What is the role of PRPF39 in cisplatin treated cancer cells?

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ABSTRACT

Chemotherapy resistance remains a challenge in treating cancer. There are many factors that can affect resistance, and it is not fully understood. PRPF39 is a pre-mRNA processing factor that plays a role in the mRNA processing of many genes. PRPF39 could be a candidate in understanding resistance because it affects many other genes. Previously, it was shown that PRPF39's baseline expression was correlated to cisplatin sensitivity and resistance. My research tested the limits of this observation by looking at the cisplatin induced expression of PRPF39 in cancer cells. I hypothesized that studying PRPF39 expression in cisplatin treated cancer cells would yield new insights. I evaluated cisplatin induced expression in colorectal, breast, and lung cancer cells using qPCR. In colorectal cancer, the expression of PRPF39 decreased 28% ($p=0.004$) and 48% ($p=0.002$) at 24 and 48 hours respectively. In breast cancer, the expression of PRPF39 was 23% downregulated at 24 hours ($p=0.02$), and it showed no change in expression at 48 hours. In lung cancer, the expression of PRPF39 was 52% upregulated at 24 hours ($p=0.002$), but it was 61% downregulated at 48 hours ($p=0.02$). Cisplatin induced gene expression of PRPF39 was shown to be highly dynamic across time and different cancer types. Of the three cancers studied, colorectal cancer is the most promising for PRPF39 modulation to

improve cisplatin treatment outcomes due to its consistent downregulation. Gaining more knowledge of PRPF39 in response to drug treatments is important in combating drug resistance.

INTRODUCTION

Currently, many people suffer from cancer. It is a disease that affects millions of people throughout the world. People are dying from this disease at a rapid rate. The side effects of chemotherapy also cause people to suffer. Chemotherapy can cause people's bodies to deteriorate and dwindle away- losing hair, losing weight, and causing severe pain. According to the *National Cancer Institute*, chemotherapy is defined as "treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing." This is one of the most common and effective ways to treat cancer. However, cancer still seems to be stronger than chemotherapy treatments. Cancer treatment comes in many different forms: radiation, drugs, injections, or infusions. Chemotherapy is designed to target cancer cells, but sometimes it recognizes normal cells as cancer cells and kills them. This is what causes the side effects of chemotherapy such as the loss of hair. Scientists want to improve chemotherapy by making it more accurate in targeting cancer cells and killing them. Chemotherapeutic treatments are extremely complex, and there are many genes that play a role in these treatments. This makes it more complicated to improve them.

When looking at chemotherapeutic drugs, there are many ways to research and study them. One way is to look at different genes in cancer cells and see how they respond to the treatment. Furthermore, does drug treatment cause a change in gene expression? Oftentimes, cells are

pulled between resistance and sensitivity, therefore understanding how genes act in the cancer cells can improve the understanding of drug treatments.

Selecting a gene to observe in cancer cells is somewhat difficult. However, there was a study conducted that looked at the gene, PRPF39, in non-cancer cells. In this study, *Functional consequences of PRPF39 on distant genes and cisplatin sensitivity*, it found that cells that showed higher baseline expression were also cells that showed sensitivity. It also found that cells that showed lower baseline expression were also cells that showed resistance. This relevant main conclusion suggests that there may be a correlation between baseline expression of PRPF39 and the cisplatin sensitivity and resistance. I wanted to test the limitations of this observation to see how PRPF39 would respond to cisplatin treatment in three different types of cancer. My study was looking to see how PRPF39 would respond to cisplatin treatment in cancer cells. It investigated the role of PRPF39 in cisplatin treated cancer cells. How can PRPF39 be used to improve cisplatin treatment.

HYPOTHESIS & METHODOLOGY

I hypothesized that the expression of PRPF39 would change in response to cisplatin treatment. The null hypothesis for this project was that the expression of PRPF39 would not change in response to cisplatin treatment. The hypothesis was evaluated by performing qPCR experiments to quantify the gene. First, the frozen cDNA from cancer cells induced with cisplatin was thawed. Then, the cDNA was taken and diluted to 12ng/ul and combined with the PRPF39 primer and MasterMix. The same reaction was prepared for a housekeeping gene, B2M. Next,

the prepared reaction was put in a qPCR machine, and it ran for 40 cycles. Finally, the fold change was calculated to determine the change in PRPF39 expression. Based on the fold change in PRPF39 expression, the role that the gene plays in sensitivity or resistance was determined. Two biological replicates were assayed in technical triplicate, which produced 6 individual results for each cancer. Then, the **ΔΔ**Ct formula was used to calculate the fold change.

