclerosis (MS), perhaps contributing to the white matter abnormalities documented in these two CNS diseases [193,194]. More specifically, clusterin is significantly upregulated within demyelinated MS lesions compared to adjacent unaffected white matter[195,196]. OPCs have recently been implicated in both of these diseases, potentially playing an active role in the pathology of MS and serving as important players in the progression of Alzheimer's disease[132,164,197]. What's more, recent sequencing data from human Alzheimer's disease patients and healthy controls demonstrated that healthy controls have three subpopulations of OPCs, with one of them expressing high levels of c*lusterin*. OPCs from AD patients clustered

separately from the healthy controls and showed an even greater enrichment of c*lusterin* than any of the control OPC clusters[198]. Given these associations with Alzheimer's disease and MS, and the known role of clusterin in multiple CNS pathologies, interrogating the role of clusterin in OPCs and, more broadly, the function of the clusterin-expressing subset of OPCs, may be particularly important in revealing novel ways OPCs help maintain homeostasis and how they subsequently may be playing an active role in contributing to or protecting against CNS pathology[199-202].

Many of the differentially expressed genes and related biological processes found in each OPC cluster complement emerging literature that indicates non-canonical roles for OPCs during homeostasis, and a more active role of this cell type in multiple diseases. While here we describe the transcriptional profile of OPCs during homeostasis, it is important to note that understanding the role of OPCs in the healthy brain will provide a necessary foundation for examining any protective or detrimental novel functions in disease pathology. We believe that the work presented here provides a critical foundation and basis for the investigation of non-canonical roles of OPCs. This dataset will not only assist the field in discovering novel roles for OPCs in both health and disease, but can also offer potential mechanistic explanations for intriguing phenotypes observed in OPC deletion paradigms [3-5,132].

Chapter IV: Clusterin and its functions

Introduction

Clusterin, also known as ApoJ, is a glycosylated apolipoprotein originally isolated from rat testes in the early 1980's and is mostly commonly described as an extracellular chaperone[203,204]. Named for its ability to cause cells to cluster together when added to culture, numerous functions have been attributed to this protein over the last 40 years, such as inducing apoptosis and promoting inflammation (Fig. 10)[204,205]. Additionally, clusterin has been found to be upregulated in multiple disease and disorders, including multiple sclerosis, Alzheimer's disease, ischemic kidney injury, and multiple types of cancers[206-209]. However, due to its multi-functional nature, it remains unclear if clusterin is beneficial or detrimental in disease and injury conditions.

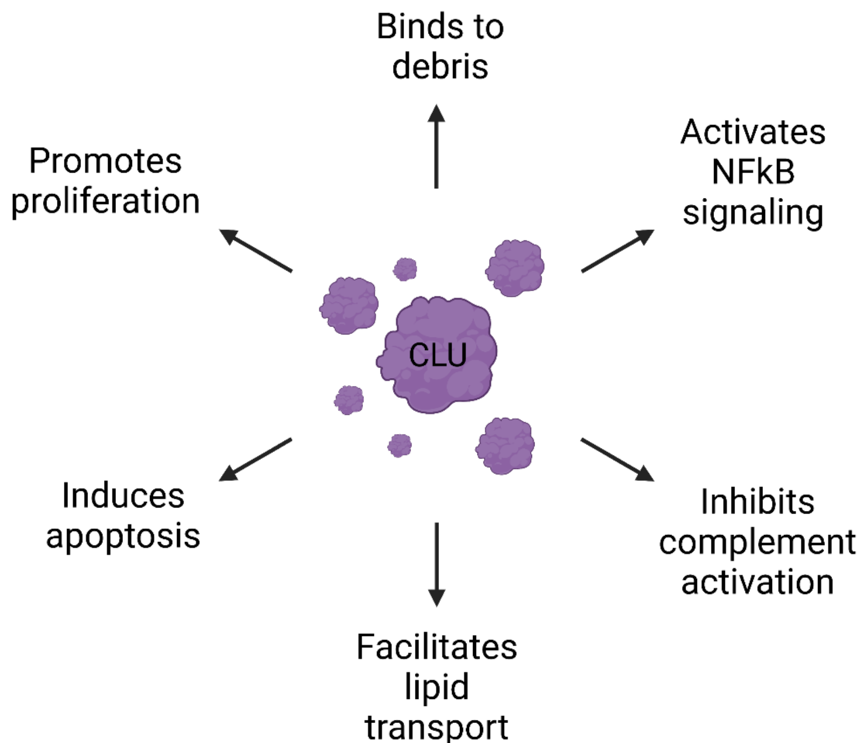

Figure 10: Graphical representation of some of the many functions of clusterin. Cartoon depicting a subset of the many, often contradicting roles of clusterin.

Clusterin was first described as a secreted protein, most commonly found extracellularly[204]. However, more recent data has demonstrated that other minor isoforms exist, including both a nuclear and cytoplasmic isoform[210,211]. While the extracellular isoform has been by-far the most well-studied, studies profiling the cytoplasmic and nuclear forms of clusterin have only made this protein more enigmatic, as these isoforms at times have the opposite function of the secreted protein [205].

Expression

Clusterin is a protein that has been conserved across all vertebrate species and is rather ubiquitously expressed in humans[212,213]. Indeed, clusterin RNA has been found in almost all tissue types and is expressed at particularly high levels in the brain, ovaries, liver, and testes[213]. Interestingly, in mice, levels of both clusterin RNA and protein have been shown to increase exponentially throughout development, with clusterin increasing from embryonic day 16 all the way through adulthood[214]. Within the brain, multiple cell types have been shown to produce clusterin. Astrocytes have been the most well-studied brain-resident cell type in terms of their production of clusterin[215]. However, other brain-resident cells, including neurons, endothelial cells, and, as described here, OPCs are also able to produce clusterin[215-218]. What's more, as the dominant isoform of clusterin is secreted into the extracellular space, clusterin expression by a single cell type can have wide-reaching effects on a variety of cells[204].

Function

Debris Clearance

Clusterin is classified as an apolipoprotein and, as such, is known to easily bind to extracellular molecules, as the main function of these proteins is to facilitate the transport of lipids and lipid-associated proteins[219]. It has been shown that the ability of clusterin to bind to extracelluar molecules allows this protein to aid in more efficient clearance of cellular debris[220]. Clusterin has been shown to bind to misfolded proteins and subsequent data demonstrates that proteins bound to clusterin are more quickly cleared than unbound proteins[220]. Clusterin has been documented to promote the clearance of insoluble Aβ, placing this protein as a central player in AD pathology[221]. Additionally, clusterin has been shown to improve the phagocytic capabilities of both professional and non-professional phagocytes alike[222,223]. Overall, this data demonstrates that clusterin is effective in assisting in extracellular debris clearance.

Immune Regulation

Clusterin has also been shown to regulate the immune system in multiple, and sometimes contradictory, ways. On one hand, clusterin has been shown to inhibit the complement cascade, which is a series of pathways used by the immune system to effectively respond to external pathogens[224,225]. From this standpoint, clusterin acts as an inhibitor of the immune system, which is supported by observations demonstrating that mice lacking clusterin are more susceptible to autoimmunity, including autoimmune myocarditis and antibody-mediated arthritis[226-228]. However, clusterin has also been shown to increase pro-inflammatory signaling through its effects on NFκB signaling.

Activation of NFκB signaling induces the production of a wide variety of pro-inflammatory cytokines and recruits immune cells to the site inflammation or injury[229]. Mechanistically, clusterin accomplishes this by potentiating the degradation of COMMD1 and IκB, two proteins that serve to inhibit NFκB signaling, resulting in an overall activation of NFκB by clusterin[230]. This pro-inflammatory signaling capacity of clusterin is supported by data showing that blocking clusterin signaling in hepatocytes using shRNA reduces the production of the pro-inflammatory cytokines IL-6 and IL-8, and that exogenous clusterin has the ability to induce nuclear translocation of NFκB in macrophages[231-233]. Since the literature indicates that clusterin can play both pro-inflammatory and anti-inflammatory roles, its function is likely dependent upon a variety of factors, such as cell-type, tissue-type, disease state, and splicing isoform.

Cell Death and Survival

Similar to clusterin's ability to be either pro-inflammatory or anti-inflammatory depending on the circumstance, clusterin has been shown to improve proliferation and protect cells from apoptosis in some instances, while increasing apoptosis under other conditions[234,235]. Clusterin has been shown to increase the proliferation and survival of multiple cell types, including astrocytes, pancreatic beta cells, tubular cells of the kidney, and many types of cancer cells[236-240]. Clusterin promotes the survival and proliferation of cell types by binding to and sequestering the pro-apoptotic protein Bax, allowing cells to escape the induction of apoptotic death[241]. More recent data has also demonstrated that clusterin can inhibit the expression of neuronal nitric oxide synthase, thereby sparing all surrounding cells from exposure to toxic nitric oxide[242]. Importantly,

the most abundant isoform of clusterin, the secreted form, is thought to be responsible for clusterin's pro-survival functions[243].

While the vast majority of data supports the anti-apoptotic effects of secreted clusterin, other data has demonstrated that one of the more minor isoforms, generally found in the nucleus, plays a pro-apoptotic role in many cell types[211]. This nuclear isoform promotes apoptosis by binding to the protein Ku70, which is a critical component of the DNA damage repair pathway. This inhibition of DNA damage repair consequently leaves cells vulnerable to DNA damage-induced apoptosis[211,244,245]. A large amount of evidence indicates that clusterin has the ability to modulate apoptosis pathways, although it seems that it can have opposing functions depending upon the isoform.

Association with Disease

While the exact functions of clusterin have been challenging to elucidate, there is a significant amount of data indicating that clusterin is upregulated in numerous disease states[246]. Clusterin has been most thoroughly studied in the context of cancer, as clusterin has been found to be upregulated in numerous types of cancer[239,240].Clusterin has been found to serve a detrimental role in cancer by increasing the survival of malignant cancer cells[247,248]. This observation has led to the development of an antisense oligonucleotide against clusterin that has progressed to Phase 3 clinical trials in the hope that reducing clusterin might improve the sensitivity of cancer cells to chemotherapy[249,250].

Clusterin has also been shown to be upregulated in patients with multiple sclerosis (MS)[251]. Interestingly, clusterin is increased in MS patients at multiple levels, as people with MS have more clusterin in their blood and cerebral spinal fluid (CSF) when compared to control patients, and clusterin has been found to be upregulated within demyelinated lesions when compared to unaffected white matter[251-253]. Importantly, clusterin has been shown to increase in multiple disorders that involve demyelination, such as amyotrophic lateral sclerosis and neuromyelitis optica, not just in MS patients[239,254,255]. While the exact role of clusterin and the consequences of its upregulation in MS and other demyelinating disorders remains unclear, the conservation of its upregulation across multiple disease states indicates that it may play an important role in disease progression or response.

Clusterin rose to the attention of the Alzheimer's Disease (AD) community a little over a decade ago when a large genome wide association study identified a single nucleotide polymorphism (SNP) in clusterin that was the third most significant risk factor for developing late-onset Alzheimer's Disease[256]. Much like patients with MS, patients with AD have increased clusterin at multiple levels, including in the blood, CSF, and the brain parenchyma[257-259]. While the literature contains conflicting reports regarding how the risk-associated SNP in clusterin effects clusterin production and function, data does indicate that higher levels of clusterin correlate with worsening disease symptoms and an increase in brain atrophy[260-264]. Mouse work investigating the effects of clusterin on AD progression and symptomatology remains divided on whether the increase in clusterin observed in AD is protective or detrimental. On one hand, data from multiple sources indicates that clusterin worsens AD symptoms, as knocking out clusterin in AD

mouse models has been shown to reduce amyloid beta (Aβ) deposition and improve memory performance[265-267]. However, other data indicates that clusterin prevents Aβ deposition and supports both synapse function and helps maintain proper neuronal spine density[268,269]. The relatively small body of literature dedicated to studying clusterin in the context of AD has not yet reached a consensus regarding how clusterin effects AD disease progression. However, the significant risk that the clusterin SNP confers for developing AD highlights the need for further study in this area.

Conclusions

Overall, clusterin has been shown to have multiple, and often contradicting, functions in areas such as debris clearance, cell survival and proliferation, and immune regulation[220,232,241]. These multiple functions, along with the fact that there are multiple splicing isoforms and detectable expression in almost every tissue type, make studying clusterin relatively challenging. However, it is clear that there is significant therapeutic value in further elucidation of clusterin's functions as it has been shown to be upregulated in a wide variety of diseases, including cancer, multiple sclerosis, and Alzheimer's disease[239,251,252]. This strong association with disease and continued lack of clarity regarding clusterin's function indicates that clusterin remains an important topic of research that could have significant implications for future therapeutics.

Chapter V: OPCs, myelin, and Alzheimer 's disease

Introduction

The disease that we now know as Alzheimer's disease (AD) was first described more than a century ago by Alois Alzheimer[270]. Since the inception of this diagnosis, millions of people have found themselves or their family members debilitated by its symptoms. In fact, the Alzheimer's Association estimated that in 2022 alone there are 6.5 million patients suffering from Alzheimer's disease[271]. In the last hundred years, a tremendous amount of time and resources have been dedicated to the study of AD etiology and the development of potential therapeutics. In this time, great strides have been made in describing and understanding the neuropathological and behavioral symptoms associated with AD. Dedicated work has allowed for the a detailed description of amyloid β plaques, tau tangles, and the overall neurodegeneration observed in AD patients, along with the resulting symptoms of dementia, reduction in executive function, depression, anxiety, and sleep disturbances[272-274]. Despite the large body of work detailing the pathology of AD, there still remains no reliable treatment to slow or stop disease progression. What's more, the accumulation of amyloid plaques has long been thought of as the key driver of AD pathology, but numerous clinical trials of anti-amyloid therapeutics have successfully decreased the Aβ plaque-burden in Alzheimer's patients but failed to reduce clinical symptoms of cognitive impairment[275]. This continued lack of effective therapeutics highlights the need for alternative treatment avenues.

Myelin alterations in Alzheimer 's disease

In his first report on Alzheimer's disease, Alois Alzheimer described disruptions to the brain's white matter, although these changes were disregarded for many decades following his publication[276]. In recent years, increasing amounts of data have pointed to white matter loss and myelin disruption as a significant pathological finding in Alzheimer's disease patients that may be of importance to disease progression[277,278]. MRI studies have shown a significant loss of white matter volume in AD patients compared to age-matched controls[193,279]. Furthermore, postmortem studies have offered a more detailed look at myelin perturbations in AD. Histopathological studies have shown an overall decrease in myelination using luxol fast blue staining, and other studies have shown an overall decrease in myelin basic protein MBP by western blot[277,280]. Additionally other publications have used immunofluorescence to demonstrate an increase in degraded MBP in AD postmortem samples[281]. These myelin perturbations are conserved in animal models of AD, as myelin degradation has been observed in the 3xTg, 5xFAD, and APP/PS1 models of Alzheimer's[282-285].

Interestingly, recent work has postulated that myelin disruption is a key event that precipitates cognitive decline in AD[286]. This hypothesis necessitates that myelin disruption and damage precedes cognitive impairment. This is supported by multiple post-mortem studies in which patients with amyloid beta plaques consistent with Alzheimer's disease showed significantly disrupted myelin and white matter atrophy despite showing little to no cognitive impairment prior to death[287,288]. Additionally, pre-clinical studies have demonstrated that mouse models of AD exhibit loss of myelin as early as 1 month of age. This myelin loss can often be observed prior to the onset of memory deficiencies or even the appearance of Aβ plaques[282-284]. This evidence

supports the notion that myelin disruption appears early in AD progression and may even influence the progression of cognitive symptoms.

Remyelination as a potential therapeutic

With the complete lack of effective treatments for AD, as well as a renewed interest in myelin degeneration in AD patients, there is some excitement around the potential use of remyeilnating therapies in the treatment of AD. This line of inquiry is supported by data showing that the generation of new oligodendrocytes is necessary for motor learning, suggesting that oligodendrocytes may play a critical role in memory formation[61]. Within the past two years, this connection between oligodendrocytes and memory has been further elucidated, with multiple studies showing that the ability to produce mature oligodendrocytes is needed to support proper spatial memory consolidation and fear memory recall[289,290]. Finally, data from Chen and colleagues published in 2021 showed that when APP/PS1 mice were treated with the pro-myelinating drug clemastine, these mice demonstrated significantly improved myelination and memory performance when compared to their untreated APP/PS1 counterparts. Importantly, improvement in cognitive abilities were observed despite no change in the plaque burden in clemastine-treated mice[285]. Taken together, these data indicate that future work should continue to pursue the promotion of remyelination as a potential treatment for AD patients.

OPC function in AD

While myelin loss has been recognized as a key component of Alzheimer's disease, it

remains unclear why oligodendrocyte progenitor cells (OPCs), a population which remains mitotically active and able to produce new oligodendrocytes throughout adulthood, fails to compensate for the loss of myelin in the context of AD[9,31,58]. In comparison to neurons and other glial subtypes, relatively little is known about how OPCs are affected by AD progression. Recent work has demonstrated a decrease in OPC density in AD mouse models, accompanied by significant changes in OPC cell volume and morphology[283,291]. Additionally, OPCs have been implicated as important mediators of memory deficits in AD. In 2019, Zhang and colleagues demonstrated that ablation of senescent OPCs that cluster around Aβ plaques improved the memory deficits normally observed in the AD model[199]. With more data emerging in recent years demonstrating that OPCs can perform functions such as clearing debris, presenting antigen, producing cytokines, and sculpting neuronal circuitry, it is possible that the AD environment induces alternative functions of OPCs that prevent this population from maintaining the proper density of mature oligodendrocytes, potentiating a reduction in functional myelin[154,161,292,293]. Whatever may be the cause of this lack of OPC differentiation, more work is needed to develop effective therapeutics to reverse myelin deficits observed in AD and to determine if this reversal will improve behavioral symptoms.

Conclusions

While white matter perturbations have been recognized as a symptom of AD for more than a century, recent work connecting oligodendrogenesis to memory consolidation and recall have spurred research into how myelin deficits might be playing an integral role in the progression of AD symptoms[276,285,289,290]. This work, along with

the recent emergence of data showing that OPCs can play many roles in the CNS, places oligolineage cells as important players in Alzheimer’s disease, and necessitates further work elucidating their role in AD pathology[294]. In sum, investigation into OPCs and oligodendrocytes in the context of Alzheimer’s disease presents an exciting opportunity for the development of novel AD therapeutics.

