Inhibition of Histone Demethylases as a Cancer Therapy

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Introduction
Cancer cells are marked by a dysregulated epigenome, which is the non-coding part of the genome responsible for gene expression. This leads to the altered expression of multiple proteins. Abnormal expression of histone modifying enzymes, including demethylases, is a key contributor to the altered epigenetic landscape in cancer. Given their role in cancer development, demethylases have recently risen as potential targets for cancer therapeutics.

Of particular note is the JARID 1 family of histone demethylases, which while present in normal cells, is overexpressed in various types of cancer cells, especially breast cancer. Interestingly, the overexpression of JARID 1 family members marks a population of slow-cycling cancer cells that are resistant to many conventional chemotherapies (Wang). By targeting the JARID 1 family with synthetic inhibitors, relapse could be greatly reduced among cancer patients.

Because certain types of cells fall under the radar in current therapies, a more advanced therapy would be one that affects characteristics in all breast cancer cells. Specifically, potential drugs have been identified that affect the overexpression of the JARID 1 family of histone demethylases. JIB-04, a synthetic pan inhibitor of the Jumonji family of demethylases was found to both work in vitro and in vivo to reduce histone demethylase activity in tumors and prolong cancer survival in mice. (Wang) While managing to regulate histone demethylation in cancer cells, this drug also did not affect normal cell proliferation, meaning that it is potentially capable of inducing specialized cell death. Similarly, GSK-J4 is another such molecule that is a histone demethylase inhibitor, which we will be testing alongside JIB-04. (Rochefort)

In this series of experiments, we compared the effects of the drugs on two different cell lines: MCF7 and MB231 breast cancer cell lines. The purpose of using multiple cell lines was to make sure that the drugs do not only work on one cell line, but rather a wide range. Ideally, a possible treatment would work on the largest population. MCF7 cells are estrogen receptor positive (ER+), whereas MB231 cells are estrogen receptor negative (ER-). This is an important difference, because ER+ breast cancers often have a better prognosis and are responsive to anti-estrogen therapy, whereas ER- breast cancers are more aggressive and do not respond to anti-estrogens. (Rochefort et al) By testing GSK-04 and JIB-04 on both types of lines, we observed if the drugs work on both ER types—a huge hurdle in current therapies.

Methods
MCF7 and MB231 cells were treated with JIB-04 and GSK-J4 and cell growth was determined using a cell proliferation assay called a Sulforhodamine B assay that measures optical density of dyed protein as a surrogate for cell number. This way, we can observe the effects of JIB-04 and GSK-J4 on cell proliferation. The reason two cell lines were tested is because we wanted to see if the drugs’ effects were cell line specific.

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Conclusions
Preliminary results show that in all trials of the experiment, cell proliferation decreased with increasing dosages of JIB-04 and GSK-J4 drug treatments as compared to untreated MCF7 and MB231 cells. This means that with higher dosages of drug, cell growth was stunted. Additionally, the DMSO vehicle appears to have minimal impact on the cells, seeing that % control cell growth remains relatively close to 100%. This indicates that it is a relatively neutral vehicle.

The IC50 of JIB-04 treated MCF7's is more than 10-fold less than that of MB231, indicating that the ER+ cell line tested was more sensitive to JIB-04 than the ER- cell line tested. The IC50 of the GSK-J4 treated MCF7s and MB231 is unable to be determined. While there was a stable plateau of cell growth at lower concentrations of GSK-J4, there was a sudden, large drop in cell growth from 1 to 3 x 10-5 M dosages. There does not appear to be a difference in sensitivity between the two cell lines either in GSK-J4 or the inactive enantiomer, GSK-J5. However, it is interesting to note that there is a more than 100-fold increase in sensitivity when cells are exposed to JIB-04 rather than GSK-J4, indicating that these drugs, though the same class of molecule, might have different defined targets and inhibit differently from each other.

References


Results

Figure 1: MCF7s and MB231 cells were treated with various concentrations of the indicated drug for 72 hours. A dose-response curve was generated from the data, indicating the effects of JIB-04 and it’s affiliated vehicles on cell proliferation.

Figure 2: MCF7s and MB231 cells were treated with various concentrations of the indicated drug for 72 hours. A dose-response curve was generated from the data, indicating the effects of GSK-J4 and it’s affiliated vehicles on cell proliferation.

Future Directions
During the upcoming school year, I will be conducting gene expression assays using lower dosages of GSK-J4 and JIB-04 for a longer treatment period on modified HeLa and MCF7 cell lines. These are both stably expressing a TMS1-GFP construct. TMS1 is a gene that is differentially methylated in cancer subtypes. The promoter of TMS1 is fused to the protein coding portion of GFP, so when the cell signals for TMS1 to be transcribed, GFP is transcribed instead, and the subsequent protein fluoresces green. The purpose of this research is to see how much the expression of GFP returns after treatment, seeing that currently, the promoter region is shut off.