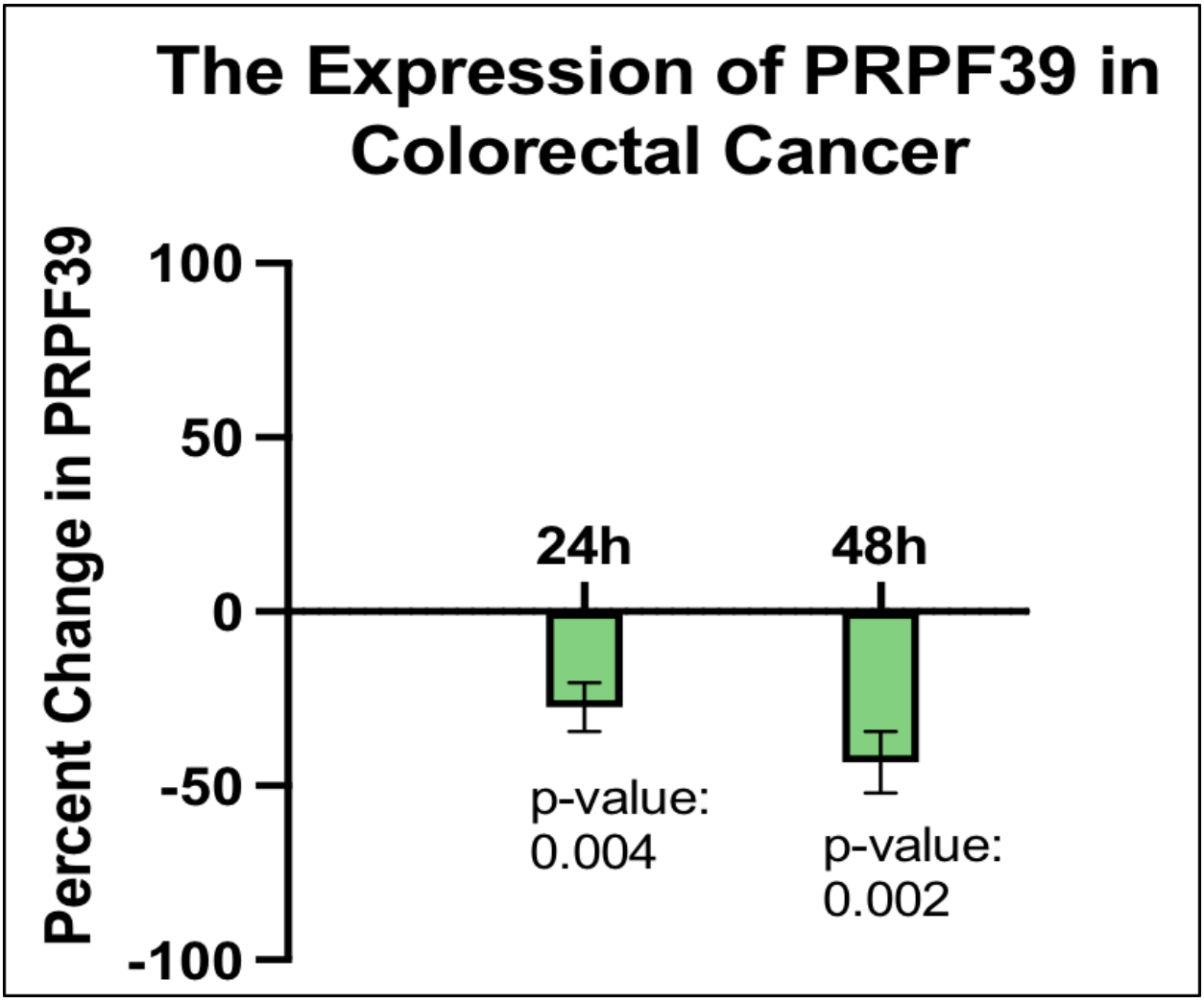
RESULTS & DISCUSSION

The role of PRPF39 was first looked at in colorectal cancer. The results showed that at 24 hours PRPF39 was 28% downregulated with a p-value of 0.004, and at 48 hours PRPF39 was 48% downregulated with a p-value of 0.002. The preliminary study suggests that PRPF39 is working through a resistance mechanism due to its lower expression at 24 and 48 hours. Furthermore, PRPF39 is not helping the cancer cells respond better to the cisplatin treatment. The cisplatin induced expression of PRPF39 in colorectal cancer is consistent at both 24 and 48 hours, and it shows the same downregulation trend at both time periods. To improve cancer cell sensitivity, the downregulation expression of PRPF39 could be blocked. Therefore, PRPF39 is a promising candidate for modulation in colorectal cancer due to its consistency in lower expression.