Chapter VI: Oligomeric amyloid beta prevents myelination in a clusterin dependent manner

Abstract

White matter loss has been described as a common occurrence in Alzheimer's disease (AD) patients for multiple decades. However, it remains unclear why oligodendrocyte progenitor cells (OPCs) fail to repair myelin deficits in these patients. Here, we show that clusterin, a risk factor for late-onset AD, is produced by OPCs and inhibits their differentiation into oligodendrocytes. Specifically, we demonstrate that a unique subset of OPCs produces clusterin. We show that phagocytosis of debris, including amyloid beta (Aβ) and myelin, drives the upregulation of clusterin in OPCs. We confirm, in vivo, that Aβ oligomers drive clusterin upregulation and that OPCs phagocytose Aβ. Furthermore, we show that clusterin is a potent inhibitor of OPC differentiation and prevents the production of myelin proteins. Finally, we demonstrate that clusterin inhibits OPC differentiation by significantly reducing the production of IL-9 by OPCs. Our data reveals that clusterin may be responsible for the lack of myelin repair observed in AD and is a promising therapeutic target for AD-associated cognitive decline.

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease that currently has no approved therapeutic to effectively prevent disease progression, and patients inevitably succumb to debilitating dementia[295]. White matter loss has been documented in AD patients, but how this myelin loss contributes to disease progression remains unclear[277]. Recent studies indicate that generation of new myelin-forming oligodendrocytes is critical for memory consolidation and recall[289,290]. Additionally, remyelination therapeutics have been shown to reduce cognitive deficits seen in an animal model of AD[285]. This evidence supports the hypothesis that the inability to generate oligodendrocytes is a significant driver of the cognitive deficits observed in AD.

The adult brain contains oligodendrocyte progenitor cells (OPCs), a highly proliferative population of glia that are maintained in a progenitor state throughout adulthood and are normally capable of generating nascent oligodendrocytes[9,26]. However, it is not understood why OPCs fail to repair myelin damage present in AD. The ability of OPCs to produce mature, myelinating oligodendrocytes during adulthood is critical, as motor learning, memory consolidation, and memory recall are all dependent on de novo production of myelin[61,289,290].

Here we investigated if clusterin, a risk factor for late-onset AD, alters the function of OPCs in AD[256]. Clusterin is a multifunctional apolipoprotein that is upregulated in multiple neurodegenerative disorders and is known to play a role in the clearance of debris[195,220,227,261]. We found that a specific subset of OPCs in the adult mouse brain express clusterin, mimicking data from humans indicating that a subset of OPCs express high levels of clusterin[198]. This same data indicates that OPCs, but not any

other brain-resident cell type, upregulates clusterin expression in the context of AD 198. Consequently, we investigated what factors might contribute to clusterin production in OPCs. We found that phagocytosis of debris, including both oligomeric Aβ and myelin debris, results in an upregulation of clusterin production by OPCs. We further discovered that clusterin is a potent inhibitor of OPC differentiation and the production of myelin proteins. At a mechanistic level, we found that clusterin reduces the production of the cytokine IL-9 by OPCs and that restoration of IL-9 levels rescues the ability of OPCs to differentiate in the presence of clusterin.

Results

Clusterin is expressed by a OPCs in the human brain

Clusterin, most commonly found as a secreted protein, is upregulated in the brains of patients with Alzheimer's disease (Fig. 11a, b)[210]. Interestingly, we found that cells expressing clusterin RNA could be found directly surrounding Aβ plaques. (Fig. 11c). This observation is conserved in pre-clinical models of AD, as clusterin is also found to be upregulated in multiple brain regions of the 5xFAD mouse model of AD (Fig. 11d, e). Because of the emerging role of OPCs and myelin in AD pathology, we investigated whether OPCs expressed clusterin in AD[199,285]. Surprisingly, we found that OPCs express clusterin both in the AD brain, as well as in normal aging (Fig. 11f). Taken together, these results show that clusterin, a risk factor for late-onset AD, is upregulated in the parenchyma of AD patients, and that this gene is expressed by OPCs in normal aging as well as in late-stage AD.

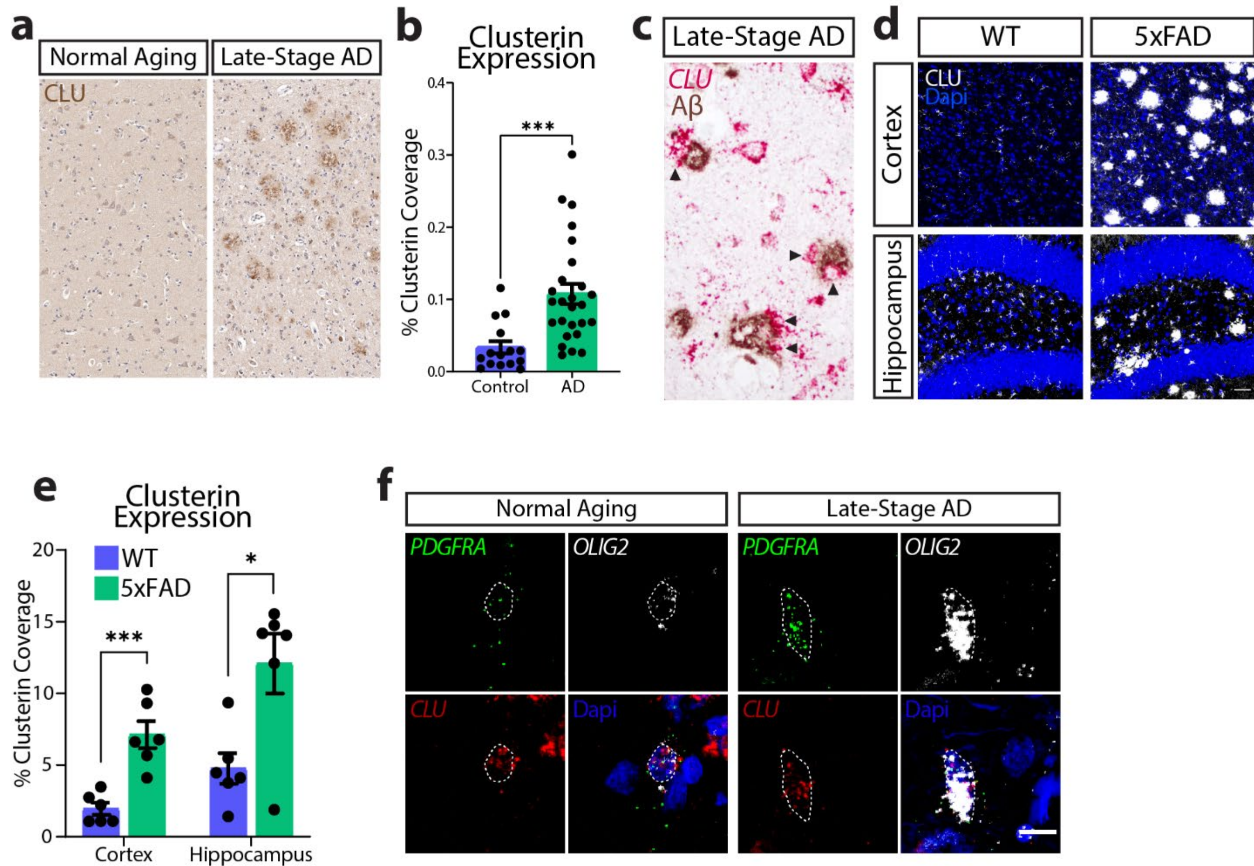

Figure 11: OPCs express the AD-risk factor clusterin. a, Representative image of Clusterin expression (immunohistochemistry, brown) in the cortex from a normal aging patient and a late-stage AD patient. **b**, Quantification of clusterin coverage in the cortex of normal aging (n=15) and AD patients (n=26, from two independent experiments, depicted in a). Data analyzed using an unpaired t-test; t(39)=3.767. **c**, Detection of clusterin RNA (in situ hybridization, red) and Aβ protein (immunohistochemistry, brown) in late-stage AD brain (late-stage AD n=2; from two independent experiment). Arrowheads indicate clusterin-expressing cells around plaques. **d**, Representative images of clusterin expression (white) in the cortex and hippocampus of WT and 5xFAD mice. Scale bar=30μm. **e**, Quantification of clusterin coverage in the cortex and hippocampus of WT and 5xFAD mice (depicted in c; WT n=6, 5xFAD n=6; from two independent experiments). Statistics calculated using an unpaired Student's t-test. Cortex: t(10)=5.049; Hippocampus: t(10)=3.119. **f**, In situ hybridization for OPCs (PDGFRA in green, OLIG2 in white) expressing Clusterin (CLU; red) in normal aging and late-stage AD brains (normal aging n=1, late-stage AD n=1; from one independent experiment). Scale bar=10μm.

Phagocytosis of oligomeric Aβ and cellular debris results in an upregulation of clusterin expression by OPCs

Given our data showing that OPCs express the AD-associated gene clusterin, we wanted to determine if OPCs interacted with Aβ plaques. Surprisingly, using 5xFAD mice, we found that OPCs were surrounding and extending their processes into Aβ plaques (Fig. 12a). Because clusterin is known to facilitate debris clearance, we next wondered if OPCs were involved in the clearance of Aβ oligomers from the brain[220]. To test this, we injected WT mice with Aβ oligomers labeled with CypHer5e, a dye that only fluoresces when it has entered the acidic environment of the lysosome. We found that OPCs around the injection site phagocytosed Aβ within 12 hours of injection (Fig. 12b). Surprisingly, we also found that injecting Aβ was sufficient to drive an upregulation of clusterin expression in the brain (Fig. 12c, d).

We next investigated whether OPCs could contribute to this Aβ-induced upregulation of clusterin. We treated primary OPCs *in vitro* with Aβ oligomers for 4 hours and observed a rapid increase in clusterin production (Fig. 12e). We subsequently confirmed that OPCs also upregulate the production of clusterin protein following treatment with Aβ (Fig. 2f). We found that phagocytosis of Aβ was necessary to drive this upregulation of clusterin, as treatment with cytochalasin D (CytoD), an actin polymerization inhibitor that blocks phagocytosis, prevented Aβ from increasing clusterin production in OPCs (Fig. 12e)[222,223,296]. Interestingly, we found that these changes in clusterin were not specific to the phagocytic clearance of Aβ oligomers, but were rather generalizable to instances in which OPCs engulfed large debris. We found that treatment of OPCs with myelin debris also produced a similar upregulation of clusterin that was

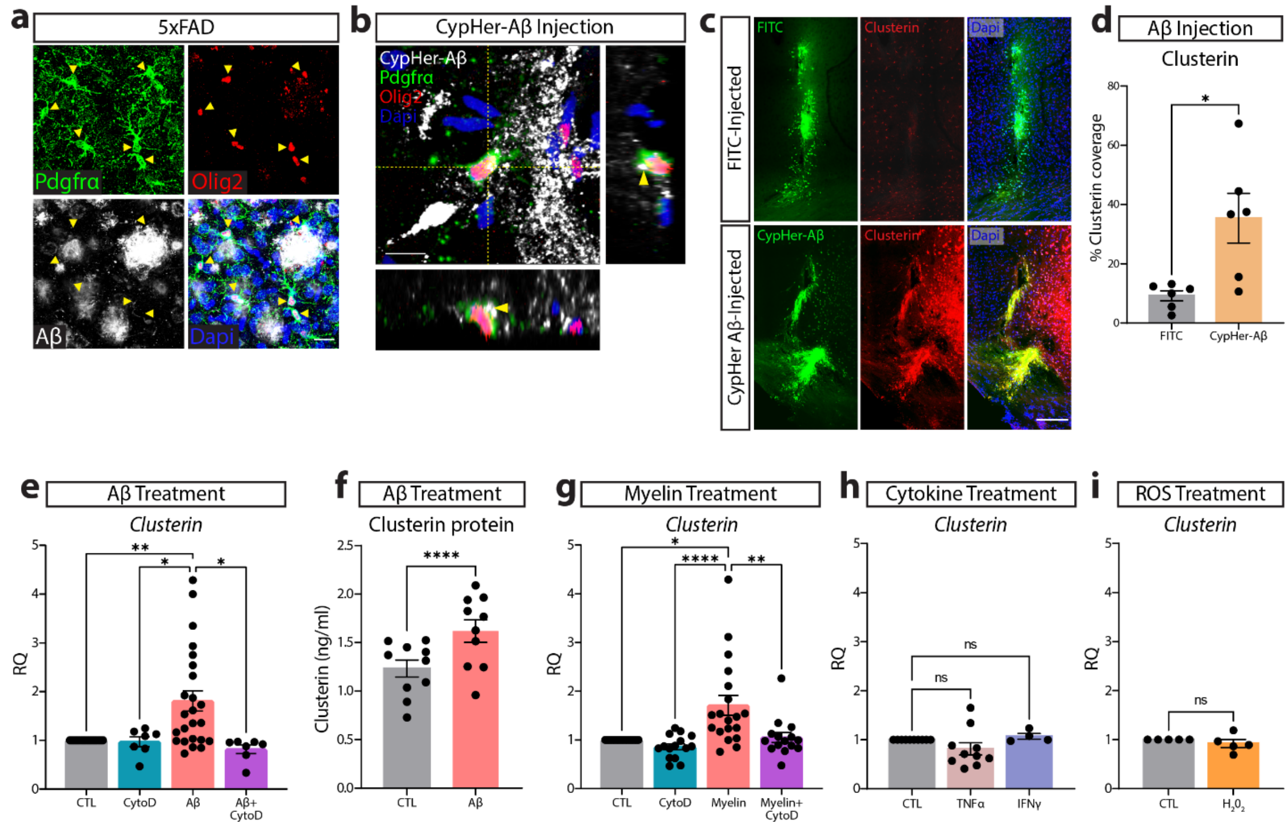

Figure 12: Phagocytosis of extracellular debris drives clusterin expression in OPCs a, Representative image of OPCs (PDGFRα in green, Olig2 in red) surrounding Aβ plaques (white). Yellow arrowheads indicate OPCs that are extending processes into areas of Aβ accumulation (*n*=4 mice; from one independent experiment). Scale bar=20µm. **b**, Representative orthogonal view of CyPher-labeled Aβ (white) inside an OPC (PDGFRα in green, Olig2 in red) following intra-parenchymal injection of Aβ (*n*=6 mice; from one independent experiment). Yellow arrowheads indicate Aβ that can be seen inside the cell body of an OPC. Scale bar=10µm. **c**, Representative images of clusterin expression (red) in the ipsilateral (CypHer-Aβ injected; green) and contralateral (FITC injected; green) hemispheres following intra-parenchymal injection. Scale bar=100µm. **d**, Quantification of clusterin expression in the ipsilateral (Aβ-injected) and contralateral (FITC-injected) hemispheres following intra-parenchymal injection. (*n*=6 mice). Statistics calculated using a paired Student's t-test; t(5)=2.979. **e**, qPCR analysis of clusterin expression in OPCs following a 4-hour *in vitro* treatment with 3µm Aβ and the phagocytosis blocker CytoD (1µm) or vehicle control (CTL *n*=24, CytoD *n*=7, Aβ *n*=24, Aβ+CytoD *n*=7; from seven independent experiments). Statistics calculated using a mixed effects analysis with a Tukey's post-hoc analysis; F(1.121, 13.08) = 8.544. **f**, Quantification of clusterin protein in OPCs following 72-hour treatment with 3µM Aβ or vehicle control (CTL *n*=10, Aβ *n*=10, from two independent experiments). Statistics calculated using a paired Student's t-test; t(9)=7.596. **g**, qPCR analysis of clusterin expression in OPCs following a 4-hour *in vitro* treatment with 100µg/ml myelin and CytoD (1µm) or vehicle control (CTL *n*=19, CytoD *n*=15, Myelin *n*=19, Myelin+CytoD *n*=15; from five independent experiments). Statistics calculated using a mixed effects analysis with a Tukey's post-hoc analysis; F (1.389, 21.29) = 10.75. **h**, qPCR analysis of clusterin expression in OPCs following a 3-hour *in vitro* treatment with 10ng/ml TNFα or 10ng/ml IFNγ (CTL *n*=10, TNFα *n*=10, IFNγ n=4; from 2 independent TNFα experiments or 1 independent IFNγ experiment). Statistics calculated using a mixed effects analysis; F (1.106, 11.61) = 1.897. **i**, qPCR analysis of clusterin expression in OPCs following a 3-hour *in vitro* treatment with 10µm H_20_2 (*n*=5; from one independent experiment). Statistics calculated using a paired Student's t-test; t(8)=0.3417. *p<0.05, **p<0.01, ****p<0.0001, ns=not significant. All error bars represent SEM.

abrogated by exposure to CytoD (Fig. 12g). In order to eliminate the possibility that this clusterin upregulation was simply a response to any cellular stressor present in AD, we treated OPCs with other factors known to be upregulated in Alzheimer's disease, including TNFα, IFNγ, and reactive oxygen species (ROS), which all failed to produce any change in clusterin expression (Fig. 12h, i)[297-299].

In previous studies, clusterin has been shown to increase the phagocytic capacity of both professional and non-professional phagocytes alike[222,223]. This led us to investigate whether clusterin altered the kinetics of phagocytosis in OPCs. Surprisingly, the addition of exogenous clusterin did not change the ability of OPCs to engulf Aβ *in vitro*

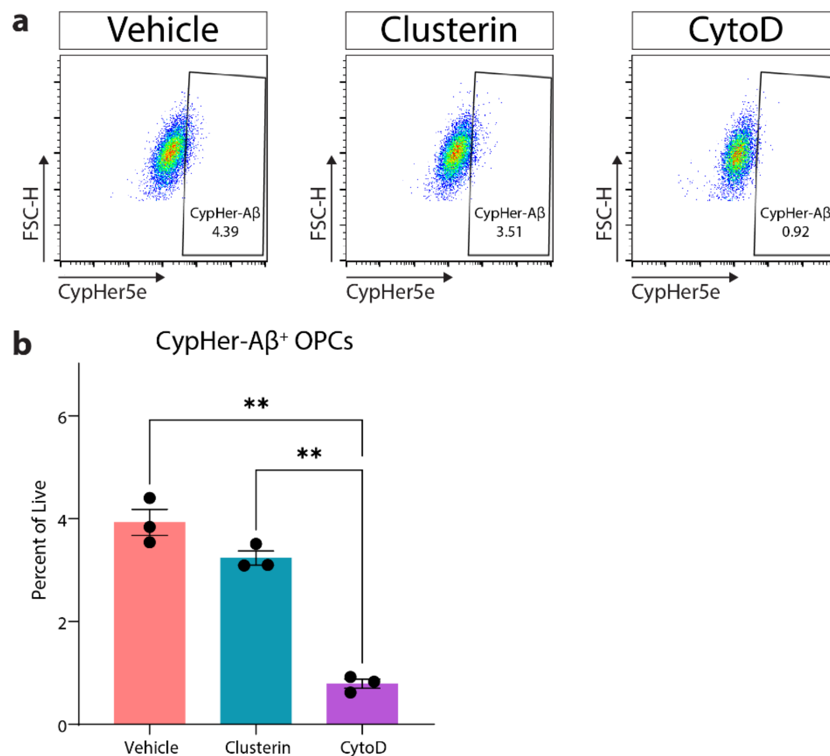

Figure 13: Clusterin does not alter OPC phagocytosis of Aβ
a, Representative flow gating (following singlets/singlets/live gates) of OPCs incubated for 90 minutes with 3μm CypHer5e-labeled Aβ oligomers (all conditions) with the addition of 8μg/ml clusterin or 1μm CytoD (Vehicle *n*=3, Clusterin *n*=3, CytoD *n*=3; from one independent experiment). **c**, Quantification of OPCs staining positive for CypHer-Aβ as depicted in **b**. Statistics calculated using a repeated measures one-way ANOVA with a Tukey's post-hoc analysis; $F(2,4)= 293.3$. **$p<0.01$. All error bars represent SEM.

