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 (APC^{KD}) were resistant to Paclitaxel (PTX).
We hypothesize that APC^{KD} cells are resistant to PTX treatment through avoidance of G_2/M arrest. This summer, my goal was to investigate the mechanism by which PTX works to arrest the cell cycle at G_2/M.

Experimental Design or Project Methods:

Cell Synchronization: To synchronize MDA-MB-157 and APC^{KD} 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 APC^{KD} 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: APC^{KD} cells cells have an increased cell population in the G_2/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 APC^{KD} cells. A double thymidine block did shorten the S phase and move MDA-MB-157 and APC^{KD} cells closer to G_0-G_1 arrest, but did not synchronize.

Paclitaxel (PTX) treatment and cell cycle analysis: MDA-MB-157 and APC^{KD} cells treated for longer intervals experienced more cell death and were further arrested in G_2/M.

Conclusion and Potential Impact: We learned that MDA-MB-157 and APC^{KD} 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, APC^{KD} cells do not avoid G2/M arrest when treated with Paclitaxel, indicating a different mechanism of PTX resistance.