Investigating Relationships Found in the Kidney Microenvironment to Aid in Understanding Malfunctions of Proteins in Chronic Kidney Disease

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Investigating Relationships Found in Kidney Microenvironment to Aid in Understanding

The Malfunctions of Proteins in Chronic Kidney Disease

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ABSTRACT

The kidneys are the filtration organ in your body that helps to filter out waste from our body. Currently, kidney disease is an ailment that causes damage to the kidney so that it cannot filter our blood and other bodily fluids properly. It is also one of the least researched illnesses in the medical field. And with about ten percent of the American population suffering from it each year, there is an urgency in finding more ways to help find a solution.

During my time of research, Dr. Zhou along with his fellow researchers, Dr. Gui and Wang and I examined the relationship between cells in the kidney. Through this research, we used this information to further understand how we could use this information to help create new treatments to different ailments found in a damaged kidney environment. We will also hoped to achieve some new understanding of damaged kidney environments by trying to find out what protein processes cause the malfunction of the kidneys. We used different devices to help us come to these conclusions such as, transgenic technology, PCR testing, western blotting, and mathematical modeling.

From the research found, I hoped to look into specific proteins that are found in kidneys and see if there is any association with race. With thirty-percent of kidney disease victims being African-American, I believe there could be a correlation with either how kidney testing is done in America or how kidneys act in microenvironments with different genetic histories. As you will see, my personal conclusion is to see if this high correlation with race and kidney disease victims can be explained through a genetic or societal issue. Through Dr. Zhou and I working together, our endgame would be to become one step closer to finding a cure for kidney disease.
INTRODUCTION

The purpose behind this project was to help close the gap that there is found in kidney disease research. About ten percent of the current American population suffers from it daily. It isn’t treated as seriously as other diseases because the victims don’t die immediately. But the life of dialysis that so many people have to live with isn’t supposed to be ignored. Dr. Zhou allowed me onto his research team, whose primary focus was to look for a therapy that could aid in slowly diminishing and then eliminating the impacts of kidney disease. They did this by looking into many subjects found in the kidney’s microenvironment. This included the impact of fibroblasts and also the subcultures that may reside in that community. The communication found between kidney microtubular cells and fibroblast cells was also discussed. The communication found here could help us understand how cells work together in the kidneys. The lab makes sure to put priority on repeating their experiments; this allows them to gain more data to discuss. It also helps the team and me to hone in on our experimental skills, including the use of western blots and PCR testing.

In the experiment, we will start by culturing the folic acid needed to mimic the effect of a damaged kidney in our rodent specimen. We then extracted RNA from the injured kidney and ran western blots on them. After the western blot helped identify the specific proteins and their functionality, we created histological stainings of the kidney injury. The histological staining helps us to view the location of the proteins and also highlight their structure. We use the information found here and place it into data graphs for analysis. The data graphs can be used further because the information found here is organized and helps us to see further conclusions. The results are also put through the mathematical approach to see if they align with the pattern in Dr. Zhou’s prior experiments.
My personal goal for this lab was to improve my experimental skills. Dr. Zhou and his team put a particular importance on understanding the procedures done in the lab. This is because it affects the results in which they put their basis of understanding. We did repeat experiments many times, and this helped me to truly understand how to use tools such as PCR testing, western blotting and immunofluorescence staining. Alongside knowing how to use each tool, I slowly understood how each worked toward finding information about the kidney microenvironment. These repetitive tasks also helped me understand a lot of scientific terminology that made me more familiar with how these tools worked. This worked in a cycle for me.

I also plan on using the information found in the lab to see if there is a connection between the genes found in the damaged injury and the genetic code found in specific ethnicities. This interests me in particular because my past research has found that about one-third of kidney disease victims in America are black. Coupling that with the fact that black people are four times as likely to develop kidney disease as white people, it made me question whether this phenomenon is genetics or a societal issue. I planned on organizing the data found in the lab by separating the functional and nonfunctional proteins and seeing what genes are prevalent in each group. With this information, I can see if a protein type is popular with a specific ethnicity. This would also help me to find out what type of proteins are more likely to cause fibrosis and other kidney disease-related disorders. In conclusion, I find that this new exploration of the information we already have will help us find new discoveries in papers that have already been published. By switching our topic interest in the studies we do, we can come up with new questions that can lead us to avenues of new answers. With new answers come new solutions to the main topic at hand: curing kidney disease.
MATERIALS/METHODS

Method 1: The first method used for the research done was transgenic technology. Transgenic technology is when foreign DNA is introduced into the genome of a specimen to create DNA sequences necessary for the research. The foreign DNA in the case of our lab is folic acid. We would apply folic acid to our rodent specimen. This would cause the rodent's kidney to become damaged. This would help us with the basis for our project. Using the now-damaged kidney, we are able to observe the relationships found between proteins and other kidney cells in the diseased kidney. The transgenic technology helps us to observe these interactions in a controlled environment and makes sure that the results are not skewed in any way.