(Fig. 13a, b), indicating that phagocytosis regulates clusterin expression in OPCs, but clusterin does not subsequently regulate phagocytic ability. In sum, these results indicate that OPCs are capable of clearing extracellular debris, including Aβ, and that phagocytosis of protein or cellular debris is responsible for driving clusterin expression in OPCs.

Exogenous clusterin inhibits OPC differentiation

The formation of new myelin has been increasingly recognized as a critical component of memory function[289,290]. In fact, a recent study demonstrated that drugs promoting nascent myelin formation results in improved memory performance in a model of AD, indicating the importance of understanding what factors are preventing the differentiation of OPCs into oligodendrocytes[285]. A multitude of studies have demonstrated that cellular debris and protein aggregation prevent OPC differentiation, although the mechanism by which this occurs remains unclear[300,301]. This data, along with our observation that debris clearance drives clusterin expression in OPCs (Fig. 12e, g), made us question whether clusterin might be inhibiting OPC differentiation. Consequently, we treated differentiating OPCs with exogenous clusterin and observed a striking decrease not only in genes encoding myelin proteins (*Mbp*, *Plp1*, *Cnp*), but also in *Myrf*, the master transcriptional regulator of the OPC differentiation program (Fig. 14a-d). We subsequently observed fewer MBP-positive oligodendrocytes when OPCs were differentiated in the presence of clusterin compared to clusterin-free media (Fig. 14e, f). Importantly, this decrease in differentiation is not due to OPC death, as there was no difference in OPC number following clusterin treatment (Fig. 14g). Overall, this data

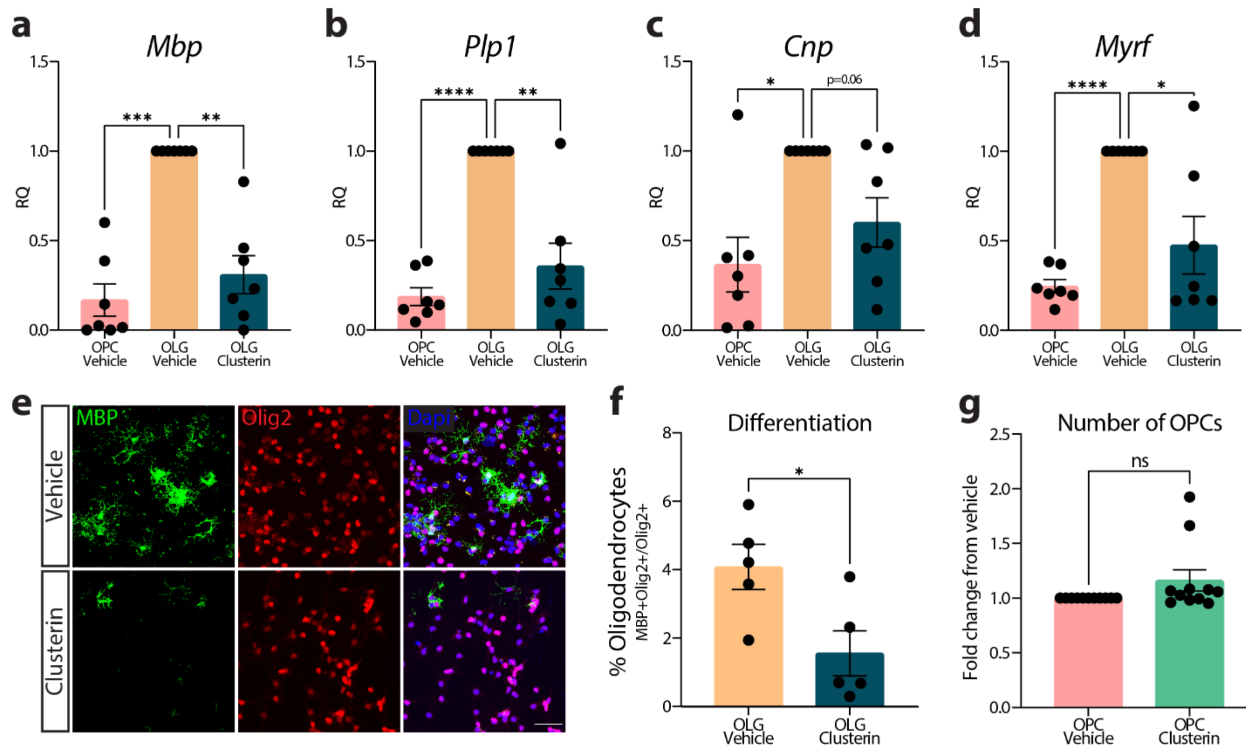

Figure 14: Exogenous clusterin inhibits OPC differentiation
Expression of *Mbp* (**a**), *Plp1* (**b**), *Cnp* (**c**), and *Myrf* (**d**), measured by qPCR in OPCs cultured in proliferation media (OPC Vehicle), differentiation media (OLG Vehicle), or differentiation media supplemented with 8µg/ml of clusterin (OLG Clusterin) for 72 hours (*n*=7 for all conditions). Statistics calculated using a repeated measures one-way ANOVA with a Tukey's post-hoc analysis; *Mbp* $F(2,6)=27.41$, *Plp1* $F(2,6)=25.72$, *Cnp* $F(2,6)=9.776$, *Myrf* $F(2,6)=16.41$. **e**, Representative images of OPCs cultured in differentiation media with or without 8µg/ml of clusterin for 72 hours and stained for oligodendrocyte markers (MBP in green, Olig2 in red). Scale bar= 50µm. **f**, Quantification of the number of OPCs that differentiated in to oligodendrocytes following treatment with clusterin (depicted in **e**; *n*=5 for all conditions; from two independent experiments). Statistics calculated using a paired Student's t-test; $t(4)=2.780$. **g**, Quantification of the number of OPCs present using a Cell Counting Kit-8 assay following a 72 hour incubation in proliferation media with or without 8µg/ml clusterin (*n*=11 for all conditions; from four independent experiments). *$p<0.05$, **$p<0.01$, ****$p<0.0001$, ns=not significant. All error bars represent SEM.

shows that clusterin is regulated by the phagocytosis of debris and is a potent inhibitor of OPC differentiation.

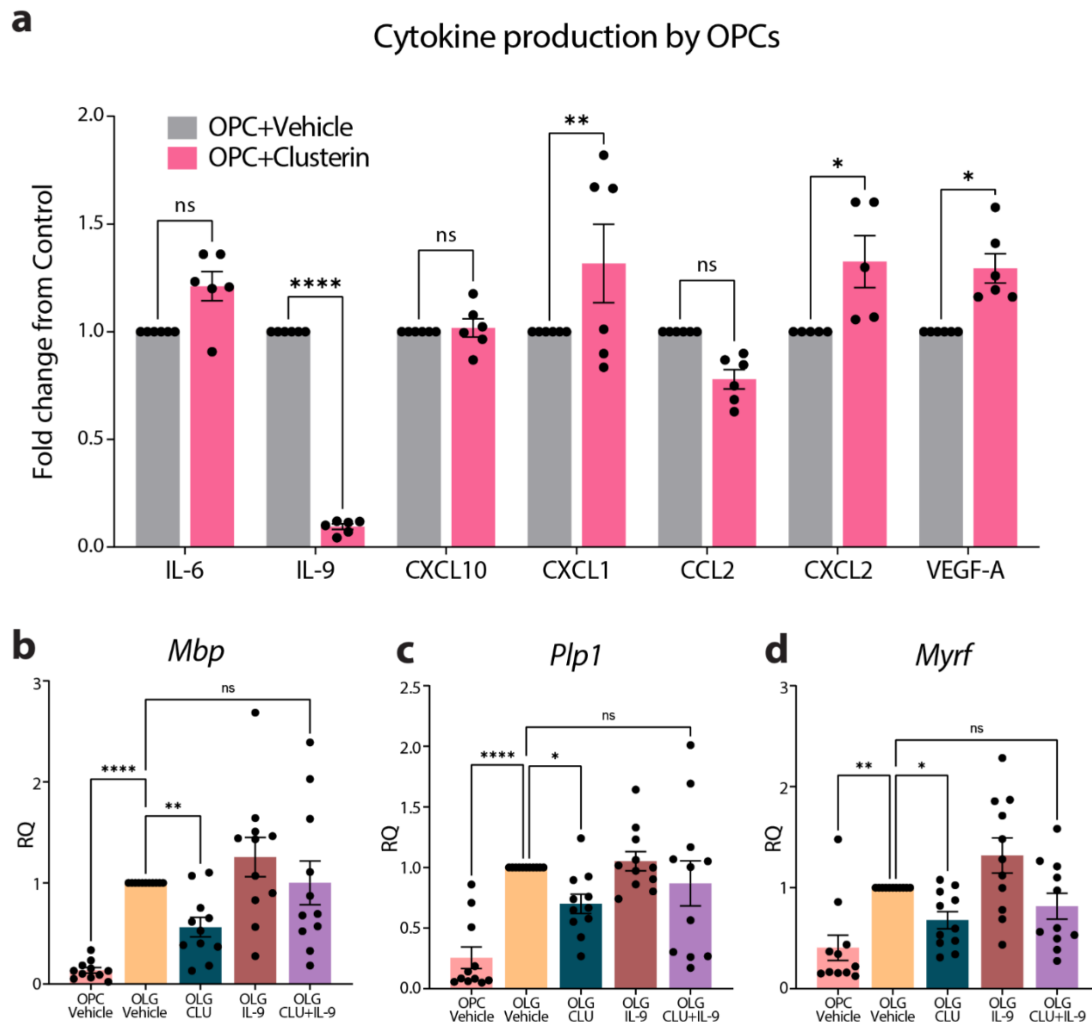

Figure 15: Clusterin inhibits differentiation by blocking IL-9 production
a, Quantification of cytokines present in the supernatant from OPCs treated with 8µg/mL clusterin or vehicle control (*n*=6 biological replicates for each condition, from two independent experiments). Data analyzed using a two-way repeated measures ANOVA with a Sidak's multiple comparison post-hoc analysis, $F(6, 34) = 23.93$. Expression of *Mbp* (**b**), *Plp1* (**c**), and *Myrf* (**d**), measured by qPCR in OPCs cultured in proliferation media (OPC Vehicle), differentiation media (OLG Vehicle), differentiation media supplemented with 8µg/ml of clusterin (OLG CLU), differentiation media supplemented with 100ng/ml IL-9 (OLG IL-9), or differentiation media supplemented with 8µg/ml of clusterin and 100ng/ml IL-9 (OLG CLU+IL-9) for 72 hours (*n*=11 for all conditions; from 3 independent experiments). Statistics calculated using a repeated measures one-way ANOVA with a Tukey's post-hoc analysis; *Mbp* $F(10,40)=10.15$, *Plp1* $F(10,40)=11.96$, *Myrf* $F(10,40)=10.32$. *$p<0.05$, **$p<0.01$, ****$p<0.0001$, ns=not significant. All error bars represent SEM.

We next investigated what factors might be mediating clusterin's inhibition of OPC differentiation. OPCs have been shown to produce a variety of growth factors and cytokines that can significantly alter their local environment[3,126,132,133,293]. Additionally, clusterin has been shown to regulate to production of cytokines[232,233,302]. Based on this data, we wondered if clusterin might be inhibiting OPC differentiation by affecting growth factor and cytokine production. We performed a Luminex Assay on the supernatant of OPCs treated with vehicle or clusterin and found that clusterin altered the secretion of multiple proteins, mostly notably increasing the production of VEGF and significantly decreasing the production of IL-9 (Fig. 15a). Since growth factors from the VEGF family have been shown to induce OPC proliferation[303], we tested whether the increase in VEGF following clusterin treatment was responsible for keeping OPCs in an undifferentiated state. However, treatment of OPCs with a combination of clusterin and an anti-VEGF antibody failed to rescue the differentiation defect observed with clusterin treatment (Fig. 16a-c).

The next candidate we assessed as the potential mechanism for the effects of clusterin on OPCs was IL-9, since its production was reduced by more than 90% following clusterin treatment (Fig. 15a). IL-9 is a relatively understudied cytokine known to be produced by T-cells[304]. Surprisingly, we found that the addition of exogenous IL-9 was sufficient to rescue the differentiation deficits induced by clusterin treatment (Fig. 15b-d). Overall, this data demonstrates that clusterin blocks the differentiation of OPCs by inhibiting the production of IL-9, and that IL-9 is an important factor in proper differentiation of OPCs.

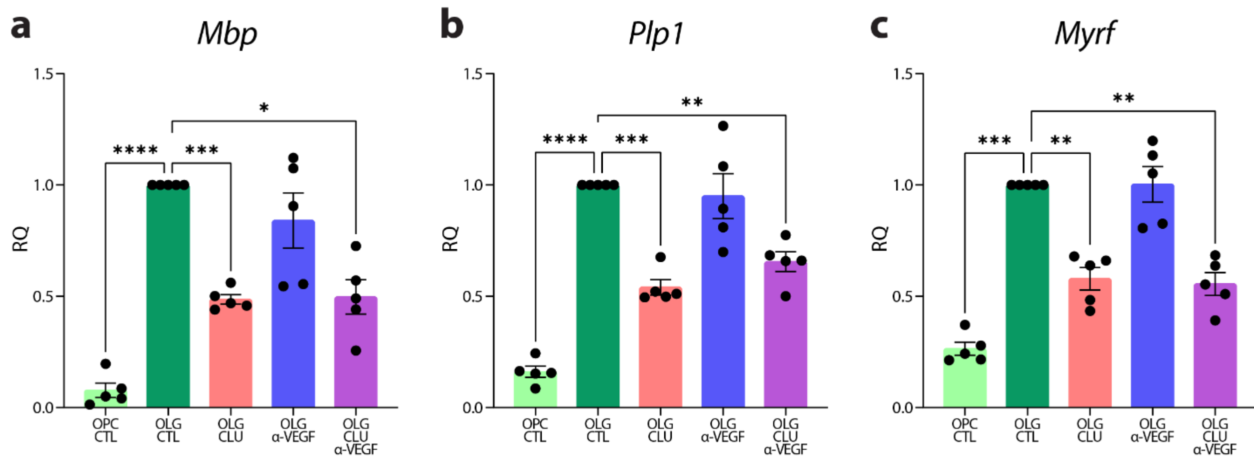

Figure 16: VEGF does not mediate the effect of clusterin on OPC differentiation Expression of *Mbp* (**a**), *Plp1* (**b**), and *Myrf* (**c**), measured by qPCR in OPCs cultured in proliferation media (OPC CTL), differentiation media (OLG CTL), differentiation media supplemented with 8µg/ml of clusterin (OLG CLU), differentiation media supplemented with 10µg/ml VEGF function-blocking antibody (OLG α-VEGF), or differentiation media supplemented with 8µg/ml of clusterin and 10µg/ml VEGF function-blocking antibody (OLG CLU α-VEGF) for 72 hours (n=5 for all conditions; from one independent experiment). Statistics calculated using a repeated measures one-way ANOVA with a Tukey's post-hoc analysis; *Mbp* $F(4,16)=28.66$, *Plp1* $F(4,16)=49.31$, *Myrf* $F(4,16)=74.58$. *$p<0.05$, **$p<0.01$, ***$p<0.001$ ****$p<0.0001$. All error bars represent SEM.

Discussion

Since the discovery of Aβ plaques in the brains of AD patients, therapeutic development has been focused on reducing plaque load. However, no therapeutic candidate, even when successful at reducing plaques, has succeeded in slowing disease progression[305]. Over recent years, a growing body of literature has speculated that other altered biological processes may be the driving force behind the clinical decline observed in AD patients[306]. Increasingly, OPCs and myelin have been implicated in the etiology and progression of AD symptoms. A recent study demonstrated that the ablation of senescent OPCs was sufficient to improve the memory impairment observed in the APP/PS1 model of AD[199]. Additionally, multiple studies have shown that inhibiting OPC

differentiation prevents memory consolidation and recall, and that therapeutically increasing myelination can improve memory performance in AD models[285,289,290,307]. Here, we offer a potential mechanism for the pervasive myelin deficits observed in Alzheimer's disease and the memory decline associated with these deficits[279,281]. We demonstrate that clusterin is upregulated in the brains of AD patients as well as in a mouse model of AD. We found that a specific subset of OPCs expresses clusterin under normal conditions, and that these clusterin-positive OPCs can be found in AD. Phagocytosis of debris, including Aβ oligomers, myelin, and apoptotic cells, efficiently drives the upregulation of clusterin in OPCs. Finally, we show that clusterin, through its effects on the production of IL-9, is a potent inhibitor of OPC differentiation and the production of myelin proteins.

A common single-nucleotide polymorphism (SNP) in clusterin has been recognized as a significant risk factor for late onset AD for over a decade[256]. While there are conflicting reports in the literature regarding how this SNP effects the function and accumulation of clusterin, there are multiple studies indicating that that increased levels of clusterin in the plasma of AD patients, regardless of the presence of a SNP at the CLU locus, correlates with a more rapid cognitive decline and an increase in brain atrophy[257,260,261,308]. These correlative studies demonstrating that excess levels of clusterin are associated with more severe AD symptoms are supported by studies indicating that removing clusterin in mouse models of AD results in a reduced plaque load as well as improved performance on memory tasks[265-267]. There are, however, reports in the literature indicting that clusterin might be involved in the clearance of Aβ plaques and the protection of neurons[268,269]. These discrepancies likely indicate that clusterin is a

multi-functional protein that may be beneficial when expressed at homeostatic levels, but could become detrimental when significantly upregulated in the context of disease pathology. Additionally, since there is an increasing amount of data indicating that the reduction of Aβ plaques in AD patients does not improve clinical symptoms, it is possible that clusterin's inhibition of OPC differentiation, as described here, may be the mechanism though which increased levels of clusterin might contribute to AD progression[309].

