Generation and Validation of HFBI-EGFR Binding Peptide Fusion Protein for Targeted Drug Delivery to Pancreatic Adenocarcinomas
(Technical Report)

Co-production of Politics and Human-Derived Biological Material Donation and Use
(STS Topic)

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Introduction

Pancreatic adenocarcinoma is the most common type of pancreatic cancer, accounting for around eighty-five percent of all pancreatic cancer cases (Rawla, Sunkara, & Gaduputi, 2019). Pancreatic adenocarcinomas cause forty-five thousand deaths per year in the United States alone and generate about three billion dollars in healthcare costs (Zimmerman & Mehr, 2013). The five-year survival rate of people with pancreatic adenocarcinoma is less than five percent (Ryan, Hong, & Bardeesy, 2014). It is one of the deadliest common cancers due to the inability to detect the cancer early and the lack of effective and targeted treatments.

To be able detect pancreatic adenocarcinomas earlier, scientists are working to find a biomarker to track the cancer. One way to find a biomarker is by sequencing tumor specimen to identify overexpressed or mutated genes specific to pancreatic adenocarcinomas. Scientists rely on donated tumor samples from patients to do this important work. Various policies and best practices have been created by academic, private, and governmental agencies to ensure that donated biological material specimens are of the highest quality so accurate data can be obtained, and patients' rights are upheld (De Souza & Greenspan, 2013). However, it is important to fully analyze how all of branches of the United States government are impacting all aspects of biological material donation, specifically regarding patient rights, such as consent to donate, ownership rights, and the use of the donated samples, not just impact on quality. Through a historical analysis, the co-production of politics and human-derived biological material will be investigated.

The other reason pancreatic adenocarcinomas are so deadly is that treatments are not effective nor targeted. Current treatments include chemotherapy, radiation therapy, and surgical tumor removal (Ryan et al., 2014). All of these treatments either have severe negative side

effects or are not always a feasible option. Due to the limited treatment options that target pancreatic adenocarcinomas, the primary goal of the technical project is to create and validate a novel fusion protein to solubilize anti-cancer drugs while targeting the drugs to the cancer cells. The fusion protein will be composed of a protein that has been shown to solubilize hydrophobic drugs with a specific targeting peptide that binds to epidermal growth factor receptors (EGFRs), an overexpressed receptor associated with pancreatic adenocarcinomas.

Technical Topic

Pancreatic adenocarcinomas start in the ductal epithelium as premalignant lesions (Hidalgo, 2010). Cancer can metastasize and spread to the liver, abdomen, and lungs (Ryan et al., 2014). One treatment option is to have surgery to remove the tumor, but due to the size and/or location of the tumor, most patients are not eligible to undergo surgery. Other options include radiation therapy and chemotherapy, but both have severe negative side effects due to the lack of specific targeting to cancer cells. Chemotherapy is a systemic treatment which negatively impacts cells throughout the body, rather than affecting only the cancerous cells. Radiation therapy is more targeted, but it still affects the healthy tissue surrounding the tumor. Only one targeted drug, Erlotinib, has been approved by the Food and Drug Administration (FDA), but this drug has increased toxicity and only increases the patient's lifespan by about two weeks (Ryan et al., 2014). Additionally, many anti-cancer drugs are hydrophobic and therefore need a surfactant for delivery (Berger & Sallada, 2019). Chemical surfactants are often used, but they can cause negative reactions in immuno-compromised patients (ibid). The goal of this technical project is to create and validate a novel, non-immunogenic fusion protein that solubilizes anti-cancer drugs while targeting the drugs to the pancreatic cancer cells.

This goal will be achieved by combining hydrophobin and a targeting peptide into a fusion protein. Hydrophobins are amphiphilic proteins characterized by four conserved disulfide bonds that cause the protein to have high surface activity (Sallada, Dunn, & Berger, 2018). This high surface activity allows hydrophobins to create stable emulsions containing hydrophobic drugs. Previous research has shown these proteins to be non-immunogenic, therefore being preferable over traditional chemical surfactants (Aimanianda et al., 2009). HFBI is a class II hydrophobin, meaning that it forms highly-ordered monolayers at air-water or water-solid interfaces (Berger & Sallada, 2019). When the HFBI is internalized into a cell, the reducing environment of the cytoplasm will cause the disulfide bonds to break. With the breaking of these bonds, the proteins will no longer self-assemble around the hydrophobic drug. The drug will then be released within the cell. Therefore, the surrounding cells should not be affected by the anti-cancer drug. The other half of the fusion protein, EGFR-binding peptide, is amino acid sequence optimized to bind to EGFRs on targeted cell lines with limited off-target effects (Kikuchi et al., 2016). Combining these two proteins into a fusion protein will allow it to solubilize the hydrophobic drug (via the HFBI) while targeting the drugs to the pancreatic cancer cells (via the EGFR-binding peptide).