Method 2: Another method used in this experiment was a mathematical approach. This approach is a tool used prior to Dr. Zhou's past labs. In the past, Dr. Zhou and his team found that there is a mathematical relationship between the shift from an acute kidney injury to a chronic kidney. They found that when the kidney is in the acute kidney injury stage, the chance of reversing the injury was meager. But in the transition window from acute kidney injury to chronic kidney disease, the chance for a reversal of the entire illness was very high. Then, again, as it became kidney disease, the chance of reversal went back down. They also found that in the transition window, the negative effects the illness had on the kidney
were very low compared to acute kidney injury and chronic kidney disease. The results from these findings created a pattern that could be used in other specimens to try to find the best time to apply treatment for the greatest chance of reversal.

**Method 3:** The lab also used a data graph. This tool helped when interpreting a protein's function. Each protein was organized into its own category over a ten-day period. The categories were color-coded, with red indicating high function, blue indicating no function, and everything in the middle showing an average of one or the other. The data is a very important tool because it helps to take away the stigma of no function, meaning negative. The data graph showed us that no function in certain places of the kidney was necessary for the health of the kidney. The data was also completely unbiased and could show us our information through an unmanipulated view.

**Method 4:** We used a protein assay in our research. The protein assay helps to find the amount of a specific protein in a sample of many different samples. In our lab, we will use together with a western blot to help us isolate all the proteins found in the kidney and identify the functionality of each protein. We then used this information to graph each protein from fully functional to
completely non-functional. The protein assay can also help us pinpoint exactly where a specific protein is found in the kidney microenvironment.

All these tools work together to help us get more information about how a damaged kidney works. We start with transgenic technology to create the basis of information needed for experiments. Then, we can organize the information found in those experiments using a protein assay. The mathematical approach and data graph then go hand in hand to help us draw conclusions on how the information shared with us actually affects the damaged kidney. This can then help us create findings on how to work against the traits that cause damage to the kidneys.
RESULTS

The main objective behind the research done this summer was to do experiments and retain this information to be understood in the future. To achieve these results, we used tools such as transgenic technology, PCR tests, western blots, and immunofluorescence histological staining. The main topic to be studied in this lab was to identify the pathogenesis of an acute kidney injury. We then organized that main idea into subtopics, such as looking into the proteins found in fibroblast cells, the sub-populations found in fibroblasts, looking into kidney microtubular cells, understanding the roles of proteins found in certain parts of the kidney, understanding the various types of acute kidney injuries, and finding therapies that could help kidney disease. Dr. Zhou made sure that I focused on mastering PCR tests, western blots and immunofluorescence staining. I then also used the information found through the project to conclude the genetics behind kidney disease.

First, we started off with some research. As a team, we looked into what caused the start of an acute kidney injury. I found that other health issues, including diabetes and high blood pressure, were both prevalent symptoms of patients who had acute kidney injuries. With diabetes and high blood pressure, they can both harm and strain the blood vessels in our kidneys, which can lead to an injury. Looking into some of the causes of kidney disease led me to a specific issue called fibrosis.

Fibrosis is the act of fibroblast cells in the kidney overpopulating themselves at a specific part of the kidney and harming the kidney by stopping that part of the kidney from working. From this information, we took a deeper look into the proteins found in these fibroblast cells. We saw that the proteins in the fibroblast cells had fibroblast activation, which is responsible for the extracellular matrix. In short, the ECM provides support and structure for the organ it occupies.
The fibroblast cells would initially be unspecialized cells and were activated at times that the kidney needed help repairing a specific part of itself.

Though this was good at first, in some cells, they would be programmed to create an excessive amount of ECM. This would, in turn, make the symptoms of fibrosis enacted faster. The extra support of the ECM would be too much for the kidney to handle and completely shut down a specific part of it. Using the protein assay, we also tried to look into some of the sub-populations found in fibroblasts. However, we came up short on that information because there is very limited knowledge about fibroblasts, which means there is minimal technology for us to study further into the topic. We know that there are multiple subpopulations of fibroblasts that act differently based on what task needs to be performed. However, we are currently unable to differentiate the fibroblast cells, so we also don’t know how many subpopulations there are.
We also took a particular interest in kidney microtubular cells using the protein assay. Since fibroblasts are our main topic when looking into acute kidney injuries, we also wanted to see how they got the information to work on the parts of the kidney that they do. We found that kidney microtubular cells help the fibroblasts to communicate with each other from across the kidney. They are very important to this organ because they are truly the glue that holds the kidney together. They make sure that early-stage activated fibroblasts can connect with each other to close any cuts in a short and quick process.

Overall, I found that with acute kidney injuries, fibroblast cells are one of the main cells at work. They are the last resort when the kidney is harmed. However, there isn’t much research on the different types of the cell. They try their best to make sure that an acute kidney injury doesn’t turn into chronic kidney disease.