It remains to be determined exactly which cell types might be contributing to the increase in clusterin observed in Alzheimer's disease. While we have demonstrated that OPCs upregulate clusterin production in response to the phagocytosis of Aβ as well as other cellular debris, and human single cell sequencing data indicates that OPCs are the only cell type in the brain that show an increase in clusterin expression in AD patients compared to healthy controls, it is known that astrocytes produce a significant amount of clusterin[198,310]. However, while the exact cellular sources of elevated levels of clusterin in AD is still an open question, the fact that the most abundant isoform of clusterin encodes for a secreted chaperone protein indicates that clusterin from any cellular source, whether OPCs or any other cell type, can impact the function and differentiation capacity of OPCs[311].

We were surprised to find that clusterin decreased the differentiation of OPCs by blocking the production of IL-9. While IL9 receptor expression has been documented in OPCs, this is the first time that OPCs have been shown to produce IL-9 and that IL-9 is important for the proper differentiation of OPCs[312]. While T-cells are known to be the main producers of IL-9, there is increasing evidence in the literature that OPCs can also

contribute to cytokine production[132,293,313,314]. The ability of OPCs to function as immunomodulatory cells is garnering increasing support in the literature, and our data indicating the importance of IL-9 in the function of OPCs offers an intriguing avenue for additional investigation[315,316].

It has been known that clusterin is increased in the brains of AD patients for over three decades[317]. However, the mechanism of clusterin's effects on the progression of AD still remains unclear. With an antisense-oligonucleotide targeting clusterin having progressed to Phase 3 clinical trials for prostate cancer, our data demonstrating that clusterin negatively affects OPCs and myelin production offers a new therapeutic avenue to potentially improve myelin integrity and memory deficits plaguing AD patients[249,318]. What's more, our data linking clusterin and myelin dysregulation is supported by MRI data demonstrating that the clusterin risk allele is associated with a decrease in white matter integrity in healthy young adults, prior to onset of any cognitive decline[319]. Overall, our data offers a mechanism for the association between clusterin and Alzheimer's disease pathology, and provides a foundation for the rapid repurposing of current drugs for the treatment of AD.

Chapter VII: Future Directions and Unanswered Questions

Introduction

There are both short-term and long-term questions that remain to be answered regarding the effects of clusterin on OPCs and their function, as well as more general questions regarding what the transcriptional heterogeneity of OPCs might mean for alternative functions of these cells. The short-term questions generally focus on further description of how clusterin is dampening the ability of OPCs to differentiate. Additionally, a critical set of experiments revolves around proof of an *in-vivo* effect of clusterin on the ability of OPCs to differentiate. Long-term experiments focus on the role of clusterin and OPCs in Alzheimer's disease (AD), as well as a range of other CNS (central nervous system) diseases and disorders, including multiple sclerosis(MS).

Short Term

Evaluate in vivo effects of clusterin on OPC differentiation

The first question that remains to be answered is whether clusterin effects the differentiation of OPCs *in vivo*. We have demonstrated multiple times that clusterin can inhibit differentiation *in vitro*. Using a clusterin knockout mouse, I have also confirmed that clusterin is not necessary for proper OPC development and myelination, as these mice do not exhibit any deficits in OPC number, oligodendrocyte number, or myelination. However, it still remains to be seen if an excess of clusterin, as seen in multiple disorders such as AD and MS, effects the differentiation of OPCs *in vivo*[195,196].

There are multiple experimental methods by which this question can be answered. One approach would be to use a virus to overexpress clusterin in the brain. There is

currently an AAV serotype available (PHP.eB) that efficiently crosses the BBB. This would allow us to administer this AAV intravenously via the tail-vein without any need for invasive intracerebral injections. Since there is not currently an AAV that is known to infect OPCs, we are planning to use a construct with a GFAP promoter to induce clusterin overexpression in astrocytes. Since clusterin is largely a secreted protein, clusterin coming from astrocytes should still be able to effect the function of OPCs. Using these clusterin-overexpressing mice, we can utilize the cuprizone model of demyelination to assess if increased levels of clusterin slow down the ability of these mice to remyelinate.

While this is one option to test our hypothesis, a second experimental approach would be to utilize lysolethicin-induced demyelination coupled with an intracerebral injection of clusterin protein. Injection of lysolethicin into the white matter results in a fully demyelinated lesion within 7 days post-injection, and a fully remyelinated lesion within 14 days post-injection. This short time period will allow us to inject a bolus of clusterin protein at the time of lysolethicin injection. Evaluation of the level of remyelination present at 10 days post-injection (the middle of the remyelination phase) in the clusterin and vehicle injected mice should address the question of whether clusterin effects the ability of OPCs to differentiate *in vivo*.

Evaluate in vivo effects of IL-9 on OPC differentiation

Another set of experiments that need to be confirmed *in vivo* is the effect of IL-9 on OPC differentiation. We have observed *in vitro* that clusterin reduces the production of IL-9 by OPCs, and that this reduction is responsible for clusterin’s inhibitory effects on OPC differentiation. However, we have not yet proven that IL-9 is able to promote OPC

differentiation *in vivo*. To answer this particular question, it would be most useful to again utilize the model of lysolethicin-induced demyelination described above. Using this method, we would inject an anti-IL-9 antibody, or IgG as a control, into the corpus callosum at the same time that we inject lysolethicin. As before, we plan on assessing the amount of myelination present at 10 days post injection by staining for MBP as well as for mature oligodendrocytes using Olig2 and CC1. This will allow us to determine if blocking IL-9 signaling *in vivo* is effective at blocking OPC differentiation.

Elucidate clusterin binding partners present on OPCs

While we have observed significant effects of clusterin on downstream OPC signaling and behavior, we still do not know what receptor clusterin binds to on OPCs that results in these effects. In past studies, clusterin has been shown to bind to multiple receptors, including ApoER2, VLDLR, and LRP2[320,321]. However, we do not know if clusterin is binding to a previously-confirmed receptor on OPCs, or if it is binding to a receptor that has not yet been identified as a binding partner for clusterin. Since we do not currently have any strong candidates, we believe that the best course of action would be to perform an unbiased mass spectrometry analysis on all proteins bound to clusterin following clusterin treatment of OPCs. To complete this experiment, we will be using recombinant clusterin that contains an HA tag, allowing us to immunoprecipitate clusterin and anything that is bound to it. We will treat OPCs *in vitro* with clusterin for a short period of time, perform immunoprecipitation, and then send the resulting protein samples for mass spectrometry. We will use samples of OPCs treated with PBS as a control for background protein detection, allowing us to generate a list of OPC proteins that are able

to bind clusterin. Since clusterin is known to bind to debris in addition to functional receptors, we will cross-reference our list of proteins with a database of known membrane-bound receptors to generate a list of potential candidates. Using this method, we will then be able to methodically block the function of each candidate receptor to assess each as a potential mediator of clusterin's effects on OPCs.

Confirm that OPCs upregulate clusterin in 5xFAD mice

The other experiment that remains to round out the OPC and clusterin story in the short-term is validating that OPCs upregulate clusterin in the 5xFAD model of AD. While we have shown that clusterin goes up in general in both the 5xFAD brain as well as the AD brain, and demonstrated that OPCs upregulate clusterin production at both the RNA and protein level in response to Aβ *in vitro*, we have not yet conclusively demonstrated that OPCs in the 5xFAD brain produce more clusterin than wildtype OPCs. The challenge with assessing clusterin production *in vivo* is that the majority of clusterin produced is secreted from the cell, making it hard to gauge exactly which cells are producing clusterin and how much of they are making. To circumvent this problem, we would ideally use RNAscope combined with protein staining, allowing us to stain OPC cell bodies with a PDGFRα protein antibody, OPC nuclei with an Olig2 protein antibody, and clusterin RNA with an RNAscope probe. This will allow us to quantify how many OPCs in the 5xFAD brain express clusterin compared to age-matched wildtype controls. Additionally, using RNAscope will allow us to easily quantify how much clusterin each OPC is making, as each puncta stained represents one unique transcript. This will allow for a more nuanced quantification of clusterin expression by OPCs in 5xFAD.

Long Term

Assess how clusterin affects myelination and behavior in the 5xFAD mouse model

Within our data thus far, we have demonstrated that clusterin is upregulated in the brains of AD patients and 5xFAD mice, that a subset of OPCs express clusterin, that OPCs upregulate the production of clusterin in response to Aβ, and that clusterin is a potent inhibitor of OPC differentiation. This data, along with recent published literature indicating that improving myelination in animal models of AD improves memory performance despite having no effect on plaque burden, has led us to hypothesize that clusterin may at least be partially responsible for inhibiting OPC differentiation, and therefore contributing to the lack of myelin repair and the increasing memory deficits observed in 5xFAD mice (Fig. 17)[285]. The first set of experiments necessary to test this hypothesis would be to breed the existing clusterin knockout mouse to 5xFAD mice to generate 5xFAD mice that do not express any clusterin (CLU KO/5xFAD). We would first examine how the loss of myelin in CLU KO/5xFAD mice compares to myelin expression in 5xFAD mice, including CLU KO mice and WT mice as relevant controls. Myelin effects could be measured in a multitude of ways, including MBP immunofluorescence, luxol fast blue histology, western blotting for a variety of myelin proteins (MBP, MAG, CNPase, etc), as well as electron microscopy to evaluate myelin thickness, demyelinated axons, and myelin integrity. Based on the data that we have already collected, we predict that

removing clusterin from 5xFAD mice will reduce the myelin loss observed in clusterin-competent 5xFAD mice.

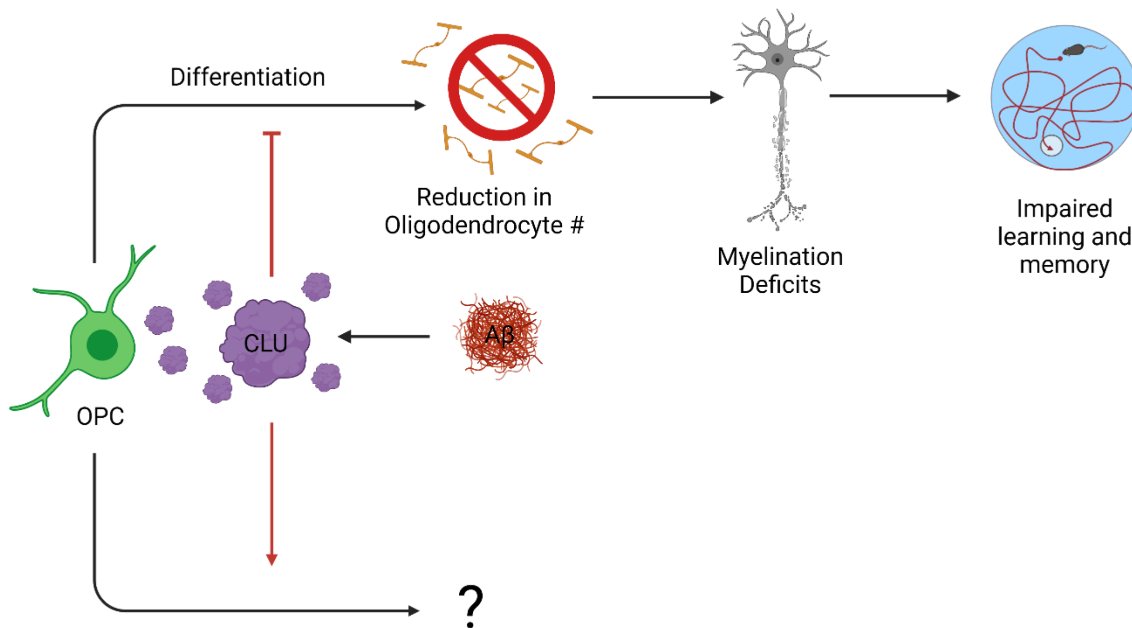

Figure 17: Graphical representation of the hypothesized effects of Aβ on myelination and memory performance in AD.
Cartoon depicting how we believe Aβ results in clusterin upregulation, which subsequently inhibits OPC differentiation, resulting in myelination deficits and impaired learning and memory. It is still unclear if clusterin promotes another function in OPCs or simply inhibits their differentiation capacity.

The other aspect of 5xFAD pathology that we predict may be altered in CLU KO/5xFAD mice is the memory deficits induced by 5xFAD pathology. As previously mentioned, a recent paper demonstrated that improving myelination is sufficient to improve memory performance in the APP/PS1 model of AD[285]. Since we hypothesize that removing clusterin will improve myelination, we also predict that CLU KO/5xFAD mice will present with improved memory performance compared to their 5xFAD counterparts. Typical experimental paradigms that we could use to test memory performance in these mice included the Morris water maze as well as the Barnes maze. Taken together, these

experiments will elucidate the role that clusterin plays in the myelination and memory deficits observed in 5xFAD mice.

Investigate how clusterin from specific cell types affects 5xFAD pathology

While the experiments listed above will reveal how clusterin effects 5xFAD pathology, the usual caveats that accompany any full-body knockout model still apply. This includes caveats such as altering normal development, allowing for potential adaptation to the loss of clusterin by other apolipoproteins, and being unable to determine if clusterin from one cell type is having a greater influence on pathology than other cell types. We have proposed to first utilize the full body knockout because a mouse line containing a floxed clusterin allele (CLU F/F) needed for Cre excision is not currently available. However, designing this mouse using CRISPR would provide the opportunity for a variety of experiments that would address the caveats of a full body knockout. We could cross this new CLU F/F mouse line with 5xFAD mice (CLU F/F 5xFAD) as well as an inducible UBC-cre line to assess the complete deletion of clusterin in adulthood and bypassing any developmental changes resulting from the lack of clusterin during development. Additionally, we could cross the CLU F/F 5xFAD mice with inducible cre drivers for OPCs (PDGFRα-Cre^{ER}), astrocytes (hGFP-Cre^{ER}), and endothelial cells (Slco1c1-Cre^{ER}). Using these new mouse lines and assessing myelination and behavior would allow us to determine if one cell type is contributing to the effects of clusterin in 5xFAD pathology and be certain that the effects we might observe are not due to lacking clusterin during development.

Assess the efficacy of an antisense oligonucleotide (ASO) against clusterin to improve myelination and behavior phenotypes in the 5xFAD model

While the CLU KO/5xFAD mouse line and any inducible knockout lines generated will address the scientific question regarding the role of clusterin in the progression of AD, it will not elucidate whether we can effectively target clusterin in a therapeutic way to reduce AD clinical symptoms. Importantly, there is already an antisense oligonucleotide against clusterin that has been shown to effectively reduce circulating clusterin levels and has made it to Phase 3 clinical trials for cancer[250,318]. This means that, should reducing clusterin be effective at reducing AD symptoms in preclinical experiments, the repurposing of this existing ASO for AD treatment would be much quicker than developing a completely novel drug. To assess the use of a clusterin ASO in the 5xFAD Alzheimer's model, we would administer the ASO or a scramble oligonucleotide for a period of time during adulthood, and then assess both myelination and memory deficits as described above. In line with our previous data, we would expect that an ASO against clusterin, if it effectively reduces clusterin in the brain, will improve both the myelination deficits and memory impairment observed in 5xFAD mice.

There will be some technical difficulties associated with attempting to utilize an ASO to reduce gene expression in the brain, as ASOs will not cross the blood brain barrier. To circumvent this limitation we will plan to administer 4 weekly doses of a clusterin ASO via intra-cisterna magna injection, as previously described in the literature[322,323]. We plan to assess the efficacy of ASO treatment at multiple disease time points to determine at what stage of disease this treatment might be effective. Ultimately,

these experiments will offer clinically relevant data to build a foundation for the use of clusterin-reducing drugs in the treatment of Alzheimer's disease.

Investigate how OPC clusters change through the course of development and disease

Finally, there remains a large amount of work to be done to further characterize and investigate the two clusters of OPCs that we describe in Chapter III of this dissertation. While this work describes their existence in the adult brain, we still do not know if these same clusters are present during development, or if they are unique to adulthood. Similarly, we do not know the dynamics of these two clusters in the context of diseases such as AD and multiple sclerosis. Open questions about the clusters during disease include if the clusters are present during disease states, if the proportions of these clusters change during disease, or if a new cluster altogether is present in the context of pathology. These questions could be addressed using RNAscope as we used previously, or by using the newly developed spatial transcriptomics that would allow us to significantly expand the number of markers we could use in the brain at any one time. Ultimately, there remains a large amount of work to do in elucidating the roles of these cluster in both homeostasis and disease, especially since we have an entire single-cell dataset that catalogues differential gene expression between these two clusters. While I have evaluated the role of clusterin in OPC function within this dissertation, there are numerous other gene that are unique to an individual cluster and may provide an insight into OPC function.

Conclusions

This dissertation has characterized the transcriptional heterogeneity of OPCs in the adult brain and has begun to elucidate how a gene found in one of the clusters, clusterin, affects the function of OPCs in Alzheimer's disease. This work, however, is just the beginning of both the investigation into the effects of clusterin on OPCs, as well as the implications of this transcriptional heterogeneity on the functions of OPCs. Until recently, OPCs have been viewed simply as progenitor cells for mature oligodendrocytes and nothing more. Therefore, the field of OPC biology that focuses on roles of OPCs outside the scope of differentiating remains in its infancy. While this dissertation has begun to investigate both transcriptional and functional heterogeneity of OPCs, I hope that this work will continue and this field will continue to grow over the coming years. I believe that elucidating the function of OPCs will provide the scientific and medical community not only with new knowledge regarding the functional biology of the CNS, but will also uncover novel therapeutic avenues for a multitude of neurobiological diseases.

Chapter VIII: References

1 Dimou, L. & Simons, M. Diversity of oligodendrocytes and their progenitors. *Current opinion in neurobiology* **47**, 73-79, doi:10.1016/j.conb.2017.09.015 (2017).

2 Leong, S. Y. *et al.* Heterogeneity of oligodendrocyte progenitor cells in adult human brain. *Ann Clin Transl Neurol* **1**, 272-283, doi:10.1002/acn3.55 (2014).

3 Birey, F. *et al.* Genetic and Stress-Induced Loss of NG2 Glia Triggers Emergence of Depressive-like Behaviors through Reduced Secretion of FGF2. *Neuron* **88**, 941-956, doi:10.1016/j.neuron.2015.10.046 (2015).

4 Nakano, M. *et al.* NG2 glial cells regulate neuroimmunological responses to maintain neuronal function and survival. *Sci Rep* **7**, 42041, doi:10.1038/srep42041 (2017).

