General Information About Prostate Cancer

- Definition: Cancer that forms in the tissue of the male prostate gland.
- Estimated new cases (2013): 238,590
- Estimated deaths (2013): 29,720 (About 12%)
- Fatality occurs when the tumor cells metastasize, meaning, the cancer spreads from one organ to other, more distant organs.
Disparity in Prostate Cancer

- Incidence Rate: 144.9 per 100,000 white men compared to 228.5 per 100,000 black men.
- Mortality Rate: 21.2 per 100,000 white men compared to 50.9 per 100,000 black men.

Chemotherapy

Chemotherapy is described as the use of drugs to destroy cancer cells. Chemotherapy drugs often interrupt the cell cycle, causing the cell to be unable to divide. When the cell is unable to divide it undergoes apoptosis, which is a programmed cell death.

*Apoptosis in a prostate cancer cell after treatment with Etoposide*

Docetaxel

- A taxane, originally natural developed from the yew tree, now synthetically developed.
- Works by disrupting microtubule function, therefore inhibiting cell division.
- Also thought to be radiosensitizing, making cells more susceptible to death after radiation treatment.

What are G Protein-Coupled Receptors?

- Family of proteins that transduce extracellular stimuli into intracellular functions.
- Three Steps: Ligand Binding, Conformational Change, and Deactivation.
- Function regulated by G Protein-Coupled Receptor Kinases (GRKs) and Arrestin.
G Protein-Coupled Receptor Kinases (GRKs)

- GRKs along with Arrestin regulate GPCRs through a process called desensitization.
- Phosphorylation of the GPCR by GRKs is the first step in down regulation and eventual desensitization of GPCRs.

Why GRK5?

- GRK5 expressed in high concentrations in aggressive prostate cancer.
- GRK5 leads to desensitization of GPCRs.
- GRK5 has an immediate role in the regulation of prostate tumor growth.
Purpose

To investigate whether or not shGRK5-PC3 cells are more responsive to treatment (more prone to apoptosis) than wild-type shGFP-PC3 by treating both cells lines with 1μM of the chemotherapy drug, Docetaxel.

Methods

Cell Culture: Maintain Cell Line
- Completed twice a week, incubated at 37°C in a F-12k medium with 10% serum and 1% penicillin. at 0.25% Trypsin-EDTA

Immunoblotting (Western Blot): Determine expression of apoptosis markers.
- 10% resolving gel, incubated with 1° antibody over night in 4°C refrigerator, incubated with 2° antibody for an hour, washed 3 times for 5 minutes each with PBST.

Confocal Microscopy: Determine the abundance of nuclear fragmentation in both cell lines.
- Cells were fixed in 2% paraformaldehyde, stained with phalloidin and DAPI, and then micrographed using a Leica SP5 confocal microscope at 63X.

Flow Cytometry: Detect percentage of cells in subG₀/G₁.
- Both cell lines were fixed in 70% chilled ethanol and stained with propidium iodide. The cell cycle was analyzed in a flow cytometer.
Confocal Microscopy

Nuclear fragmentation using DAPI and Phalloidin. Nuclear fragmentation is an apoptosis indicator. The treated shGFP-PC3 cells show minimal nuclear fragmentation while the shGRK5-PC3 cells show ample nuclear fragmentation as indicated by the white arrows.
Additionally, the knockdown cells had a greater number of detached cells than the wild type, indicating a greater amount of apoptosis or cell death.
Flow Cytometry

shGFP-PC3 without Docetaxel  shGFP-PC3 with Docetaxel

The percentage of cells in subG₀/G₁ after Docetaxel was added is 3.54%.

Flow Cytometry

shGRK5-PC3 without Docetaxel  shGRK5-PC3 with Docetaxel

The percentage of shGRK5 cells in subG₀/G₁ after Docetaxel was added is 38.86%.
Flow Cytometry

The percentage of knockdown cells in subG₀/G₁ after Docetaxel was added is 38.86% which is significantly higher than the percentage of wild type cells in subG₀/G₁, which was only 3.54%.

Immunoblotting

The shGRK5 cells displayed a greater amount of PARP cleavage after treatment as compared to the shGFP cells after treatment.
Conclusions

- The shGRK5-PC3 cells treated with Docetaxel had a greater amount of cleaved PARP, a greater amount of fragmented DNA, a greater amount of fragmented nuclei as compared to the wild-type shGFP-PC3 cells treated with Docetaxel.
- The shGRK5-PC3 cells were sensitized, or more responsive to the Docetaxel treatment as compared to the wild-type shGRF-PC3 cells.

Further Research

- Investigate the role of Arrestin in the desensitization of GPCRs and ultimately prostate cancer cell proliferation.
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References