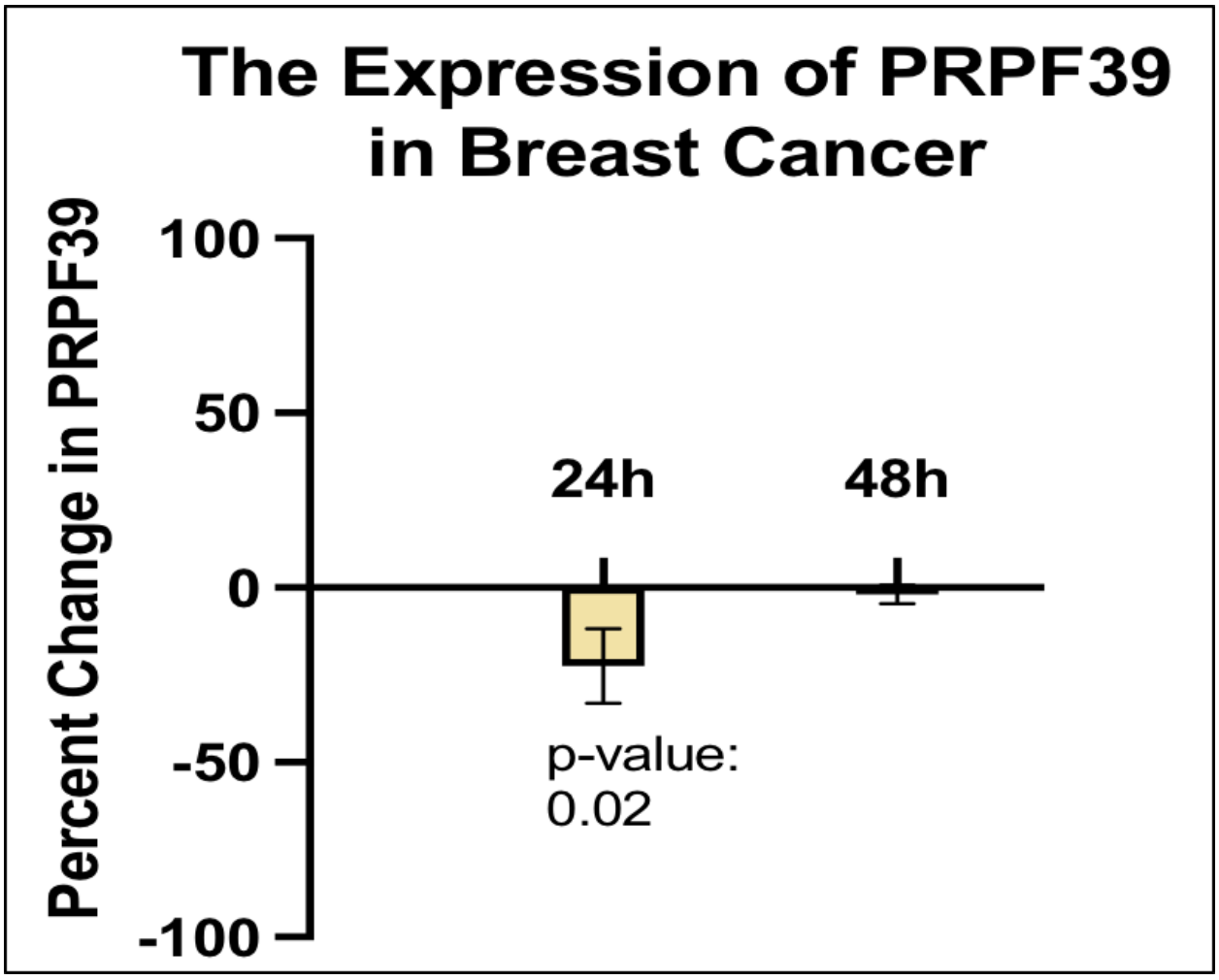
After observing the role of PRPF39 in colorectal cancer, the role of PRPF39 in breast cancer was analyzed. The results showed that at 24 hours PRPF39 was 23% downregulated with a p-value of 0.02, and at 48 hours it showed no change in expression. According to the preliminary study, the lower expression of PRPF39 at 24 hours suggests that PRPF39 is working through a resistance mechanism. However, at 48 hours there was no change in the expression of PRPF39. This

