Hypoxia, or a condition characterized by a deficiency in oxygen’s ability to reach cell tissues, is known to increase insulin resistance in cells, contributing to diabetes. Hypoxia-inducible factors (HIFs) regulate hypoxia within cells and have been implicated in insulin resistance, while prolyl hydroxylase protein isofoms (Phd1, Phd2, Phd3) degrade HIFs. It was shown that knocking out the Phd3 gene in the mouse hepatocytes stabilizes HIF-2a in murine hepatocytes, which in turn leads to increased insulin sensitivity and improvement of diabetes.

The purpose of my mentor’s project is to investigate the effects of PHD modulation with respect to HIF stabilization and insulin sensitivity in human hepatocytes. My role in the project was to use a process called qRT-PCR, or real-time reverse transcriptase polymerase chain reaction, on extracts from HepG2 cells (human hepatocytes), so that it can be verified that the correct gene was knocked down, and to see if other genes are being affected by the knockdown procedure.

The Knockdown

PHD3 was knocked down using siRNA, or silencing RNA, which is a double-stranded RNA molecule that prevents specific genes’ expression by breaking down mRNA before it is synthesized into protein.

RNA is isolated from cells using a commercially available kit that uses a phenol-chloroform extraction. Using the enzyme reverse transcriptase, the RNA is reverse transcribed to cDNA, which is the double-stranded DNA complementary to the RNA, so that it can be more accurately measured by the qPCR machine. This cDNA is diluted, and then is mixed with primer and SYBR Green (the fluorescent dye for the qPCR). The samples are then loaded into wells and fed into a qPCR machine, which performs the PCR and produces the data. As the machine cycles, SYBR green intercalates into the PCR product, which produces a fluorescent signal for the machine to read. The raw data is provided as cycle threshold, which is then normalized using the 2Δct method. This normalizes changes in gene expression to the changes of the loading control (RP2) and the control sample.

Results

PHD3 Knockdown in HepG2 cells

<table>
<thead>
<tr>
<th>Method</th>
<th>PCR Image</th>
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<tbody>
<tr>
<td>SCRM</td>
<td><img src="https://en.wikipedia.org/wiki/Polymerase_chain_reaction" alt="PCR Image" /></td>
</tr>
<tr>
<td>PHD3KD - 50μM</td>
<td><img src="https://en.wikipedia.org/wiki/Polymerase_chain_reaction" alt="PCR Image" /></td>
</tr>
<tr>
<td>PHD3KD - 100μM</td>
<td><img src="https://en.wikipedia.org/wiki/Polymerase_chain_reaction" alt="PCR Image" /></td>
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</tbody>
</table>

Discussion

The goal of this part of the project was to knock down Phd3 gene expression. If the knockdown was successful, we should now see a marked decrease in Phd3 mRNA abundance and no change in the expression of Phd1 and Phd2.

As expected, the graph shows marked decrease in Phd3 for the knockdown sample. As the amount of a strand of DNA increases exponentially, the intensity of fluorescence increases. After each cycle, a detector measures the fluorescence until the PCR is complete. Afterwards, it gathers all of the data for analysis by the experimenter.

References

- PCR Image: [https://en.wikipedia.org/wiki/Polymerase_chain_reaction](https://en.wikipedia.org/wiki/Polymerase_chain_reaction)