Next, we looked into the various types of acute kidney injuries, and we wanted to make sure I could identify what protein was prominent within various acute kidney injuries. We took a look into the main three injuries. This included sepsis, ischemic and drug-induced models. Using western blot techniques, I could study each model and see the protein types. I could also study the functionality of each protein in each model. This then allowed me to see how each type of acute kidney injury model affected the kidney and their severity.
We then looked into finding therapies that could help kidney disease. We understand that proteins should be our target in studying kidney disease. To simplify, we concluded that we need to target the harmful proteins and inhibit their activities so that they can stop repopulating and affecting the kidney negatively. Precisely, we needed to get rid of the bad fibroblasts. They are indeed the root of the issue we focused on because they would be inhibiting the actions of a part of the kidney that needed to work to keep the whole organ in homeostasis. To get rid of the bad fibroblasts, we need to be able to identify what proteins are harmful. Therefore, there needed to be a criteria of what was meant by harmful and how we could separate those from the good proteins. This opened up the conversations of finding these “bad” characteristics.
This information was very valuable for the lab and the research we were conducting there. It also helped me to make some conclusions about the genetics behind kidney disease. This lab gave me a foundational understanding of the concept of an acute kidney injury. By mastering the techniques of PCR testing and western blotting, I became fluent in the language of the lab. This information helped me to expand further on my personal project. Through these repetitive tasks, I learned that kidney disease does not rely entirely on your genetic code but includes other factors such as your diet and other health issues you may have. However, there have been some factors that I will expand further on in the discussion section that show that genetics definitely plays a role in kidney disease.

All of these findings were only able to be done through the tools I used every day in the lab. From culturing my own cells to using immunofluorescence histological staining, I expanded my knowledge. Looking into the PCR tests, I could almost expand the little information I had in a cultured cell by duplicating a specific target sequence so that I could see more of a particular part of the rodent DNA we were looking at. The western blot technique helped by allowing me to see what antibody was used in the observed protein. It worked by creating a gel line for each protein. With the gel line length increasing, so did its color. This is genuinely how you could see how heavy each antibody was. With the weights differing for each antibody, you would then be able to differentiate which is which and know where each is populated. Immunofluorescence staining helped pinpoint the exact location of the proteins we were looking at. These tools all worked together to give us the information needed to help combat kidney disease.
DISCUSSION

The entire lab experience truly taught me that science is a language that needs to be studied to unearth the information being taught entirely. Every day, so many of us face language barriers that stop us from communicating with each other. But I’ve learned that science as a language can actually bring together different cultures because of the common interest in exploring ourselves and our environment around this. When we choose to take that extra step to learn this new culture of science, we can then create breakthroughs in research that have already been found. It can bring different perspectives together like puzzle pieces to create a development waiting to be discovered. By stepping out of my comfort zone and joining this lab, I was able to draw connections in the lab between our rodent specimen and how it affects different populations of people in real life.

After understanding the effects that diabetes and high blood pressure can have on the likelihood of a person developing kidney disease, I was led to the conclusion that these factors can also affect a specific race. Both diabetes and high blood pressure are very high ailments in the Black community. This answered part of the question as to why kidney disease was so prevalent in this community. With diabetes and high blood pressure being dominant in this community, these symptoms would increase their chance of developing kidney disease. This would conclude that kidney disease could be genetic as the symptoms that cause it run through specific groups of people through their DNA.

There is also another factor that backs up the claim that kidney disease could be developed because of genetics. In the past, Dr. Zhou experimented using a white mouse and a black rodent. He removed one kidney from each rodent and found that the white mouse went to live normally for ten days, while the black rodent died after two days of removal. The singular
change in their fur caused an entirely different reaction to the same procedure for both organisms. This means I could look further into the genes that are responsible for this phenotype to see how it affects each rodent’s health status. I plan to look into this further by studying research articles that separated their patients into different races and seeing if the phenomenon also occurs in humans.

However, with this conclusion comes more questions and theories. Which includes; what makes diabetes and high blood pressure genetically popular for the black community? Could we look into the diet of specific black communities and see if it ties back to diabetes or high blood pressure? Could there be cultural values in different black communities that could increase high blood pressure, such as the fear of going to a doctor that is prevalent in African-American communities? Is there a specific gene that ties back to diabetes for black people? Is there a particular gene for race that also correlates with the health of the kidney, or could it be connected to symptoms that help to develop kidney disease? All these questions take me back to my original inquiry, where I ask, “are kidney disorders related to genetic or societal factors?” and I add, “or cultural factors or a mixture of each?”

I plan to work further with Dr. Zhou in his lab in the upcoming semester, and my future focus is to have the focal point of understanding the difference between the two rodents and what were the factors that caused their difference in health. Through this, I would need to zone in on the different departments of the kidney. This includes the interstitial, fibroblast and microtubule proteins. Each of these cell types has its own specific markers, in which we need to determine what kind of cell these proteins are expressed.

I’d also like to move further with Dr. Zhou on his understanding of fibrosis, and I’d like to look into the specific genes that are known to cause fibrosis. I would do this to see if it is
found to be popular among a particular race. If it is, I’d like to see if there is a difference in
protein expression when comparing different ethnicities. At the end of the day, I am trying to
unearth truths that are already there by looking at them in a different light. A new perspective is
needed to expand our vocabulary in a language fluent to all.
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