supports the null hypothesis which states that PRPF39 would not change in expression in response to cisplatin treatment, and it suggests that PRPF39 is not having an effect on the cisplatin treatment. Furthermore, at one time period PRPF39 is showing a response, and at another time it is showing no response. This shows how dynamic and complex PRPF39 is. It is a gene that can have many downstream effects and can give different types of feedback. At 24 hours, PRPF39 has the potential to be modulated at 24 hours by blocking the downregulation expression to induce cancer cell sensitivity.

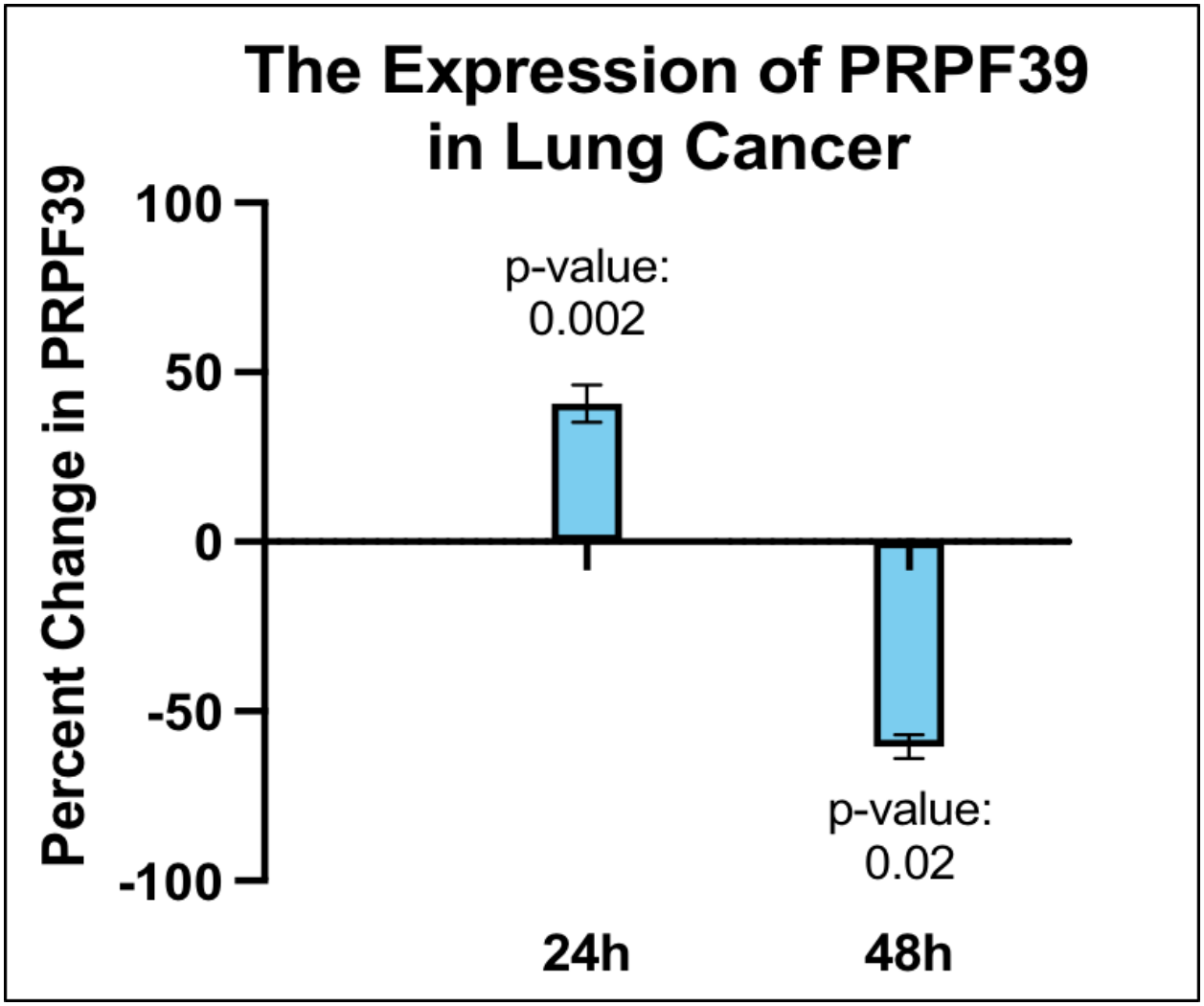
Following breast cancer, the role of PRPF39 was investigated in lung cancer cells. The results showed that at 24 hours PRPF39 was 52% upregulated with a p-value of 0.002, and at 48 hours PRPF39 was 61% downregulated with a p-value of 0.02. The preliminary study suggests that PRPF39 is working through a sensitivity mechanism at 24 hours due to its higher expression. Furthermore, PRPF39 is helping the cancer cells respond better to the drug treatment at 24 hours. However, the lower expression of PRPF39 at 48 hours shows that PRPF39 is working through a resistance mechanism. In lung cancer, PRPF39 exhibits a role in a sensitivity mechanism to exhibiting a role in a resistance mechanism. This is a drastic change and exemplifies how dynamic PRPF39 is. Because PRPF39 shows inconsistency in its expression in lung cancer, it is a less promising candidate for PRPF39 modulation.