5 Djogo, T. *et al.* Adult NG2-Glia Are Required for Median Eminence-Mediated Leptin Sensing and Body Weight Control. *Cell Metab* **23**, 797-810, doi:10.1016/j.cmet.2016.04.013 (2016).

6 Raff, M. C., Miller, R. H. & Noble, M. A Glial Progenitor-Cell That Develops Invitro into an Astrocyte or an Oligodendrocyte Depending on Culture-Medium. *Nature* **303**, 390-396, doi:DOI 10.1038/303390a0 (1983).

7 Espinosa de los Monteros, A., Zhang, M., & De Vellis, J. O2A progenitor cells transplanted into the neonatal rat brain develop into oligodendrocytes but not astrocytes. *Proceedings of the National Academy of Sciences of the United States of America* **50**, 50-54 (1993).

8 Zhu, X., Bergles, D. E. & Nishiyama, A. NG2 cells generate both oligodendrocytes and gray matter astrocytes. *Development* **135**, 145-157, doi:10.1242/dev.004895 (2008).

9 Kang, S. H., Fukaya, M., Yang, J. K., Rothstein, J. D. & Bergles, D. E. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. *Neuron* **68**, 668-681, doi:10.1016/j.neuron.2010.09.009 (2010).

10 Doering, L. C. & Fedoroff, S. Isolation and transplantation of oligodendrocyte precursor cells. *J Neurol Sci* **63**, 183-196 (1984).

11 Nishiyama, A., Yu, M., Drazba, J. A. & Tuohy, V. K. Normal and reactive NG2+ glial cells are distinct from resting and activated microglia. *J Neurosci Res* **48**, 299-312 (1997).

12 Nishiyama, A., Watanabe, M., Yang, Z. & Bu, J. Identity, distribution, and development of polydendrocytes: NG2-expressing glial cells. *J Neurocytol* **31**, 437-455 (2002).

13 Pozniak, C. D. *et al.* Sox10 directs neural stem cells toward the oligodendrocyte lineage by decreasing Suppressor of Fused expression. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 21795-21800, doi:10.1073/pnas.1016485107 (2010).

14 Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. & Wegner, M. Sox10, a novel transcriptional modulator in glial cells. *J Neurosci* **18**, 237-250 (1998).

15 Lu, Q. R., Dong-in Yuk, John A Alberta, Zhimin Zhu, Inka Pawlitzky, Joanne Chan, Andrew P McMahon, Charles D Stiles, David H Rowitch. Sonic Hedgehog–Regulated Oligodendrocyte Lineage Genes Encoding bHLH Proteins in the Mammalian Central Nervous System. *Neuron* **25**, 317-329 (2000).

16 Yao, R. Q. *et al.* Olig2 overexpression accelerates the differentiation of mouse embryonic stem cells into oligodendrocyte progenitor cells in vitro. *Dev Growth Differ* **56**, 511-517, doi:10.1111/dgd.12150 (2014).

17 Nishiyama, A., Lin, X. H., Giese, N., Heldin, C. H. & Stallcup, W. B. Co-localization of NG2 proteoglycan and PDGF alpha-receptor on O2A progenitor cells in the developing rat brain. *J Neurosci Res* **43**, 299-314, doi:10.1002/(SICI)1097-4547(19960201)43:3<299::AID-JNR5>3.0.CO;2-E (1996).

18 Hall, A., Giese, N. A. & Richardson, W. D. Spinal cord oligodendrocytes develop from ventrally derived progenitor cells that express PDGF alpha-receptors. *Development* **122**, 4085-4094 (1996).

19 Aghajanian H, C. Y., Rizer NW, Wang Q, Li L, Degenhardt K, Jain R. Pdgfrα functions in endothelial-derived cells to regulate neural crest cells and the development of the great arteries. *Disease Models & Mechanisms* **10**, 1101-1108 (2017).

20 Ozerdem, U., Grako, K., Dahlin-Huppe, K., Monosov, E. & Stallcup, W. The NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis. *Developmental Dynamics*, 218-227 (2001).

21 Jones, L. L., Yamaguchi, Y., Stallcup, W. B. & Tuszynski, M. H. NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. *Journal of Neuroscience* **22**, 2792-2803 (2002).

22 Lu, Q. R. *et al.* Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. *Cell* **109**, 75-86 (2002).

23 Williams, B. P., Read, J. & Price, J. The generation of neurons and oligodendrocytes from a common precursor cell. *Neuron* **7**, 685-693 (1991).

24 Kessaris, N. *et al.* Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nature neuroscience* **9**, 173-179, doi:10.1038/nn1620 (2006).

25 Ponti, G. *et al.* Cell cycle and lineage progression of neural progenitors in the ventricular-subventricular zones of adult mice. *Proceedings of the National Academy of Sciences of the United States of America* **110**, E1045-1054, doi:10.1073/pnas.1219563110 (2013).

26 Dawson, M. R., Polito, A., Levine, J. M. & Reynolds, R. NG2-expressing glial progenitor cells: an abundant and widespread population of cycling cells in the adult rat CNS. *Mol Cell Neurosci* **24**, 476-488 (2003).

27 Hughes, E. G., Kang, S. H., Fukaya, M. & Bergles, D. E. Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nature neuroscience* **16**, 668-676, doi:10.1038/nn.3390 (2013).

28 Choe, Y., Huynh, T. & Pleasure, S. Migration of Oligodendrocyte Progenitor Cells Is Controlled by Transforming Growth Factor β Family Proteins during Corticogenesis. *Journal of Neuroscience* **35**, 14973–14983 (2014).

29 Schmidt, C. *et al.* Analysis of motile oligodendrocyte precursor cells in vitro and in brain slices. *Glia* **20**, 284-298 (1997).

30 Hill, R. A., Patel, K. D., Goncalves, C. M., Grutzendler, J. & Nishiyama, A. Modulation of oligodendrocyte generation during a critical temporal window after NG2 cell division. *Nature neuroscience* **17**, 1518-1527, doi:10.1038/nn.3815 (2014).

31 Rivers, L. E. *et al.* PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. *Nature neuroscience* **11**, 1392-1401, doi:10.1038/nn.2220 (2008).

32 Geha, S. *et al.* NG2+/Olig2+ cells are the major cycle-related cell population of the adult human normal brain. *Brain Pathol* **20**, 399-411, doi:10.1111/j.1750-3639.2009.00295.x (2010).

33 Simon, C., Gotz, M. & Dimou, L. Progenitors in the adult cerebral cortex: cell cycle properties and regulation by physiological stimuli and injury. *Glia* **59**, 869-881, doi:10.1002/glia.21156 (2011).

34 Richardson, W. D., Young, K. M., Tripathi, R. B. & McKenzie, I. NG2-glia as multipotent neural stem cells: fact or fantasy? *Neuron* **70**, 661-673, doi:10.1016/j.neuron.2011.05.013 (2011).

35 Psachoulia, K., Jamen, F., Young, K. M. & Richardson, W. D. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. *Neuron Glia Biol* **5**, 57-67, doi:10.1017/S1740925X09990354 (2009).

36 Young, K. M. *et al.* Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. *Neuron* **77**, 873-885, doi:10.1016/j.neuron.2013.01.006 (2013).

37 Savas, J. N., Toyama, B. H., Xu, T., Yates, J. R., 3rd & Hetzer, M. W. Extremely long-lived nuclear pore proteins in the rat brain. *Science* **335**, 942, doi:10.1126/science.1217421 (2012).

38 Toyama, B. H. *et al.* Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell* **154**, 971-982, doi:10.1016/j.cell.2013.07.037 (2013).

39 Mackenzie, S. *et al.* Depression and suicide ideation among students accessing campus health care. *The American journal of orthopsychiatry* **81**, 101-107, doi:10.1111/j.1939-0025.2010.01077.x (2011).

40 Tripathi, R. B. *et al.* Remarkable Stability of Myelinating Oligodendrocytes in Mice. *Cell Rep* **21**, 316-323, doi:10.1016/j.celrep.2017.09.050 (2017).

41 Yeung, M. S. *et al.* Dynamics of oligodendrocyte generation and myelination in the human brain. *Cell* **159**, 766-774, doi:10.1016/j.cell.2014.10.011 (2014).

42 Ivanova, A. *et al.* Evidence for a second wave of oligodendrogenesis in the postnatal cerebral cortex of the mouse. *J Neurosci Res* **73**, 581-592, doi:10.1002/jnr.10717 (2003).

43 Winterbottom, E. F., Illes, J. C., Faas, L. & Isaacs, H. V. Conserved and novel roles for the Gsh2 transcription factor in primary neurogenesis. *Development* **137**, 2623-2631, doi:10.1242/dev.047159 (2010).

44 Tripathi, R. B. *et al.* Dorsally and Ventrally Derived Oligodendrocytes Have Similar Electrical Properties but Myelinate Preferred Tracts. *Journal of Neuroscience* **31**, 6809-6819, doi:10.1523/Jneurosci.6474-10.2011 (2011).

45 Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M. & Noble-Haeusslein, L. J. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* **106-107**, 1-16, doi:10.1016/j.pneurobio.2013.04.001 (2013).
46 Cai, J. *et al.* Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. *Neuron* **45**, 41-53, doi:10.1016/j.neuron.2004.12.028 (2005).
47 Vallstedt, A., Klos, J. M. & Ericson, J. Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. *Neuron* **45**, 55-67, doi:10.1016/j.neuron.2004.12.026 (2005).
48 Winkler, C. C. *et al.* The Dorsal Wave of Neocortical Oligodendrogenesis Begins Embryonically and Requires Multiple Sources of Sonic Hedgehog. *J Neurosci*, doi:10.1523/JNEUROSCI.3392-17.2018 (2018).
49 Liu, R. *et al.* Region-specific and stage-dependent regulation of Olig gene expression and oligodendrogenesis by Nkx6.1 homeodomain transcription factor. *Development* **130**, 6221-6231, doi:10.1242/dev.00868 (2003).
50 Southwood, C. *et al.* CNS myelin paranodes require Nkx6-2 homeoprotein transcriptional activity for normal structure. *J Neurosci* **24**, 11215-11225, doi:10.1523/JNEUROSCI.3479-04.2004 (2004).
51 Takebayashi, H. *et al.* The basic helix-loop-helix factor Olig2 is essential for the development of motoneuron and oligodendrocyte lineages. *Current Biology* **12**, 1157-1163, doi:Pii S0960-9822(02)00926-0

Doi 10.1016/S0960-9822(02)00926-0 (2002).
52 Zhou, Q. & Anderson, D. J. The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. *Cell* **109**, 61-73 (2002).
53 Pattyn, A. Complementary roles for Nkx6 and Nkx2 class proteins in the establishment of motoneuron identity in the hindbrain. *Development* **130**, 4149-4159, doi:10.1242/dev.00641 (2003).
54 Levine, J. M., Stincone, F. & Lee, Y. S. Development and differentiation of glial precursor cells in the rat cerebellum. *Glia* **7**, 307-321, doi:10.1002/glia.440070406 (1993).
55 Reynolds, R. & Hardy, R. Oligodendroglial progenitors labeled with the O4 antibody persist in the adult rat cerebral cortex in vivo. *J Neurosci Res* **47**, 455-470 (1997).
56 Guo, F., Ma, J., McCauley, E., Bannerman, P. & Pleasure, D. Early postnatal proteolipid promoter-expressing progenitors produce multilineage cells in vivo. *J Neurosci* **29**, 7256-7270, doi:10.1523/JNEUROSCI.5653-08.2009 (2009).
57 Raff, M. C., Williams, B. P. & Miller, R. H. The in vitro differentiation of a bipotential glial progenitor cell. *Embo J* **3**, 1857-1864 (1984).
58 Dimou, L., Simon, C., Kirchhoff, F., Takebayashi, H. & Gotz, M. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. *J Neurosci* **28**, 10434-10442, doi:10.1523/JNEUROSCI.2831-08.2008 (2008).
59 Marques, S. *et al.* Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science* **352**, 1326-1329, doi:10.1126/science.aaf6463 (2016).

60 Gibson, E. M. *et al.* Neuronal Activity Promotes Oligodendrogenesis and Adaptive Myelination in the Mammalian Brain. *Science* **344** (2014).

61 McKenzie, I. A. *et al.* Motor skill learning requires active central myelination. *Science* **346**, 318-322, doi:10.1126/science.1254960 (2014).

62 Xiao, L. *et al.* Rapid production of new oligodendrocytes is required in the earliest stages of motor-skill learning. *Nature neuroscience* **19**, 1210-1217, doi:10.1038/nn.4351 (2016).

63 Mitew, S. *et al.* Pharmacogenetic stimulation of neuronal activity increases myelination in an axon-specific manner. *Nat Commun* **9**, 306, doi:10.1038/s41467-017-02719-2 (2018).

64 Bergles, D. E., Roberts, J. D., Somogyi, P. & Jahr, C. E. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* **405**, 187-191, doi:10.1038/35012083 (2000).

65 Kondo, T. & Raff, M. Oligodendrocyte precursor cells reprogrammed to become multipotential CNS stem cells. *Science* **289**, 1754-1757 (2000).

66 Franklin, R. J. M., Bayley, S. A., Milner, R., Ffrenchconstant, C. & Blakemore, W. F. Differentiation of the O-2a Progenitor-Cell Line Cg-4 into Oligodendrocytes and Astrocytes Following Transplantation into Glia-Deficient Areas of Cns White-Matter. *Glia* **13**, 39-44, doi:DOI 10.1002/glia.440130105 (1995).

67 Belachew, S. *et al.* Postnatal NG2 proteoglycan–expressing progenitor cells are intrinsically multipotent and generate functional neurons. *The Journal of Cell Biology* **161**, 169-186, doi:10.1083/jcb.200210110 (2003).

68 Alonso, G. NG2 proteoglycan-expressing cells of the adult rat brain: possible involvement in the formation of glial scar astrocytes following stab wound. *Glia* **49**, 318-338, doi:10.1002/glia.20121 (2005).

69 Zhu, X. *et al.* Age-dependent fate and lineage restriction of single NG2 cells. *Development* **138**, 745-753, doi:10.1242/dev.047951 (2011).

70 Guo, F. *et al.* Pyramidal neurons are generated from oligodendroglial progenitor cells in adult piriform cortex. *J Neurosci* **30**, 12036-12049, doi:10.1523/JNEUROSCI.1360-10.2010 (2010).

71 Robins, S. C. *et al.* Evidence for NG2-glia derived, adult-born functional neurons in the hypothalamus. *PLoS One* **8**, e78236, doi:10.1371/journal.pone.0078236 (2013).

72 Clarke, L. E. *et al.* Properties and fate of oligodendrocyte progenitor cells in the corpus callosum, motor cortex, and piriform cortex of the mouse. *J Neurosci* **32**, 8173-8185, doi:10.1523/JNEUROSCI.0928-12.2012 (2012).

73 Chittajallu, R., Aguirre, A. & Gallo, V. NG2-positive cells in the mouse white and grey matter display distinct physiological properties. *J Physiol* **561**, 109-122, doi:10.1113/jphysiol.2004.074252 (2004).

74 Vue, T. Y., Kim, E. J., Parras, C. M., Guillemot, F. & Johnson, J. E. Ascl1 controls the number and distribution of astrocytes and oligodendrocytes in the gray matter and white matter of the spinal cord. *Development* **141**, 3721-3731, doi:10.1242/dev.105270 (2014).

75 Vigano, F., Mobius, W., Gotz, M. & Dimou, L. Transplantation reveals regional differences in oligodendrocyte differentiation in the adult brain. *Nature neuroscience* **16**, 1370-1372, doi:10.1038/nn.3503 (2013).

76 Nave, K. A., Lai, C., Bloom, F. E. & Milner, R. J. Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 9264-9268 (1986).

77 Thomson, C. E. *et al.* The early phenotype associated with the jimpy mutation of the proteolipid protein gene. *J Neurocytol* **28**, 207-221 (1999).

78 Baracskay, K. L., Duchala, C. S., Miller, R. H., Macklin, W. B. & Trapp, B. D. Oligodendrogenesis is differentially regulated in gray and white matter of jimpy mice. *Journal of Neuroscience Research* **70**, 645-654, doi:10.1002/jnr.10418 (2002).

79 Hua, K., Schindler, M. K., McQuail, J. A., Forbes, M. E. & Riddle, D. R. Regionally distinct responses of microglia and glial progenitor cells to whole brain irradiation in adult and aging rats. *PLoS One* **7**, e52728, doi:10.1371/journal.pone.0052728 (2012).

80 Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. & Riddle, P. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562, doi:10.1038/333560a0 (1988).

81 Hill, R. A., Patel, K. D., Medved, J., Reiss, A. M. & Nishiyama, A. NG2 Cells in White Matter But Not Gray Matter Proliferate in Response to PDGF. *Journal of Neuroscience* **33**, 14558-+, doi:10.1523/Jneurosci.2001-12.2013 (2013).

82 Ciana, P. *et al.* The orphan receptor GPR17 identified as a new dual uracil nucleotides/cysteinyl-leukotrienes receptor. *Embo J* **25**, 4615-4627, doi:10.1038/sj.emboj.7601341 (2006).

83 Chen, Y. *et al.* The oligodendrocyte-specific G protein–coupled receptor GPR17 is a cell-intrinsic timer of myelination. *Nature neuroscience* **12**, 1398, doi:10.1038/nn.2410 (2009).

84 Vigano, F. *et al.* GPR17 expressing NG2-Glia: Oligodendrocyte progenitors serving as a reserve pool after injury. *Glia* **64**, 287-299, doi:10.1002/glia.22929 (2016).

85 Fumagalli, M., Lecca, D., Coppolino, G. T., Parravicini, C. & Abbracchio, M. P. Pharmacological Properties and Biological Functions of the GPR17 Receptor, a Potential Target for Neuro-Regenerative Medicine. *Adv Exp Med Biol* **1051**, 169-192, doi:10.1007/5584_2017_92 (2017).

86 Marucci, G. *et al.* The G Protein-Coupled Receptor GPR17: Overview and Update. *ChemMedChem* **11**, 2567-2574, doi:10.1002/cmdc.201600453 (2016).

87 Boda, E. *et al.* Early phenotypic asymmetry of sister oligodendrocyte progenitor cells after mitosis and its modulation by aging and extrinsic factors. *Glia* **63**, 271-286, doi:10.1002/glia.22750 (2015).

88 Fumagalli, M. *et al.* Phenotypic changes, signaling pathway, and functional correlates of GPR17-expressing neural precursor cells during oligodendrocyte differentiation. *J Biol Chem* **286**, 10593-10604, doi:10.1074/jbc.M110.162867 (2011).

