The Effects of PDK4 on Drug Sensitivity

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Breast Cancer

• Increase in recent years
• Cancer is the highest leading cause of death
• Breast cancer has the second highest mortality rate in cancer patients
• 12% of American women will contract breast cancer
• One in six will die from it
• Even higher if it runs in the family
Goals

• To detect drug resistance in early stages of cancer development
  ▫ Improve patient care by avoiding antiestrogen therapy and the risks associated with it
• To better understand cancer cell metabolism
  ▫ Potential to synthesize new drugs
Model System

woman with metastatic breast cancer

Michigan Cancer Foundation

MCF-7 cell line

Masonic Cancer Center

MCC-MCF-7 cell line

in vitro selection for resistance to 4-OH Tamoxifen

TamR-MCF-7 cell line
Model System

- Drug sensitive breast cancer cells: MCC-MCF7
- Drug resistant breast cancer cells: MCF7-TamR. Selected for tamoxifen resistance.
Preliminary Data

RT² Profiler PCR Array: Human Glucose Metabolism

Comparison of gene expression in estradiol treated breast cancer cells.
Observation: PDK-4 mRNA is elevated in TamR

We measured PDK4 mRNA and 18s rRNA in TamR and MCC cells using qPCR. Each bar represents the average of four qPCR reactions; error bars indicate 95% confidence interval (n = 3). The p values are from two tailed student t tests.
Setting the Stage

• Investigate the effects of miferpristone and dexamethasone on breast cancer cells and correlate it to drug resistance

• Hypothesis: Dexamethasone treatment and the drug resistant TamR phenotype are dependent on PDK4 expression and activity
Why mifepristone and dexamethasone?

Dexamethasone is an agonist of the glucocorticoid receptor, and mifepristone is an antagonist.
Our (Theoretical) Approach

- First step: Determine the effects of dexamethasone and mifepristone on breast cancer cells to confirm their (ant)-agonistic roles
  - qPCR (mRNA level)
- Second step: Use PDK4 siRNA to knock down PDK4
  - Confirm that the siRNA is knocking down PDK4
  - SRB assay (total cellular abundance)
- Third step: Confirm the effects of PDK4 manipulation on the protein level
  - Confirm that we get a PDK4 band
  - Repeat step 2 using Western Blots
First Step: Manipulation of PDK4 expression by glucocorticoid receptor ligands

PDK4 mRNA expression is regulated by dexamethasone (+) and mifepristone (-) in drug resistant cells, but not in drug sensitive cells.
Second step: Use PDK-4 siRNA to knock down PDK4

PDK4 mRNA is successfully knocked down in TamR cells after transfection with anti-PDK4 siRNA.
Second step: Use PDK-4 siRNA to knock down PDK4

Transfection with PDK4 siRNA increases TamR sensitivity to ICI. MCC and TamR cells were transfected with PDK4 siRNA, then treated with 0, 1 or 5 nm ICI. Four days later cell abundance was measured by SRB staining. The average SRB staining relative to control is shown; error bars indicate one standard deviation (n = 4). Treatments that do not share the same letters are significantly different (one way → Tukey-Kramer HSD, p < 0.05).

Knocking down PDK-4 shows a significant change in drug sensitivity in the drug resistant cells.
Third step: Confirm the effects of PDK4 manipulation on the protein level

We were able to confirm that there is an elevated PDK-4 band in dex treated TamR cells.
Problems

- Unable to replicate clean PDK4 bands
- Unable to get a sample concentrated enough for an siRNA experiment
What’s Next?

- Currently working on improving Western Blotting conditions
  - e.g. stringent antibody and blocking buffers
  - Try other primary antibodies
Credits

• Dr. Andrew Skildum, department of biomedical sciences, University of Minnesota Duluth
  ▫ For working closely with me on the project and overcoming some of the bumps we had along the road

• UROP
  ▫ Funding
  ▫ Opportunity to make this project possible