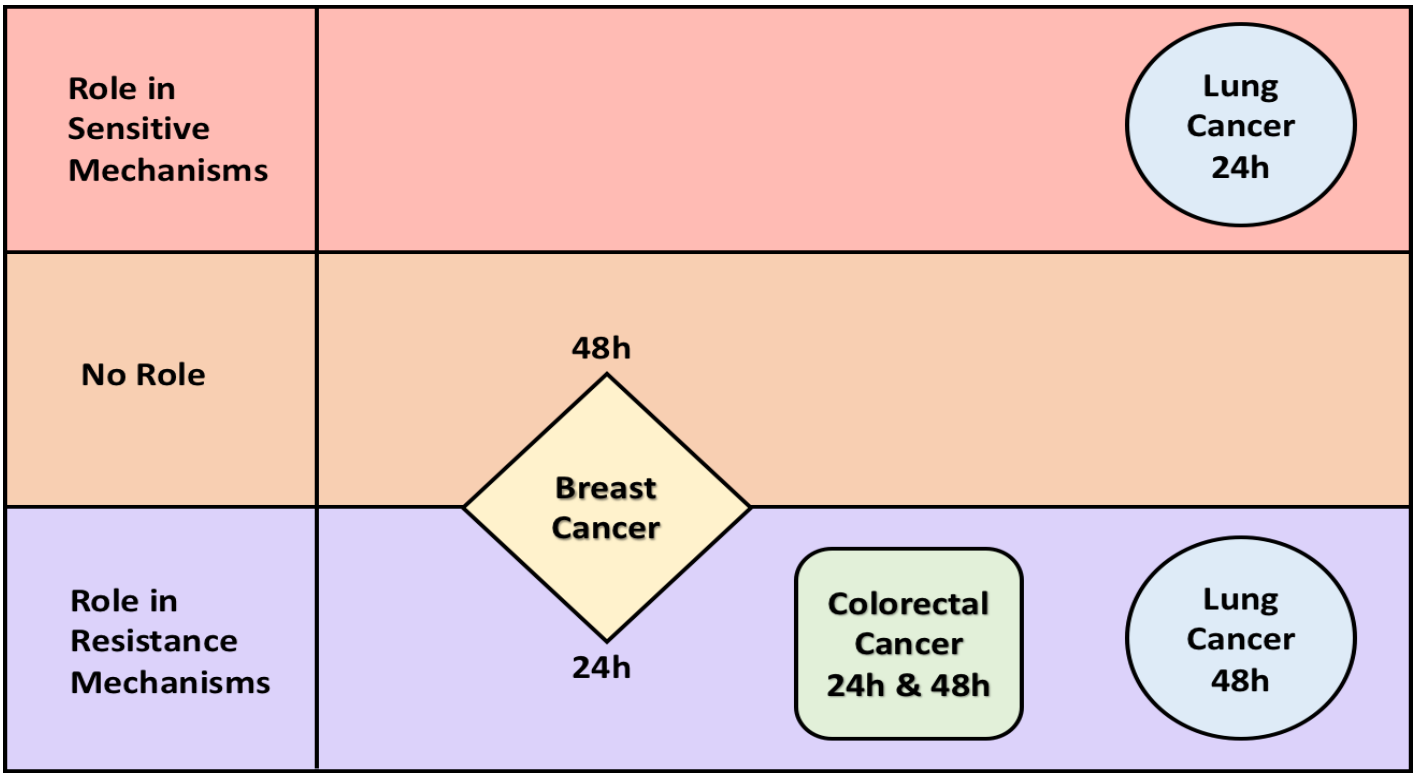
** This graph displays the percent change in PRPF39 in colorectal cancer at 24 and 48 hours. It shows that at 24 hours the expression of PRPF39 is 28% downregulated, and at 48 hours the expression of PRPF39 is 48% downregulated. The p-value for each result is presented beside the bar representing the percent change. In colorectal cancer, the percent change for both time periods is statistically significant.



