The Impact of MTAP Deficiency on PRMT5 Inhibition

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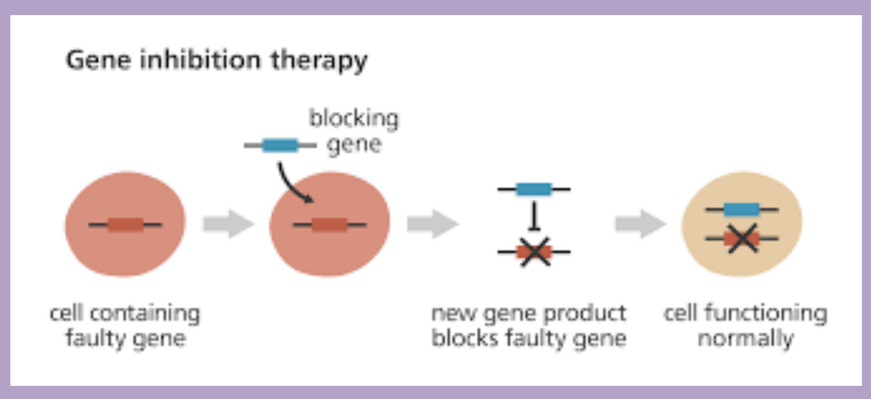
Introduction

Research context:

PRMT5 (protein arginine methyltransferase 5) is a gene that is overexpressed in a wide variety of cancers. Deficiency of the MTAP (methylthioadenosine phosphorylase) gene in certain breast cancer cell lines has been shown to impair PRMT5 activity. My goal was to investigate how susceptible MCF-7 (which expresses MTAP) and MDA-MB-231 (which lacks MTAP) are to inhibition. Therefore, I treated with a selective PRMT5 inhibitor—known as CMP5—to compare cell viability.

This research was conducted in Dr. Shawn He's Multiscale Biomaterials Engineering Laboratory.



Investigation

Research question:

Does MTAP deficiency impact PRMT5 inhibition in breast cancer cell lines?

Hypothesis:

The MTAP deficient MDA-MB-231 cells will be less resilient to PRMT5 inhibition than MCF-7 cells, and will therefore have fewer viable cells after being treated with CMP5.

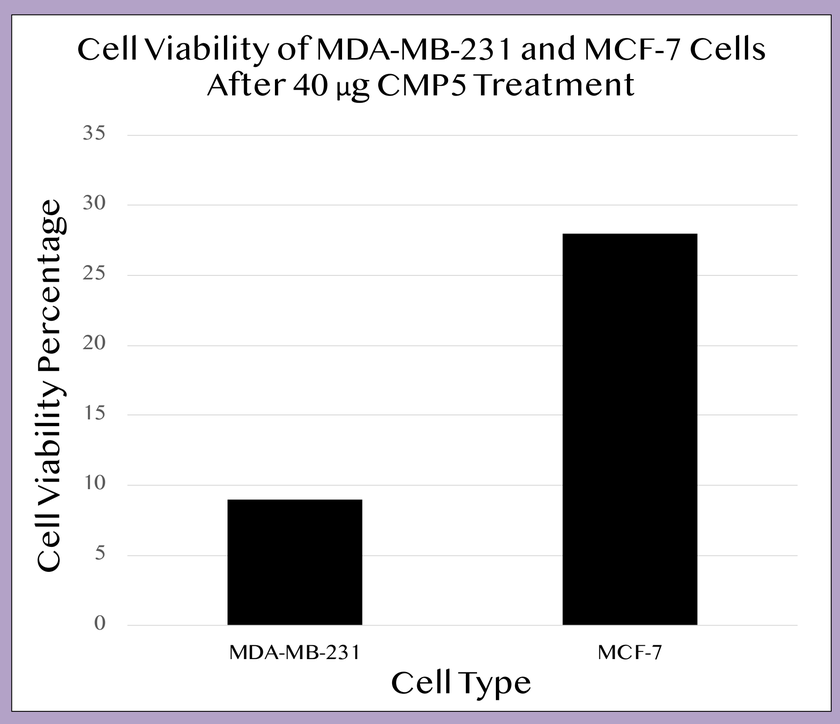
Methodology

Experiments and Procedures:

In order to properly compare the two breast cancer cell lines, both MDA-MB-231 and MCF-7 were treated under the same conditions by performing the following tasks:

- Cultured in DMEM growth medium every 3-4 days
- Used a hemocytometer to count cells
- Diluted cell solutions to achieve 5,000 cells per well
- Seeded cells in 96-well plates
- Treated cells with DMEM solution with 40 micrograms (μg) concentration of CMP5
- Incubated cells for 72 hours
- Treated the cells with CCK-8 (Cell Counting Kit-8)
- Measured absorbance using a microplate reader
- Used absorbance reading to calculate cell viability

Data



Conclusions

Analysis:

The bar graph displays that 9% of MDA-MB-231 cells were viable after treatment, and MCF7s had about 28% viability. This data supports my hypothesis, because the MTAP deficient MDA-MB-231 cells were less resilient to the PRMT5 inhibition. It is likely that a greater percentage of MCF7 cells could withstand the treatment due to MTAP expression.

Although my hypothesis was correct according to my first trial, there is still plenty of data for me to gather. Due to the short duration of this project, I was unable to perform multiple experiments with varying concentrations of CMP5 which could further demonstrate the effects of MTAP deficiency. Further research would have to be conducted to confirm the significant impact of MTAP deficiency on PRMT5 inhibition that I observed.

Reflection

Potential human error:

It is possible that made errors in measuring solution quantities with pipettes. Due to the quarantine circumstances, I was unable to perform multiple trials that could have minimized the effect of human error.

Suggestions for future research:

PRMT5 inhibition could be tested with nanotechnology as well. Nanoparticles could be formulated to treat cancer cells with CMP5. PRMT5 expression is prevalent in several other cancer types which could be similarly examined as well. This could be a novel approach to drug delivery for cancer treatment. I plan on continuing to explore the human genome and cancer treatment in my ongoing undergraduate research.

A special thanks to my Principal Investigator Dr. Shawn He for giving me this research opportunity, and to my mentor James Shamul for his guidance and support.