This fusion protein will be made recombinantly in *Pichia pastoris.* *Pichia pastoris* is used because it is better than bacteria at forming disulfide bonds, which are critical to create the surface activity (Cregg, Cereghino, Shi, & Higgins, 2000). Since the self-assembly of HFBI depends on its high surface activity, it is important that the disulfide bonds are properly formed. Additionally, *Pichia pastoris* can secrete proteins that are inserted after its Alcohol Oxidase I promoter site instead of having to lyse the cell to release the protein. This provides a pre-purification step as the HFBI only has to be separated from the cell culture media during

chromatography. Lysing the cell releases every single protein within the cell, so it is a much more complicated process to separate out the protein of interest (Cregg et al., 2000). The fusion protein will then be subject to experiments that confirm the function of the HFBI and EGFR-binding peptide. After the functionalities are confirmed, the fusion proteins will be combined with anti-cancer drugs and tested on human pancreatic cancer cells.

The early stages of research for this project will be completed in the Berger Lab in the Department of Chemical Engineering. This includes the molecular biology work required to create the fusion protein gene vector and the production of the fusion protein in *Pichia pastoris*. The fusion protein will be purified using a high-pressure liquid chromatography system in the Berger Lab. The binding affinity of the fusion protein for pancreatic cancer cells will be quantified using the flow cytometer instrument in the Flow Cytometry Core Facility in Pinn Hall. Subsequent testing of the fusion protein with human pancreatic cancer cells will be done in the Lazzara Lab in the Chemical Engineering Department.

Donated Biological Material and Biobanks

The technical project only addresses one issue causing high death rates, the lack of an effective and targeted treatment for pancreatic adenocarcinomas. It does not address the need for an early detection system. One way to find a biomarker for early detection is to sequence tumors from patients to find mutations in genes or overexpressed genes. To be able sequence the tumors, scientists are relying on donated tumor samples from patients which are stored in biobanks. Biobanks are often associated with actual physical samples, but they can also hold genetic data from people (De Souza & Greenspan, 2013).

Biobanks are very important for medical advancement because of the data and specimens they provide to researchers. Using animal cells and tissue models can only mimic the behavior of humans and human cells to a certain point, so having human-derived materials is necessary to gather accurate data. Biological samples, like fibroblasts and blood cells from a patient, can be used to derive induced pluripotent stem cells, a growing area of research that has importance in tissue engineering and regenerative medicine. Biopsies from cancerous tissues can be used in the identification of biomarkers for a variety of cancers which can provide essential information in creating immunotherapies and for early stage detection of cancer.

Socio-Technical Elements of Donated Biological Material

Many groups of people are affected by the donation and use of human-derived biological materials such as patients, scientists, scientific institutions, and the federal government. The patients are the most obvious group affected as they are the ones who are donating their own biological material. Scientists rely on these donations to be able to perform experiments and gather data to better inform medical advances. Scientific institutions have become involved in ownership disputes with scientists over the donated materials. The federal government plays a critical role in creating regulations for the use, various levels of consent, and privacy laws regarding donated material.

It is crucial that biobanks themselves, as well as the samples contained in them, are properly regulated through laws and policies. Lack of regulation can harm the research being done using the biological samples, as subpar samples could have negative impacts on the research. It can also harm patients who donate samples if their identity and personal information is revealed. For example, research may show that a certain ethnic group is more likely to suffer from a certain disease which can lead to stigmatization or discrimination of the group.

Furthermore, ethical and legal questions can be raised when the proper policy system is not in place.

The framework that will be used in my analysis of biobanks and donated biological materials is co-production. This theory was proposed by Sheila Jasanoff in her 2004 book, "The States of Knowledge: The Co-Production of Science and the Social Order". Jasanoff argues that science and society are jointly created and influence the creation of one another (Jasanoff, 2004). In my analysis, the science is specifically in relation to the donation, storage, and use of human-derived biological materials. As for society, I will concentrate on political structures and ethical underpinnings of the institutions, formal rules, that govern this socio-technical system. Furthermore, I will focus on the *interactionist* strand of co-production. The *interactionist* strand of co-production highlights the processes of altering power and order (Jasanoff, 2004).

