Cell cycle analysis of MDA-MB-157 and APC knockdown cells
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Background and Hypothesis:

A majority of sporadic breast cancers include deficits in the expression of Adenomatous Polyposis Coli (APC), a tumor suppressor. Deficits in APC are more common in patients with TNBC (triple negative breast cancer), which is also a cancer prone to chemotherapy resistance. The Prosperi lab previously found that APC knockdown cells (APCKD) were resistant to Paclitaxel (PTX). We hypothesize that APCKD cells are resistant to PTX treatment through avoidance of G2/M arrest. This summer, my goal was to investigate the mechanism by which PTX works to arrest the cell cycle at G2/M.

Experimental Design or Project Methods:

Cell Synchronization: To synchronize MDA-MB-157 and APCKD cells, we first tried serum starvation for 24-72 hours. Second, we tried synchronizing the cells using a double thymidine block as described (Bostock, 1971). In either protocol, cells were then stained with Propidium Iodide (PI) and flow cytometry was performed.

Paclitaxel (PTX) treatment and cell cycle analysis: MDA-MB-157 and APCKD cells were grown to confluency and then treated with 0.078µM PTX for 12, 24, and 48 hours. Cells were stained with PI and flow cytometry was performed.

Results:

Cell synchronization: APCKD cells cells have an increased cell population in the G2/M phase than the parental cells after serum starvation. Importantly, APC knockdown cells are not impacted by serum starvation up to 72 hours. Additionally, a double thymidine block is insufficient to synchronize MDA-MB-157 and APCKD cells. A double thymidine block did shorten the S phase and move MDA-MB-157 and APCKD cells closer to G0-G1 arrest, but did not synchronize.

Paclitaxel (PTX) treatment and cell cycle analysis: MDA-MB-157 and APCKD cells treated for longer intervals experienced more cell death and were further arrested in G2/M.

Conclusion and Potential Impact: We learned that MDA-MB-157 and APCKD cannot be easily synchronized using serum starving or a double thymidine block. Future investigations will require alternative methods of synchronization or will proceed without synchronization. Furthermore, APCKD cells do not avoid G2/M arrest when treated with Paclitaxel, indicating a different mechanism of PTX resistance.