89 Fumagalli, M. *et al.* The ubiquitin ligase Mdm2 controls oligodendrocyte maturation by intertwining mTOR with G protein-coupled receptor kinase 2 in the

regulation of GPR17 receptor desensitization. *Glia* **63**, 2327-2339, doi:10.1002/glia.22896 (2015).

90 Ceruti, S. *et al.* Expression of the new P2Y-like receptor GPR17 during oligodendrocyte precursor cell maturation regulates sensitivity to ATP-induced death. *Glia* **59**, 363-378, doi:10.1002/glia.21107 (2011).

91 Lecca, D. *et al.* The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. *PLoS One* **3**, e3579, doi:10.1371/journal.pone.0003579 (2008).

92 Bonfanti, E. *et al.* The role of oligodendrocyte precursor cells expressing the GPR17 receptor in brain remodeling after stroke. *Cell Death Dis* **8**, e2871, doi:10.1038/cddis.2017.256 (2017).

93 Franke, H. *et al.* Changes of the GPR17 receptor, a new target for neurorepair, in neurons and glial cells in patients with traumatic brain injury. *Purinergic Signal* **9**, 451-462, doi:10.1007/s11302-013-9366-3 (2013).

94 Ceruti, S. *et al.* The P2Y-like receptor GPR17 as a sensor of damage and a new potential target in spinal cord injury. *Brain* **132**, 2206-2218, doi:10.1093/brain/awp147 (2009).

95 Boccazzi, M. *et al.* A new role for the P2Y-like GPR17 receptor in the modulation of multipotency of oligodendrocyte precursor cells in vitro. *Purinergic Signal* **12**, 661-672, doi:10.1007/s11302-016-9530-7 (2016).

96 Raposo, A. A. *et al.* Ascl1 Coordinately Regulates Gene Expression and the Chromatin Landscape during Neurogenesis. *Cell Rep*, doi:10.1016/j.celrep.2015.02.025 (2015).

97 Nakatani, H. *et al.* Ascl1/Mash1 promotes brain oligodendrogenesis during myelination and remyelination. *J Neurosci* **33**, 9752-9768, doi:10.1523/JNEUROSCI.0805-13.2013 (2013).

98 Parras, C. M. *et al.* The proneural gene Mash1 specifies an early population of telencephalic oligodendrocytes. *J Neurosci* **27**, 4233-4242, doi:10.1523/JNEUROSCI.0126-07.2007 (2007).

99 Szuchet, S. *et al.* The genetic signature of perineuronal oligodendrocytes reveals their unique phenotype. *European Journal of Neuroscience* **34**, 1906-1922, doi:10.1111/j.1460-9568.2011.07922.x (2011).

100 Sugimori, M. *et al.* Ascl1 is required for oligodendrocyte development in the spinal cord. *Development* **135**, 1271-1281, doi:10.1242/dev.015370 (2008).

101 Kelenis, D. P., Hart, E., Edwards-Fligner, M., Johnson, J. E. & Vue, T. Y. ASCL1 regulates proliferation of NG2-glia in the embryonic and adult spinal cord. *Glia*, doi:10.1002/glia.23344 (2018).

102 Kim, E. J., Leung, C. T., Reed, R. R. & Johnson, J. E. In vivo analysis of Ascl1 defined progenitors reveals distinct developmental dynamics during adult neurogenesis and gliogenesis. *J Neurosci* **27**, 12764-12774, doi:10.1523/JNEUROSCI.3178-07.2007 (2007).

103 Fields, R. D. & Stevens-Graham, B. New Insights into Neuron-Glia Communication. *Science (New York, N.Y.)* **298**, 556-562, doi:10.1126/science.298.5593.556 (2002).

104 Barres, B. A. & Raff, M. C. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. *Nature* **361**, 258-260, doi:10.1038/361258a0 (1993).

105 Karadottir, R., Hamilton, N. B., Bakiri, Y. & Attwell, D. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. *Nature neuroscience* **11**, 450-456, doi:10.1038/nn2060 (2008).

106 De Biase, L. M., Nishiyama, A. & Bergles, D. E. Excitability and synaptic communication within the oligodendrocyte lineage. *J Neurosci* **30**, 3600-3611, doi:10.1523/JNEUROSCI.6000-09.2010 (2010).

107 Gautier, H. O. *et al.* Neuronal activity regulates remyelination via glutamate signalling to oligodendrocyte progenitors. *Nat Commun* **6**, 8518, doi:10.1038/ncomms9518 (2015).

108 Fannon, J., Tarmier, W. & Fulton, D. Neuronal activity and AMPA-type glutamate receptor activation regulates the morphological development of oligodendrocyte precursor cells. *Glia* **63**, 1021-1035, doi:10.1002/glia.22799 (2015).

109 Harlow, D. E., Saul, K. E., Komuro, H. & Macklin, W. B. Myelin Proteolipid Protein Complexes with αv Integrin and AMPA Receptors In Vivo and Regulates AMPA-Dependent Oligodendrocyte Progenitor Cell Migration through the Modulation of Cell-Surface GluR2 Expression. *The Journal of Neuroscience* **35**, 12018 (2015).

110 Li, C. *et al.* A functional role of NMDA receptor in regulating the differentiation of oligodendrocyte precursor cells and remyelination. *Glia* **61**, 732-749, doi:10.1002/glia.22469 (2013).

111 Mensch, S. *et al.* Synaptic vesicle release regulates myelin sheath number of individual oligodendrocytes in vivo. *Nature neuroscience* **18**, 628-630, doi:10.1038/nn.3991 (2015).

112 Faulkner, J. R. *et al.* Reactive astrocytes protect tissue and preserve function after spinal cord injury. *J Neurosci* **24**, 2143-2155, doi:10.1523/JNEUROSCI.3547-03.2004 (2004).

113 McTigue, D. M., Wei, P. & Stokes, B. T. Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. *J Neurosci* **21**, 3392-3400 (2001).

114 Zai, L. J. & Wrathall, J. R. Cell proliferation and replacement following contusive spinal cord injury. *Glia* **50**, 247-257, doi:10.1002/glia.20176 (2005).

115 Buss, A. *et al.* NG2 and phosphacan are present in the astroglial scar after human traumatic spinal cord injury. *BMC Neurology* **9**, 32, doi:10.1186/1471-2377-9-32 (2009).

116 Hesp, Z. C. *et al.* Proliferating NG2-Cell-Dependent Angiogenesis and Scar Formation Alter Axon Growth and Functional Recovery After Spinal Cord Injury in Mice. *The Journal of Neuroscience* **38**, 1366 (2018).

117 Rhodes, K. E., Moon, L. D. & Fawcett, J. W. Inhibiting cell proliferation during formation of the glial scar: effects on axon regeneration in the CNS. *Neuroscience* **120**, 41-56 (2003).

118 Tang, X., Davies, J. E. & Davies, S. J. Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican

V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. *J Neurosci Res* **71**, 427-444, doi:10.1002/jnr.10523 (2003).

119 Bradbury, E. J. *et al.* Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* **416**, 636-640, doi:10.1038/416636a (2002).

120 Tan, A. M., Zhang, W. & Levine, J. M. NG2: a component of the glial scar that inhibits axon growth. *Journal of Anatomy* **207**, 717-725, doi:10.1111/j.1469-7580.2005.00452.x (2005).

121 Rodriguez, J. P. *et al.* Abrogation of beta-catenin signaling in oligodendrocyte precursor cells reduces glial scarring and promotes axon regeneration after CNS injury. *J Neurosci* **34**, 10285-10297, doi:10.1523/JNEUROSCI.4915-13.2014 (2014).

122 Hesp, Z. C., Goldstein, E. Z., Miranda, C. J., Kaspar, B. K. & McTigue, D. M. Chronic oligodendrogenesis and remyelination after spinal cord injury in mice and rats. *J Neurosci* **35**, 1274-1290, doi:10.1523/JNEUROSCI.2568-14.2015 (2015).

123 Honsa, P. *et al.* Generation of reactive astrocytes from NG2 cells is regulated by sonic hedgehog. *Glia* **64**, 1518-1531, doi:10.1002/glia.23019 (2016).

124 Zhao, J. W., Raha-Chowdhury, R., Fawcett, J. W. & Watts, C. Astrocytes and oligodendrocytes can be generated from NG2+ progenitors after acute brain injury: intracellular localization of oligodendrocyte transcription factor 2 is associated with their fate choice. *European Journal of Neuroscience* **29**, 1853-1869, doi:10.1111/j.1460-9568.2009.06736.x (2009).

125 Jakel, S. & Dimou, L. Glial Cells and Their Function in the Adult Brain: A Journey through the History of Their Ablation. *Front Cell Neurosci* **11**, 24, doi:10.3389/fncel.2017.00024 (2017).

126 Zhang, Y. W., Denham, J. & Thies, R. S. Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. *Stem Cells Dev* **15**, 943-952, doi:10.1089/scd.2006.15.943 (2006).

127 Seo, J. H. *et al.* Oligodendrocyte Precursor Cells Support Blood-Brain Barrier Integrity via TGF-β Signaling. *PLOS ONE* **9**, e103174, doi:10.1371/journal.pone.0103174 (2014).

128 Sypecka, J. & Sarnowska, A. The Neuroprotective Effect Exerted by Oligodendroglial Progenitors on Ischemically Impaired Hippocampal Cells. *Molecular neurobiology* **49**, 685-701, doi:10.1007/s12035-013-8549-9 (2014).

129 Psachoulia, K. *et al.* IL4I1 augments CNS remyelination and axonal protection by modulating T cell driven inflammation. *Brain* **139**, 3121-3136, doi:10.1093/brain/aww254 (2016).

130 Peymani, M. *et al.* Ameliorating the Effect of Pioglitazone on LPS-Induced Inflammation of Human Oligodendrocyte Progenitor Cells. *Cellular and Molecular Neurobiology* **38**, 517-527, doi:10.1007/s10571-017-0500-6 (2018).

131 Valentin-Torres, A., Savarin, C., Barnett, J. & Bergmann, C. C. Blockade of sustained tumor necrosis factor in a transgenic model of progressive autoimmune encephalomyelitis limits oligodendrocyte apoptosis and promotes oligodendrocyte maturation. *J Neuroinflammation* **15**, 121, doi:10.1186/s12974-018-1164-y (2018).

132 Kang, Z. *et al.* Act1 mediates IL-17-induced EAE pathogenesis selectively in NG2+ glial cells. *Nature neuroscience* **16**, 1401-1408, doi:10.1038/nn.3505 (2013).

133 Wang, C. *et al.* IL-17 induced NOTCH1 activation in oligodendrocyte progenitor cells enhances proliferation and inflammatory gene expression. *Nature Communications* **8**, 15508, doi:10.1038/ncomms15508 (2017).

134 Deshmane, S. L., Kremlev, S., Amini, S. & Sawaya, B. E. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res* **29**, 313-326, doi:10.1089/jir.2008.0027 (2009).

135 Moyon, S. *et al.* Demyelination Causes Adult CNS Progenitors to Revert to an Immature State and Express Immune Cues That Support Their Migration. *The Journal of Neuroscience* **35**, 4 (2015).

136 Seo, J. H. *et al.* Oligodendrocyte precursors induce early blood-brain barrier opening after white matter injury. *J Clin Invest* **123**, 782-786, doi:10.1172/JCI65863 (2013).

137 Van den Steen, P. E. *et al.* Biochemistry and Molecular Biology of Gelatinase B or Matrix Metalloproteinase-9 (MMP-9). *Critical Reviews in Biochemistry and Molecular Biology* **37**, 375-536, doi:10.1080/10409230290771546 (2002).

138 Ferrara, G. *et al.* NG2, a common denominator for neuroinflammation, blood–brain barrier alteration, and oligodendrocyte precursor response in EAE, plays a role in dendritic cell activation. *Acta Neuropathologica* **132**, 23-42, doi:10.1007/s00401-016-1563-z (2016).

139 Williams, M. *et al.* Fibrillary astrocytes are decreased in the subgenual cingulate in schizophrenia. *Eur Arch Psychiatry Clin Neurosci* **264**, 357-362, doi:10.1007/s00406-013-0482-4 (2014).

140 Ongur, D., Drevets, W. C. & Price, J. L. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 13290-13295 (1998).

141 Yang, Y., Zhang, Y., Luo, F. & Li, B. Chronic stress regulates NG2+ cell maturation and myelination in the prefrontal cortex through induction of death receptor 6. *Experimental Neurology* **277**, 202-214, doi:https://doi.org/10.1016/j.expneurol.2016.01.003 (2016).

142 Czéh, B. *et al.* Chronic Social Stress Inhibits Cell Proliferation in the Adult Medial Prefrontal Cortex: Hemispheric Asymmetry and Reversal by Fluoxetine Treatment. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **32**, 1490, doi:10.1038/sj.npp.1301275 (2006).

143 Banasr, M. *et al.* Chronic Unpredictable Stress Decreases Cell Proliferation in the Cerebral Cortex of the Adult Rat. *Biological Psychiatry* **62**, 496-504, doi:https://doi.org/10.1016/j.biopsych.2007.02.006 (2007).

144 Liu, J., Dietz, K., Hodes, G. E., Russo, S. J. & Casaccia, P. Widespread transcriptional alternations in oligodendrocytes in the adult mouse brain following chronic stress. *Dev Neurobiol* **78**, 152-162, doi:10.1002/dneu.22533 (2018).

145 Li, Y. *et al.* cxcl12-engineered endothelial progenitor cells enhance neurogenesis and angiogenesis after ischemic brain injury in mice. *Stem Cell Res Ther* **9**, 139, doi:10.1186/s13287-018-0865-6 (2018).

146 Lehmann, M. L., Weigel, T. K., Elkahloun, A. G. & Herkenham, M. Chronic social defeat reduces myelination in the mouse medial prefrontal cortex. *Scientific Reports* **7**, 46548, doi:10.1038/srep46548

https://www.nature.com/articles/srep46548#supplementary-information (2017).

147 Aston, C., Jiang, L. & Sokolov, B. P. Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Molecular psychiatry* **10**, 309, doi:10.1038/sj.mp.4001565

https://www.nature.com/articles/4001565#supplementary-information (2004).

148 Sibille, E. *et al.* A molecular signature of depression in the amygdala. *The American journal of psychiatry* **166**, 1011-1024, doi:10.1176/appi.ajp.2009.08121760 (2009).

149 Liu, J. *et al.* Impaired adult myelination in the prefrontal cortex of socially isolated mice. *Nature neuroscience* **15**, 1621, doi:10.1038/nn.3263

https://www.nature.com/articles/nn.3263#supplementary-information (2012).

150 Baxi, E. G. *et al.* A selective thyroid hormone beta receptor agonist enhances human and rodent oligodendrocyte differentiation. *Glia* **62**, 1513-1529, doi:10.1002/glia.22697 (2014).

151 Oakley, H. *et al.* Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* **26**, 10129-10140, doi:10.1523/jneurosci.1202-06.2006 (2006).

152 Pedraza, C. E., Monk, R., Lei, J., Hao, Q. & Macklin, W. B. Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors. *Glia* **56**, 1339-1352, doi:10.1002/glia.20702 (2008).

153 Stine, W. B., Jungbauer, L., Yu, C. & LaDu, M. J. in *Alzheimer's Disease and Frontotemporal Dementia: Methods and Protocols* (ed Erik D. Roberson) 13-32 (Humana Press, 2011).

154 Gaultier, A. *et al.* Low-density lipoprotein receptor-related protein 1 is an essential receptor for myelin phagocytosis. *J Cell Sci* **122**, 1155-1162, doi:10.1242/jcs.040717 (2009).

155 Pluvinage, J. V. *et al.* CD22 blockade restores homeostatic microglial phagocytosis in ageing brains. *Nature* **568**, 187-192, doi:10.1038/s41586-019-1088-4 (2019).

156 Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol* **36**, 411-420, doi:10.1038/nbt.4096 (2018).

157 Stuart, T. *et al.* Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902 e1821, doi:10.1016/j.cell.2019.05.031 (2019).

158 Buettner, F. *et al.* Computational analysis of cell-to-cell heterogeneity in single-cell RNA-sequencing data reveals hidden subpopulations of cells. *Nat Biotechnol* **33**, 155-160, doi:10.1038/nbt.3102 (2015).

159 Erben, L., He, M.-X., Laeremans, A., Park, E. & Buonanno, A. A Novel Ultrasensitive In Situ Hybridization Approach to Detect Short Sequences and

Splice Variants with Cellular Resolution. *Molecular neurobiology* **55**, 6169-6181, doi:10.1007/s12035-017-0834-6 (2018).
160 Hrvatin, S. *et al.* Single-cell analysis of experience-dependent transcriptomic states in the mouse visual cortex. *Nature neuroscience* **21**, 120-129, doi:10.1038/s41593-017-0029-5 (2018).
161 Orduz, D. *et al.* Developmental cell death regulates lineage-related interneuron-oligodendroglia functional clusters and oligodendrocyte homeostasis. *Nat Commun* **10**, 4249, doi:10.1038/s41467-019-11904-4 (2019).
162 Spitzer, S. O. *et al.* Oligodendrocyte Progenitor Cells Become Regionally Diverse and Heterogeneous with Age. *Neuron*, doi:10.1016/j.neuron.2018.12.020 (2019).
163 Falcão, A. M. *et al.* Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. *Nature Medicine*, doi:10.1038/s41591-018-0236-y (2018).
164 Fernández-Castañeda, A. *et al.* The active contribution of OPCs to neuroinflammation is mediated by LRP1. *Acta Neuropathologica*, doi:10.1007/s00401-019-02073-1 (2019).
165 Kirby, L. *et al.* Oligodendrocyte precursor cells present antigen and are cytotoxic targets in inflammatory demyelination. *Nature Communications* **10**, 3887, doi:10.1038/s41467-019-11638-3 (2019).
166 Aghajanian, H. *et al.* Pdgfrα functions in endothelial-derived cells to regulate neural crest cells and the development of the great arteries. *Disease Models & Mechanisms* **10**, 1101-1108 (2017).
167 Li, R. *et al.* Pdgfra marks a cellular lineage with distinct contributions to myofibroblasts in lung maturation and injury response. *eLife* **7**, e36865, doi:10.7554/eLife.36865 (2018).
168 Endale, M. *et al.* Temporal, spatial, and phenotypical changes of PDGFRα expressing fibroblasts during late lung development. *Developmental biology* **425**, 161-175, doi:10.1016/j.ydbio.2017.03.020 (2017).
169 Marques, S. *et al.* Transcriptional Convergence of Oligodendrocyte Lineage Progenitors during Development. *Developmental Cell* (2018).
170 Witmer, A. N., van Blijswijk, B. C., van Noorden, C. J. F., Vrensen, G. F. J. M. & Schlingemann, R. O. In vivo angiogenic phenotype of endothelial cells and pericytes induced by vascular endothelial growth factor-A. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society* **52**, 39-52, doi:10.1177/002215540405200105 (2004).
171 Xiao, W. *et al.* Ozone oil promotes wound healing by increasing the migration of fibroblasts via PI3K/Akt/mTOR signaling pathway. *Biosci Rep* **37**, BSR20170658, doi:10.1042/BSR20170658 (2017).
172 Bondjers, C. *et al.* Transcription profiling of platelet-derived growth factor-B-deficient mouse embryos identifies RGS5 as a novel marker for pericytes and vascular smooth muscle cells. *The American journal of pathology* **162**, 721-729, doi:10.1016/S0002-9440(10)63868-0 (2003).
173 Smyth, L. C. D. *et al.* Markers for human brain pericytes and smooth muscle cells. *J Chem Neuroanat* **92**, 48-60, doi:10.1016/j.jchemneu.2018.06.001 (2018).
174 Falcao, A. M. *et al.* Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. *Nat Med*, doi:10.1038/s41591-018-0236-y (2018).