** This graph displays the percent change in PRPF39 in breast cancer at 24 and 48 hours. It shows that at 24 hours the expression of PRPF39 is 23% downregulated, and at 48 hours there is no change in the expression of PRPF39. The p-value for each result is presented beside the bar representing the percent change. In breast cancer, the percent change for 24 hours is statistically significant.



** This graph displays the percent change in PRPF39 in lung cancer at 24 and 48 hours. It shows that at 24 hours the expression of PRPF39 is 52% upregulated, and at 48 hours the expression of PRPF39 is 61% downregulated. The p-value for each result is presented beside the bar representing the percent change. In lung cancer, the percent change for both time periods is statistically significant.



** This figure shows the results for all three cancers. This graph represents the similarities and differences in the role of PRPF39 in all three cancers. It also highlights that colorectal cancer is the most promising candidate for PRPF39 modulation due to its consistent role in a resistance mechanism.

CONCLUSION

In conclusion, the study's results support the hypothesis that the expression of PRPF39 would change in response to cisplatin treatment. Decreased expression of PRPF39 suggests that PRPF39 is working through resistance mechanisms. PRPF39 exhibited consistent lower expression in colorectal cancer at both 24 and 48 hours. This indicates that of the three cancers

researched, colorectal cancer is the most promising candidate for PRPF39 modulation. In comparison, the expression of PRPF39 in breast and lung cancer showed to be working in two different ways. The inconsistency in a single trend in these two cancers shows that it would be less straightforward for PRPF39 modulation. The expression of PRPF39 in breast and lung cancer is a less promising candidate for PRPF39 modulation because it does not stay constant. The statistical analysis supports that the expression of PRPF39 is not due to chance because the p-values of the results show to be significant as defined as 0.05. The statistical analysis also supports that PRPF39 is a gene that affects many genes and can give many types of feedback. To improve cancer cell sensitivity,the downregulation expression of PRPF39 in colorectal cancer could be blocked. There is the potential for PRPF39 to be modulated in colorectal and breast cancer, however, the expression of PRPF39 in breast shows to be a less promising candidate due to its inconsistency. These results help to further the understanding of PRPF39's response to cisplatin treatment and will help scientists to improve drug treatments to be more effective.

In the future, this project could be extended to looking at the role of PRPF39 in other cancers treated with cisplatin, such as ovarian and testicular cancer. Ovarian and testicular cancer are two other cancers that are treated with cisplatin. Therefore, PRPF39 would be investigated to see if it would exhibit similar trends in ovarian and testicular cancer. Expanding the understanding of the role of PRPF39 will help scientists to improve drug treatments. Furthermore, looking at the trends of PRPF39 in more cancers could help new trends, different trends, and similar trends. Therefore, the same approach to improve drug treatments could be used in cancers that show similar trends in PRPF39 expression. Next, the genes connected to PRPF39 would be investigated to understand their connection to PRPF39. In the preliminary study, it found that

there are more than 60 genes connected to PRPF39. The focus of researching these genes would be to see how they change when PRPF39 changes. Developing more knowledge on PRPF39 and its response to cisplatin treatment will help researchers to improve drug treatments and combat drug resistance.

WORKS CITED

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