Precedent surrounding the ownership of donated biological materials was set by several court cases in the United States. In one case, *Moore v. Regents of the University of California*, a patient sued researchers over the fact that they took samples from him to make a patented cell line (Schleiter, 2009). The court ruled that the researchers must disclose their interests (research or economic), but that the cell line was an invention and therefore patentable without the patient having any ownership rights. In a second case, *Greenberg v. Miami Children's Hospital Research Institute, Inc.*, patients sued over the use of their tissues to gain a patent for a disease testing kit, as the tissues were originally donated for research purposes only. Fearing the halt of research, the courts ruled that the institute should have warned the patients, but the patients still have no ownership (Schleiter, 2009). In these cases, the political structure influenced science. Because of judges' rulings, scientists are able to commercialize their research without providing benefits to the donor.

In the legislative branch, laws like the Code of Federal Regulations Title 45 § 46.101 are in place to attempt to ensure the anonymity of patients donating biological materials (Andrews, 2005). Without this law, biobanks can put the patients who donated samples at risk because of the personal information that can be revealed. In this case, policy is affecting science, especially the donation aspect of human-derived biological material. Scientists now have to go through more steps and paperwork before using the donated samples. This has the potential to slow down research, but it is more beneficial to the donors.

In 2001, then-President George W. Bush signed an executive order banning the funding of human embryonic stem cells (Murugan, 2009). In this case, science influenced politics. Scientists first discovered that they could make stem cells from human embryos. This discovery inspired an ethical debate which led to the executive order. The executive order then in turn influenced science, as United States researchers could no longer use the human embryonic stem cells in their laboratories located in the United States.

The above cases offer evidence of the co-production of science, the donation and use of human-derived biological material, and society, the political structure in place. Each example is from a different branch of the United States government, illustrating that all three branches have an influence on human-derived biological material, or are influenced by it. These are just a few examples of what I plan to further investigate to create a more complete picture of science and society's co-production over time.

Research Question and Methods

The question I aim to answer is: How have United States government's policies and the donation of biological materials influenced one another over time? This question allows us to

view science and politics as the dynamic, interwoven socio-technical systems that they are. It is important to analyze political impacts on regulation at all stages of the process: donation or collection, storage, and use in research. This question is also important to ensure that the donation and use of biological materials and the policies surrounding them are created to benefit the public.

I will use secondary evidence, specifically sources from all three branches of the United States government. From the executive branch, I will look at executive orders, patents, and agency policies. For the legislative branch, I will look at laws issued, policy documents, and congressional testimonies. For the judicial branch, I will focus on case laws. This evidence will be gathered by doing a literature search for each of the different sources from the late twentieth century to present day.

For my data analysis, I will be taking a historical approach. This analysis style will allow me to see the growth of politics and science over time. In my analysis, I will create a timeline. This timeline will contain significant events derived from the evidence for all three branches. Additionally, it will contain significant scientific developments in the donation, storage, and use of human-derived biological materials. This timeline will allow me to full analyze the co-production of science and politics. I will be able to analyze how scientific events created political responses, and how political events created scientific responses by looking at the organization of the timeline. On the timeline, I also intend to add positive and negative effects on the United States' populace for the various events. This will allow me to be able to analysis the impact of human-derived biological material donation and use on the general public.

Conclusion

Biobanks and donated biological materials provide information that can be used to guide research to find biomarkers, as well as providing many other beneficial uses in medical research. However, it is important to analyze the politics that influence and are influenced by the donation and use of biological materials. Policies need to be enacted so that the public receives the benefits from donated biological materials. I expect to see the heavily interwoven nature of politics and donated biological material technology that causes them to change and adapt over time. I also expect to see policies that were intended to benefit the public, but do not always succeed in that goal.

To complete this analysis, I will use the following timeline. First, I will complete my literature search by mid-to-late January to collect all of my evidence. By mid-February, I will have created an annotated bibliography for my sources of evidence. I will make my timeline by the end of February and will begin to synthesize my argument. By mid-March, I will have submitted a preliminary draft of my thesis. I will submit the final draft of my thesis by early April.

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