175 Gu, H. *et al.* Association of Clusterin Gene Polymorphisms with Late-Onset Alzheimer's Disease. *Dementia and Geriatric Cognitive Disorders* **32**, 198-201, doi:10.1159/000331276 (2011).
176 Villa, A. *et al.* Sex-Specific Features of Microglia from Adult Mice. *Cell reports* **23**, 3501-3511, doi:10.1016/j.celrep.2018.05.048 (2018).
177 Santos-Galindo, M., Acaz-Fonseca, E., Bellini, M. J. & Garcia-Segura, L. M. Sex differences in the inflammatory response of primary astrocytes to lipopolysaccharide. *Biology of sex differences* **2**, 7, doi:10.1186/2042-6410-2-7 (2011).
178 van den Brink, S. C. *et al.* Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. *Nature methods* **14**, 935-936, doi:10.1038/nmeth.4437 (2017).
179 Marinov, G. K. *et al.* From single-cell to cell-pool transcriptomes: stochasticity in gene expression and RNA splicing. *Genome research* **24**, 496-510, doi:10.1101/gr.161034.113 (2014).
180 Grün, D., Kester, L. & van Oudenaarden, A. Validation of noise models for single-cell transcriptomics. *Nature methods* **11**, 637-640, doi:10.1038/nmeth.2930 (2014).
181 DeSisto, J. *et al.* Single-Cell Transcriptomic Analyses of the Developing Meninges Reveal Meningeal Fibroblast Diversity and Function. *Developmental Cell* **54**, 43-59.e44, doi:https://doi.org/10.1016/j.devcel.2020.06.009 (2020).
182 Vanlandewijck, M. *et al.* A molecular atlas of cell types and zonation in the brain vasculature. *Nature* **554**, 475-480, doi:10.1038/nature25739 (2018).
183 Hammond, T. R. *et al.* Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* **50**, 253-271.e256, doi:10.1016/j.immuni.2018.11.004 (2019).
184 Masuda, T. *et al.* Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. *Nature* **566**, 388-392, doi:10.1038/s41586-019-0924-x (2019).
185 Xin, W. & Bonci, A. Functional Astrocyte Heterogeneity and Implications for Their Role in Shaping Neurotransmission. *Frontiers in cellular neuroscience* **12**, 141-141, doi:10.3389/fncel.2018.00141 (2018).
186 John Lin, C.-C. *et al.* Identification of diverse astrocyte populations and their malignant analogs. *Nature neuroscience* **20**, 396-405, doi:10.1038/nn.4493 (2017).
187 Mackie, M., Hughes, D. I., Maxwell, D. J., Tillakaratne, N. J. & Todd, A. J. Distribution and colocalisation of glutamate decarboxylase isoforms in the rat spinal cord. *Neuroscience* **119**, 461-472 (2003).
188 Marisca, R. *et al.* Functionally distinct subgroups of oligodendrocyte precursor cells integrate neural activity and execute myelin formation. *Nature neuroscience*, doi:10.1038/s41593-019-0581-2 (2020).
189 Perlman, K. *et al.* Developmental trajectory of oligodendrocyte progenitor cells in the human brain revealed by single cell RNA sequencing. *Glia* **n/a**, doi:10.1002/glia.23777 (2020).

190 Chamling, X. *et al.* Single-cell transcriptomic reveals molecular diversity and developmental heterogeneity of human stem cell-derived oligodendrocyte lineage cells. *Nat Commun* **12**, 652, doi:10.1038/s41467-021-20892-3 (2021).
191 Foerster, S., Hill, M. F. E. & Franklin, R. J. M. Diversity in the oligodendrocyte lineage: Plasticity or heterogeneity? *Glia* **67**, 1797-1805, doi:10.1002/glia.23607 (2019).
192 Zheng, K. *et al.* Molecular and Genetic Evidence for the PDGFRα-Independent Population of Oligodendrocyte Progenitor Cells in the Developing Mouse Brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **38**, 9505-9513, doi:10.1523/JNEUROSCI.1510-18.2018 (2018).
193 Salat, D. H. *et al.* White matter pathology isolates the hippocampal formation in Alzheimer's disease. *Neurobiology of aging* **31**, 244-256, doi:https://doi.org/10.1016/j.neurobiolaging.2008.03.013 (2010).
194 Franklin, R. J. Why does remyelination fail in multiple sclerosis? *Nature reviews. Neuroscience* **3**, 705-714, doi:10.1038/nrn917 (2002).
195 Loveless, S. *et al.* Tissue microarray methodology identifies complement pathway activation and dysregulation in progressive multiple sclerosis. *Brain Pathology* **28**, 507-520, doi:10.1111/bpa.12546 (2018).
196 Miners, J. S., Clarke, P. & Love, S. Clusterin levels are increased in Alzheimer's disease and influence the regional distribution of Aβ. *Brain pathology (Zurich, Switzerland)* **27**, 305-313, doi:10.1111/bpa.12392 (2017).
197 Zhang, P. *et al.* Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. *Nature neuroscience* **22**, 719-728, doi:10.1038/s41593-019-0372-9 (2019).
198 Grubman, A. *et al.* A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nature neuroscience* **22**, 2087-2097, doi:10.1038/s41593-019-0539-4 (2019).
199 Zhang, P. *et al.* Senolytic therapy alleviates Aβ-associated oligodendrocyte progenitor cell senescence and cognitive deficits in an Alzheimer's disease model. *Nature neuroscience*, doi:10.1038/s41593-019-0372-9 (2019).
200 Green, A. J. *et al.* Clemastine fumarate as a remyelinating therapy for multiple sclerosis (ReBUILD): a randomised, controlled, double-blind, crossover trial. *Lancet (London, England)* **390**, 2481-2489, doi:10.1016/S0140-6736(17)32346-2 (2017).
201 Cadavid, D. *et al.* Safety and efficacy of opicinumab in patients with relapsing multiple sclerosis (SYNERGY): a randomised, placebo-controlled, phase 2 trial. *The Lancet. Neurology* **18**, 845-856, doi:10.1016/S1474-4422(19)30137-1 (2019).
202 Schwartzbach, C. J. *et al.* Lesion remyelinating activity of GSK239512 versus placebo in patients with relapsing-remitting multiple sclerosis: a randomised, single-blind, phase II study. *Journal of neurology* **264**, 304-315, doi:10.1007/s00415-016-8341-7 (2017).
203 Wilson, M. R. & Easterbrook-Smith, S. B. Clusterin is a secreted mammalian chaperone. *Trends in Biochemical Sciences* **25**, 95-98, doi:10.1016/s0968-0004(99)01534-0 (2000).

204 Blaschuk, O., Burdzy, K. & Fritz, I. B. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. *Journal of Biological Chemistry* **258**, 7714-7720, doi:https://doi.org/10.1016/S0021-9258(18)32238-5 (1983).
205 Rodríguez-Rivera, C., Garcia, M. M., Molina-Álvarez, M., González-Martín, C. & Goicoechea, C. Clusterin: Always protecting. Synthesis, function and potential issues. *Biomedicine & Pharmacotherapy* **134**, 111174, doi:10.1016/j.biopha.2020.111174 (2021).
206 Rosenberg, M. E. & Paller, M. S. Differential gene expression in the recovery from ischemic renal injury. *Kidney International* **39**, 1156-1161, doi:https://doi.org/10.1038/ki.1991.146 (1991).
207 Makridakis, M. *et al.* Analysis of Secreted Proteins for the Study of Bladder Cancer Cell Aggressiveness. *Journal of proteome research* **9**, 3243-3259, doi:10.1021/pr100189d (2010).
208 Shapiro, B., Tocci, P., Haase, G., Gavert, N. & Ben-Ze'Ev, A. Clusterin, a gene enriched in intestinal stem cells, is required for L1-mediated colon cancer metastasis. *Oncotarget* **6**, 34389-34401, doi:10.18632/oncotarget.5360 (2015).
209 Jongbloed, W. *et al.* Clusterin Levels in Plasma Predict Cognitive Decline and Progression to Alzheimer's Disease. *Journal of Alzheimer's Disease* **46**, 1103-1110, doi:10.3233/JAD-150036 (2015).
210 Herring, S. K., Moon, H.-J., Rawal, P., Chhibber, A. & Zhao, L. Brain clusterin protein isoforms and mitochondrial localization. *eLife* **8**, e48255, doi:10.7554/eLife.48255 (2019).
211 Leskov, K. S., Klokov, D. Y., Li, J., Kinsella, T. J. & Boothman, D. A. Synthesis and Functional Analyses of Nuclear Clusterin, a Cell Death Protein *. *Journal of Biological Chemistry* **278**, 11590-11600, doi:10.1074/jbc.M209233200 (2003).
212 Siltberg-Liberles, J. Evolution of Structurally Disordered Proteins Promotes Neostructuralization. *Molecular Biology and Evolution* **28**, 59-62, doi:10.1093/molbev/msq291 (2011).
213 de Silva, H. V., Harmony, J. A., Stuart, W. D., Gil, C. M. & Robbins, J. Apolipoprotein J: structure and tissue distribution. *Biochemistry* **29**, 5380-5389, doi:10.1021/bi00474a025 (1990).
214 Charnay, Y. *et al.* Clusterin expression during fetal and postnatal CNS development in mouse. *Neuroscience* **155**, 714-724, doi:https://doi.org/10.1016/j.neuroscience.2008.06.022 (2008).
215 Cordero-Llana, O. *et al.* Clusterin secreted by astrocytes enhances neuronal differentiation from human neural precursor cells. *Cell Death Differ* **18**, 907-913, doi:10.1038/cdd.2010.169 (2011).
216 Gregory, J. M. *et al.* Neuronal clusterin expression is associated with cognitive protection in amyotrophic lateral sclerosis. *Neuropathology and Applied Neurobiology* **46**, 255-263, doi:10.1111/nan.12575 (2020).
217 Zandl-Lang, M. *et al.* Regulatory effects of simvastatin and apoJ on APP processing and amyloid-β clearance in blood-brain barrier endothelial cells. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1863**, 40-60, doi:10.1016/j.bbalip.2017.09.008 (2018).

218 Urbich, C., Fritzenwanger, M., Zeiher, A. M. & Dimmeler, S. Laminar Shear Stress Upregulates the Complement-Inhibitory Protein Clusterin. *Circulation* **101**, 352-355, doi:10.1161/01.cir.101.4.352 (2000).
219 Semenkovich, C. F. in *Goldman's Cecil Medicine (Twenty Fourth Edition)* (eds Lee Goldman & Andrew I. Schafer) 1346-1354 (W.B. Saunders, 2012).
220 Wyatt, A. R. *et al.* Clusterin facilitates in vivo clearance of extracellular misfolded proteins. *Cellular and Molecular Life Sciences* **68**, 3919-3931, doi:10.1007/s00018-011-0684-8 (2011).
221 Bell, R. D. *et al.* Transport Pathways for Clearance of Human Alzheimer's Amyloid β-Peptide and Apolipoproteins E and J in the Mouse Central Nervous System. *Journal of Cerebral Blood Flow & Metabolism* **27**, 909-918, doi:10.1038/sj.jcbfm.9600419 (2007).
222 Weng, X. *et al.* Clusterin regulates macrophage expansion, polarization and phagocytic activity in response to inflammation in the kidneys. *Immunology & Cell Biology* **99**, 274-287, doi:https://doi.org/10.1111/imcb.12405 (2021).
223 Bartl, M. M., Luckenbach, T., Bergner, O., Ullrich, O. & Koch-Brandt, C. Multiple receptors mediate apoJ-dependent clearance of cellular debris into nonprofessional phagocytes. *Exp Cell Res* **271**, 130-141, doi:10.1006/excr.2001.5358 (2001).
224 Sarma, J. V. & Ward, P. A. The complement system. *Cell and Tissue Research* **343**, 227-235, doi:10.1007/s00441-010-1034-0 (2011).
225 Tschopp, J., Chonn, A., Hertig, S. & French, L. E. Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 beta, and the b domain of C9. *The Journal of Immunology* **151**, 2159 (1993).
226 McLaughlin, L. *et al.* Apolipoprotein J/clusterin limits the severity of murine autoimmune myocarditis. *Journal of Clinical Investigation* **106**, 1105-1113, doi:10.1172/jci9037 (2000).
227 Falgarone, G. & Chiocchia, G. Chapter 8: Clusterin: A multifacet protein at the crossroad of inflammation and autoimmunity. *Advances in cancer research* **104**, 139-170, doi:10.1016/s0065-230x(09)04008-1 (2009).
228 Carpanini, S. M., Torvell, M. & Morgan, B. P. Therapeutic Inhibition of the Complement System in Diseases of the Central Nervous System. *Frontiers in Immunology* **10**, doi:10.3389/fimmu.2019.00362 (2019).
229 Liu, T., Zhang, L., Joo, D. & Sun, S.-C. NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy* **2**, 17023, doi:10.1038/sigtrans.2017.23 (2017).
230 Zoubeidi, A. *et al.* Clusterin facilitates COMMD1 and I-kappaB degradation to enhance NF-kappaB activity in prostate cancer cells. *Mol Cancer Res* **8**, 119-130, doi:10.1158/1541-7786.MCR-09-0277 (2010).
231 Sikora, J. P., Chlebna-Sokół, D. & Krzyzańska-Oberbek, A. Proinflammatory cytokines (IL-6, IL-8), cytokine inhibitors (IL-6sR, sTNFRII) and anti-inflammatory cytokines (IL-10, IL-13) in the pathogenesis of sepsis in newborns and infants. *Arch Immunol Ther Exp (Warsz)* **49**, 399-404 (2001).
232 Liang, Y. *et al.* Role of Clusterin/NF-κB in the secretion of senescence-associated secretory phenotype in Cr(VI)-induced premature senescent L-02

hepatocytes. *Ecotoxicology and environmental safety* **219**, 112343, doi:10.1016/j.ecoenv.2021.112343 (2021).

233 Shim, Y. J. *et al.* Clusterin induces matrix metalloproteinase-9 expression via ERK1/2 and PI3K/Akt/NF-κB pathways in monocytes/macrophages. *J Leukoc Biol* **90**, 761-769, doi:10.1189/jlb.0311110 (2011).

234 Ammar, H. & Closset, J. L. Clusterin activates survival through the phosphatidylinositol 3-kinase/Akt pathway. *J Biol Chem* **283**, 12851-12861, doi:10.1074/jbc.M800403200 (2008).

235 Chen, T. *et al.* Clusterin-Mediated Apoptosis Is Regulated by Adenomatous Polyposis Coli and Is p21 Dependent but p53 Independent. *Cancer Research* **64**, 7412-7419, doi:10.1158/0008-5472.can-04-2077 (2004).

236 Nguan, C. Y. C., Guan, Q., Gleave, M. E. & Du, C. Promotion of cell proliferation by clusterin in the renal tissue repair phase after ischemia-reperfusion injury. *American Journal of Physiology-Renal Physiology* **306**, F724-F733, doi:10.1152/ajprenal.00410.2013 (2014).

237 Shin, Y.-J. *et al.* Clusterin enhances proliferation of primary astrocytes through extracellular signal-regulated kinase activation. *Neuroreport* **17** (2006).

238 Kaya-Dagistanli, F. & Ozturk, M. The role of clusterin on pancreatic beta cell regeneration after exendin-4 treatment in neonatal streptozotocin administrated rats. *Acta Histochem* **115**, 577-586, doi:10.1016/j.acthis.2012.12.007 (2013).

239 Liu, Y. *et al.* Clusterin promotes growth and invasion of clear cell renal carcinoma cell by upregulation of S100A4 expression. *Cancer Biomark* **21**, 915-923, doi:10.3233/cbm-171018 (2018).

240 Fu, Y. *et al.* Lentivirus-mediated shRNA interference of clusterin blocks proliferation, motility, invasion and cell cycle in the ovarian cancer cells. *J Ovarian Res* **8**, 59, doi:10.1186/s13048-015-0173-z (2015).

241 Zhang, H. *et al.* Clusterin inhibits apoptosis by interacting with activated Bax. *Nature cell biology* **7**, 909-915, doi:10.1038/ncb1291 (2005).

242 Vargas, A., Yamamoto, K. L., Craft, C. M. & Lee, E.-J. Clusterin enhances cell survival by suppressing neuronal nitric-oxide synthase expression in the rhodopsin S334ter-line3 retinitis pigmentosa model. *Brain Research* **1768**, 147575, doi:https://doi.org/10.1016/j.brainres.2021.147575 (2021).

243 Huang, H., Wang, L., Li, M., Wang, X. & Zhang, L. Secreted clusterin (sCLU) regulates cell proliferation and chemosensitivity to cisplatin by modulating ERK1/2 signals in human osteosarcoma cells. *World J Surg Oncol* **12**, 255, doi:10.1186/1477-7819-12-255 (2014).

244 Shannan, B., Seifert, M., Boothman, D. A., Tilgen, W. & Reichrath, J. Clusterin and DNA repair: a new function in cancer for a key player in apoptosis and cell cycle control. *Journal of Molecular Histology* **37**, 183-188, doi:10.1007/s10735-006-9052-7 (2006).

245 Yang, C. R. *et al.* Nuclear clusterin/XIP8, an x-ray-induced Ku70-binding protein that signals cell death. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 5907-5912, doi:10.1073/pnas.97.11.5907 (2000).

246 Rosenberg, M. E. & Silkensen, J. Clusterin: Physiologic and pathophysiologic considerations. *The International Journal of Biochemistry & Cell Biology* **27**, 633-645, doi:https://doi.org/10.1016/1357-2725(95)00027-M (1995).

247 Trougakos, I. P., So, A., Jansen, B., Gleave, M. E. & Gonos, E. S. Silencing Expression of the Clusterin/Apolipoprotein J Gene in Human Cancer Cells Using Small Interfering RNA Induces Spontaneous Apoptosis, Reduced Growth Ability, and Cell Sensitization to Genotoxic and Oxidative Stress. *Cancer Research* **64**, 1834-1842, doi:10.1158/0008-5472.can-03-2664 (2004).
248 Peng, M. *et al.* The role of Clusterin in cancer metastasis. *Cancer Manag Res* **11**, 2405-2414, doi:10.2147/CMAR.S196273 (2019).
249 Chi, K. N. *et al.* Custirsen in combination with docetaxel and prednisone for patients with metastatic castration-resistant prostate cancer (SYNERGY trial): a phase 3, multicentre, open-label, randomised trial. *The Lancet Oncology* **18**, 473-485, doi:https://doi.org/10.1016/S1470-2045(17)30168-7 (2017).
250 Beer, T. M. *et al.* Custirsen (OGX-011) combined with cabazitaxel and prednisone versus cabazitaxel and prednisone alone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel (AFFINITY): a randomised, open-label, international, phase 3 trial. *The Lancet Oncology* **18**, 1532-1542, doi:10.1016/S1470-2045(17)30605-8 (2017).
251 Stoop, M. P. *et al.* Multiple sclerosis-related proteins identified in cerebrospinal fluid by advanced mass spectrometry. *PROTEOMICS* **8**, 1576-1585, doi:10.1002/pmic.200700446 (2008).
252 Van Luijn, M. M. *et al.* Elevated Expression of the Cerebrospinal Fluid Disease Markers Chromogranin A and Clusterin in Astrocytes of Multiple Sclerosis White Matter Lesions. *Journal of Neuropathology & Experimental Neurology* **75**, 86-98, doi:10.1093/jnen/nlv004 (2016).
253 Rithidech, K. *et al.* Protein expression profiles in pediatric multiple sclerosis: potential biomarkers. *Multiple Sclerosis Journal* **15**, 455-464, doi:10.1177/1352458508100047 (2009).
254 Polihronis, M., Paizis, K., Carter, G., Sedal, L. & Murphy, B. Elevation of human cerebrospinal fluid clusterin concentration is associated with acute neuropathology. *Journal of the Neurological Sciences* **115**, 230-233, doi:https://doi.org/10.1016/0022-510X(93)90230-V (1993).
255 Qiao, L. *et al.* Serum Clusterin and Complement Factor H May Be Biomarkers Differentiate Primary Sjögren's Syndrome With and Without Neuromyelitis Optica Spectrum Disorder. *Frontiers in Immunology* **10**, doi:10.3389/fimmu.2019.02527 (2019).
256 Harold, D. *et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* **41**, 1088-1093, doi:10.1038/ng.440 (2009).
257 Schrijvers, E. M., Koudstaal, P. J., Hofman, A. & Breteler, M. M. Plasma clusterin and the risk of Alzheimer disease. *Jama* **305**, 1322-1326, doi:10.1001/jama.2011.381 (2011).
258 Deming, Y. *et al.* A potential endophenotype for Alzheimer's disease: cerebrospinal fluid clusterin. *Neurobiology of aging* **37**, 208.e201-208.e209, doi:10.1016/j.neurobiolaging.2015.09.009 (2016).
259 Lidström, A. *et al.* Clusterin (apolipoprotein J) protein levels are increased in hippocampus and in frontal cortex in Alzheimer's disease. *Experimental neurology* **154**, 511-521 (1998).

260 Thambisetty, M. *et al.* Plasma clusterin concentration is associated with longitudinal brain atrophy in mild cognitive impairment. *NeuroImage* **59**, 212-217, doi:10.1016/j.neuroimage.2011.07.056 (2012).

261 Thambisetty, M. *et al.* Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Archives of general psychiatry* **67**, 739-748, doi:10.1001/archgenpsychiatry.2010.78 (2010).

262 Mullan, G. M. *et al.* Plasma clusterin levels and the rs11136000 genotype in individuals with mild cognitive impairment and Alzheimer's disease. *Curr Alzheimer Res* **10**, 973-978, doi:10.2174/15672050113106660162 (2013).

263 Xing, Y.-Y. *et al.* Blood Clusterin Levels, rs9331888 Polymorphism, and the Risk of Alzheimer's Disease. *Journal of Alzheimer's Disease* **29**, 515-519, doi:10.3233/JAD-2011-111844 (2012).

264 Ling, I. F., Bhongsatiern, J., Simpson, J. F., Fardo, D. W. & Estus, S. Genetics of clusterin isoform expression and Alzheimer's disease risk. *PloS one* **7**, e33923-e33923, doi:10.1371/journal.pone.0033923 (2012).

265 Demattos, R. B. *et al.* Clusterin promotes amyloid plaque formation and is critical for neuritic toxicity in a mouse model of Alzheimer's disease. *Proceedings of the National Academy of Sciences* **99**, 10843-10848, doi:10.1073/pnas.162228299 (2002).

266 Oh, S.-B. *et al.* Clusterin contributes to early stage of Alzheimer's disease pathogenesis. *Brain Pathology* **29**, 217-231, doi:10.1111/bpa.12660 (2019).

267 Wojtas, A. M. *et al.* Loss of clusterin shifts amyloid deposition to the cerebrovasculature via disruption of perivascular drainage pathways. *Proceedings of the National Academy of Sciences* **114**, E6962-E6971, doi:10.1073/pnas.1701137114 (2017).

268 Chen, F. *et al.* Clusterin secreted from astrocyte promotes excitatory synaptic transmission and ameliorates Alzheimer's disease neuropathology. *Mol Neurodegener* **16**, doi:10.1186/s13024-021-00426-7 (2021).

269 Wojtas, A. M. *et al.* Astrocyte-derived clusterin suppresses amyloid formation in vivo. *Mol Neurodegener* **15**, doi:10.1186/s13024-020-00416-1 (2020).

270 Dahm, R. Alzheimer's discovery. *Curr Biol* **16**, R906-910, doi:10.1016/j.cub.2006.09.056 (2006).

271 Association, A. s. 2022 Alzheimer's disease facts and figures. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **18**, 700-789, doi:10.1002/alz.12638 (2022).

272 DeTure, M. A. & Dickson, D. W. The neuropathological diagnosis of Alzheimer's disease. *Mol Neurodegener* **14**, 32, doi:10.1186/s13024-019-0333-5 (2019).

273 Lanctôt, K. L. *et al.* Neuropsychiatric signs and symptoms of Alzheimer's disease: New treatment paradigms. *Alzheimer's & Dementia: Translational Research & Clinical Interventions* **3**, 440-449, doi:10.1016/j.trci.2017.07.001 (2017).

274 Lafleche, G. & Albert, M. S. Executive function deficits in mild Alzheimer's disease. *Neuropsychology* **9**, 313 (1995).

275 Huang, L.-K., Chao, S.-P. & Hu, C.-J. Clinical trials of new drugs for Alzheimer disease. *J Biomed Sci* **27**, 18-18, doi:10.1186/s12929-019-0609-7 (2020).

276 Möller, H. J. & Graeber, M. B. The case described by Alois Alzheimer in 1911. *European Archives of Psychiatry and Clinical Neuroscience* **248**, 111-122, doi:10.1007/s004060050027 (1998).

277 Roher, A. E. *et al.* Increased Aβ Peptides and Reduced Cholesterol and Myelin Proteins Characterize White Matter Degeneration in Alzheimer's Disease. *Biochemistry* **41**, 11080-11090, doi:10.1021/bi026173d (2002).

278 Kurihara, S., Kato, K., Asada, K., Kumagai, H. & Suzuki, H. A putrescine-inducible pathway comprising PuuE-YneI in which gamma-aminobutyrate is degraded into succinate in Escherichia coli K-12. *Journal of bacteriology* **192**, 4582-4591, doi:10.1128/JB.00308-10 (2010).

279 Guo, X. *et al.* Voxel-based assessment of gray and white matter volumes in Alzheimer's disease. *Neuroscience letters* **468**, 146-150, doi:https://doi.org/10.1016/j.neulet.2009.10.086 (2010).

280 Ihara, M. *et al.* Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies. *Acta neuropathologica* **119**, 579-589, doi:10.1007/s00401-009-0635-8 (2010).

281 Zhan, X. *et al.* Myelin Injury and Degraded Myelin Vesicles in Alzheimer's Disease. *Current Alzheimer Research* **11**, 232-238 (2014).

282 Desai, M. K. *et al.* Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. *Glia* **57**, 54-65, doi:10.1002/glia.20734 (2009).

283 Vanzulli, I. *et al.* Disruption of oligodendrocyte progenitor cells is an early sign of pathology in the triple transgenic mouse model of Alzheimer's disease. *Neurobiology of aging* **94**, 130-139, doi:https://doi.org/10.1016/j.neurobiolaging.2020.05.016 (2020).

284 Wu, D. *et al.* LINGO-1 antibody ameliorates myelin impairment and spatial memory deficits in the early stage of 5XFAD mice. *CNS neuroscience & therapeutics* **24**, 381-393, doi:10.1111/cns.12809 (2018).

285 Chen, J.-F. *et al.* Enhancing myelin renewal reverses cognitive dysfunction in a murine model of Alzheimer’s disease. *Neuron* **109**, 2292-2307.e2295, doi:https://doi.org/10.1016/j.neuron.2021.05.012 (2021).

286 Bartzokis, G. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. *Neurobiology of aging* **32**, 1341-1371, doi:10.1016/j.neurobiolaging.2009.08.007 (2011).

287 Han, X., M. Holtzman, D., W. Mckeel, D., Kelley, J. & Morris, J. C. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. *Journal of Neurochemistry* **82**, 809-818, doi:10.1046/j.1471-4159.2002.00997.x (2002).

288 de la Monte, S. M. Quantitation of cerebral atrophy in preclinical and end-stage Alzheimer's disease. *Ann Neurol* **25**, 450-459, doi:10.1002/ana.410250506 (1989).

289 Pan, S., Mayoral, S. R., Choi, H. S., Chan, J. R. & Kheirbek, M. A. Preservation of a remote fear memory requires new myelin formation. *Nature neuroscience* **23**, 487-499, doi:10.1038/s41593-019-0582-1 (2020).

290 Steadman, P. E. *et al.* Disruption of Oligodendrogenesis Impairs Memory Consolidation in Adult Mice. *Neuron* **105**, 150-164.e156, doi:https://doi.org/10.1016/j.neuron.2019.10.013 (2020).

291 Chacon-De-La-Rocha, I. *et al.* Accelerated Dystrophy and Decay of Oligodendrocyte Precursor Cells in the APP/PS1 Model of Alzheimer's-Like Pathology. *Frontiers in Cellular Neuroscience* **14**, doi:10.3389/fncel.2020.575082 (2020).

292 Fernández-Castañeda, A. *et al.* The active contribution of OPCs to neuroinflammation is mediated by LRP1. *Acta neuropathologica* **139**, 365-382, doi:10.1007/s00401-019-02073-1 (2020).

293 Moyon, S. *et al.* Demyelination causes adult CNS progenitors to revert to an immature state and express immune cues that support their migration. *J Neurosci* **35**, 4-20, doi:10.1523/JNEUROSCI.0849-14.2015 (2015).

294 Fernandez-Castaneda, A. & Gaultier, A. Adult oligodendrocyte progenitor cells – Multifaceted regulators of the CNS in health and disease. *Brain, Behavior, and Immunity* **57**, 1-7, doi:https://doi.org/10.1016/j.bbi.2016.01.005 (2016).

295 Association, A. s. 2021 Alzheimer's disease facts and figures. *Alzheimer's & Dementia* **17**, 327-406, doi:https://doi.org/10.1002/alz.12328 (2021).

296 Ribes, S. *et al.* Toll-like receptor stimulation enhances phagocytosis and intracellular killing of nonencapsulated and encapsulated Streptococcus pneumoniae by murine microglia. *Infect Immun* **78**, 865-871, doi:10.1128/IAI.01110-09 (2010).

297 Browne, T. C. *et al.* IFN-γ Production by Amyloid β–Specific Th1 Cells Promotes Microglial Activation and Increases Plaque Burden in a Mouse Model of Alzheimer's Disease. *The Journal of Immunology* **190**, 2241, doi:10.4049/jimmunol.1200947 (2013).

298 Bona, D. D. *et al.* Systematic review by meta-analyses on the possible role of TNF-α polymorphisms in association with Alzheimer's disease. *Brain Res Rev* **61**, 60-68, doi:https://doi.org/10.1016/j.brainresrev.2009.05.001 (2009).

299 Yang, J. *et al.* Oxalate-curcumin–based probe for micro- and macroimaging of reactive oxygen species in Alzheimer's disease. *Proceedings of the National Academy of Sciences* **114**, 12384, doi:10.1073/pnas.1706248114 (2017).

300 Kotter, M. R., Li, W. W., Zhao, C. & Franklin, R. J. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *J Neurosci* **26**, 328-332, doi:10.1523/JNEUROSCI.2615-05.2006 (2006).

301 Stoffels, J. M. J. *et al.* Fibronectin aggregation in multiple sclerosis lesions impairs remyelination. *Brain* **136**, 116-131, doi:10.1093/brain/aws313 (2013).

302 Shim, Y.-J., Kang, B.-H., Choi, B.-K., Park, I.-S. & Min, B.-H. Clusterin induces the secretion of TNF-α and the chemotactic migration of macrophages. *Biochem Bioph Res Co* **422**, 200-205, doi:https://doi.org/10.1016/j.bbrc.2012.04.162 (2012).

303 Hiratsuka, D., Kurganov, E., Furube, E., Morita, M. & Miyata, S. VEGF- and PDGF-dependent proliferation of oligodendrocyte progenitor cells in the medulla oblongata after LPC-induced focal demyelination. *J Neuroimmunol* **332**, 176-186, doi:10.1016/j.jneuroim.2019.04.016 (2019).

304 Perumal, N. B. & Kaplan, M. H. Regulating Il9 transcription in T helper cells. *Trends in Immunology* **32**, 146-150, doi:10.1016/j.it.2011.01.006 (2011).

305 Holmes, C. *et al.* Long-term effects of Aβ42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *The Lancet* **372**, 216-223, doi:10.1016/s0140-6736(08)61075-2 (2008).

306 Mullane, K. & Williams, M. Alzheimer's disease beyond amyloid: Can the repetitive failures of amyloid-targeted therapeutics inform future approaches to dementia drug discovery? *Biochem Pharmacol* **177**, 113945, doi:10.1016/j.bcp.2020.113945 (2020).

307 Wang, F. *et al.* Myelin degeneration and diminished myelin renewal contribute to age-related deficits in memory. *Nature neuroscience* **23**, 481-486, doi:10.1038/s41593-020-0588-8 (2020).

308 Schürmann, B. Association of the Alzheimer's Disease Clusterin Risk Allele with Plasma Clusterin Concentration. *Journal of Alzheimer's disease* **25**, 421-424.

309 Ackley, S. F. *et al.* Effect of reductions in amyloid levels on cognitive change in randomized trials: instrumental variable meta-analysis. *BMJ*, n156, doi:10.1136/bmj.n156 (2021).

310 DeMattos, R. B. *et al.* Purification and characterization of astrocyte-secreted apolipoprotein E and J-containing lipoproteins from wild-type and human apoE transgenic mice. *Neurochemistry International* **39**, 415-425, doi:https://doi.org/10.1016/S0197-0186(01)00049-3 (2001).

311 Jones, S. E. & Jomary, C. Clusterin. *The International Journal of Biochemistry & Cell Biology* **34**, 427-431, doi:https://doi.org/10.1016/S1357-2725(01)00155-8 (2002).

312 Ding, X. *et al.* IL-9 signaling affects central nervous system resident cells during inflammatory stimuli. *Experimental and Molecular Pathology* **99**, 570-574, doi:10.1016/j.yexmp.2015.07.010 (2015).

313 Wang, C. *et al.* IL-17 induced NOTCH1 activation in oligodendrocyte progenitor cells enhances proliferation and inflammatory gene expression. *Nat Commun* **8**, 15508, doi:10.1038/ncomms15508 (2017).

314 Goswami, R. & Kaplan, M. H. A Brief History of IL-9. *The Journal of Immunology* **186**, 3283-3288, doi:10.4049/jimmunol.1003049 (2011).

315 Harrington, E. P., Bergles, D. E. & Calabresi, P. A. Immune cell modulation of oligodendrocyte lineage cells. *Neuroscience letters* **715**, 134601, doi:10.1016/j.neulet.2019.134601 (2020).

316 Kirby, L. & Castelo-Branco, G. Crossing boundaries: Interplay between the immune system and oligodendrocyte lineage cells. *Seminars in Cell & Developmental Biology* **116**, 45-52, doi:https://doi.org/10.1016/j.semcdb.2020.10.013 (2021).

317 May, P. C. *et al.* Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. *Neuron* **5**, 831-839, doi:10.1016/0896-6273(90)90342-d (1990).

318 Chi, K. N. *et al.* A phase I pharmacokinetic and pharmacodynamic study of OGX-011, a 2'-methoxyethyl antisense oligonucleotide to clusterin, in patients with localized prostate cancer. *Journal of the National Cancer Institute* **97**, 1287-1296, doi:10.1093/jnci/dji252 (2005).

319 Braskie, M. N. *et al.* Common Alzheimer's disease risk variant within the CLU gene affects white matter microstructure in young adults. *J Neurosci* **31**, 6764-6770, doi:10.1523/jneurosci.5794-10.2011 (2011).
320 Leeb, C., Eresheim, C. & Nimpf, J. Clusterin is a ligand for apolipoprotein E receptor 2 (ApoER2) and very low density lipoprotein receptor (VLDLR) and signals via the Reelin-signaling pathway. *J Biol Chem* **289**, 4161-4172, doi:10.1074/jbc.M113.529271 (2014).
321 Zlokovic, B. V. *et al.* Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. *Proceedings of the National Academy of Sciences* **93**, 4229-4234, doi:10.1073/pnas.93.9.4229 (1996).
322 Moore, L. R. *et al.* Evaluation of Antisense Oligonucleotides Targeting ATXN3 in SCA3 Mouse Models. *Molecular Therapy - Nucleic Acids* **7**, 200-210, doi:https://doi.org/10.1016/j.omtn.2017.04.005 (2017).
323 Dutra, M. R. H. *et al.* Protective Role of UCP2 in Oxidative Stress and Apoptosis during the Silent Phase of an Experimental Model of Epilepsy Induced by Pilocarpine. *Oxid Med Cell Longev* **2018**, 6736721, doi:10.1155/2018/6736721 (2018).