Subcellular translocalization of Cdx2 protein mediated by Notch inhibition

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Introduction

- Caudal type homeobox 2 (Cdx2) is one of three caudal-related homeodomain transcription factors (Cdx1, Cdx2, Cdx4) (Witek, 2010)
- In adult humans, Cdx2 is specifically expressed only in the epithelial cells of the small and large intestines
- Cdx2 has been studied for its contribution to embryonic development and colon cancer, but much is still unknown about Cdx2 behavior in signaling pathways
- Cdx2 has been shown to play a role in the Notch signaling pathway

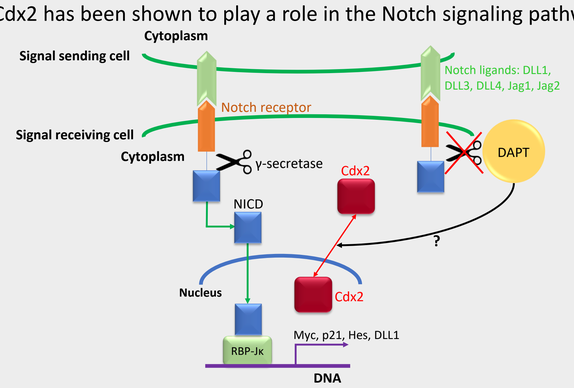

Figure 1. Schematic illustration of Notch signaling pathway and proposed interaction with Cdx2. Modified from Jiang C, et al. (2016)

- The Notch pathway is crucial for cell differentiation and proliferation through communication between adjacent cells (Fre, 2005)
- Colorectal colon cancer cells, SW480 (pre-metastatic colon cancer tumor cell line) and SW620 (patient-matched metastatic colon cancer cell line) were used to explore Cdx2 behavior when the γ-secretase component of the Notch signaling pathway is inhibited
- Using a γ-secretase inhibitor, N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), Notch signaling can be inhibited. Subcellular fractionation and western blotting is then used to determine the subcellular localization of Cdx2.

Objectives

The aim of this project was to:

- Determine if the Notch pathway inhibitor DAPT affects Cdx2 subcellular localization in colorectal colon cancer cells
- Determine if inhibition of the Notch pathway changes the subcellular localization of Cdx2 between pre- and post-metastatic colon cancer tumor cells

Methodology

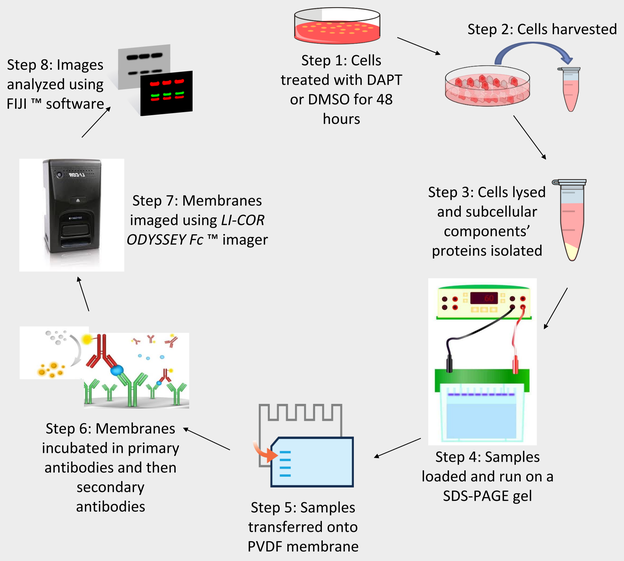

Figure 2. Pictorial representation of western blotting experiment.

Results

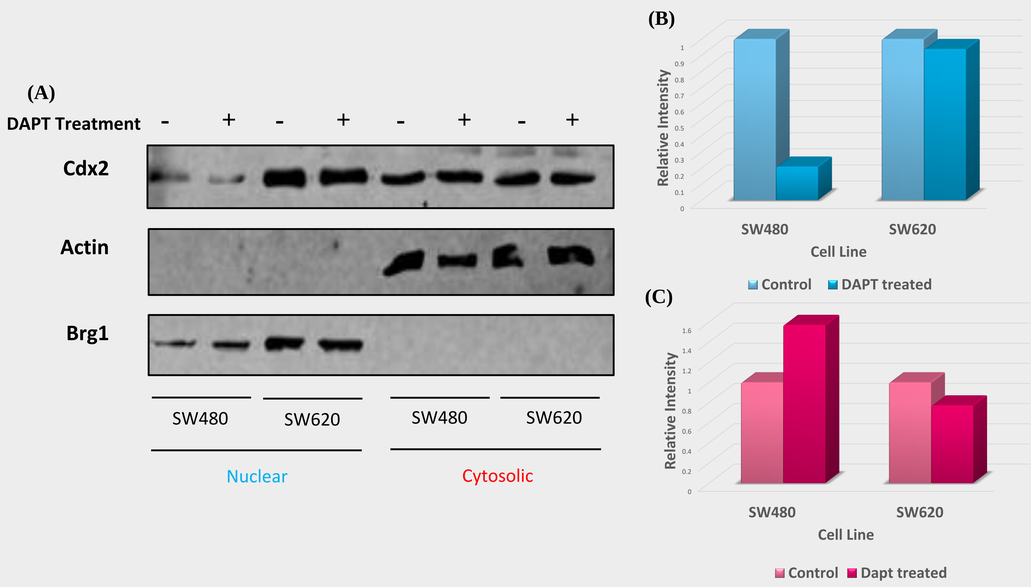

Figure 3. First trial of Notch inhibition with 48 hour DAPT treatment on SW480 and SW620 cells. (A) Western blots of Cdx2, actin and Brg1 from nuclear and cytosolic fractions of SW480 and SW620 cell lines. Actin and Brg1 were the cytosolic and nuclear loading controls, respectively. Cells were either incubated with DMSO (-) (control) or 100μM DAPT (+) for 48 hours. **(B)** Quantification of relative nuclear Cdx2 intensity of SW480 (left) untreated versus treated and SW620 (right) untreated vs treated. **(C)** Quantification of relative cytosolic Cdx2 intensity of SW480 (left) untreated versus treated with DAPT and SW620 (right) untreated vs treated with DAPT.

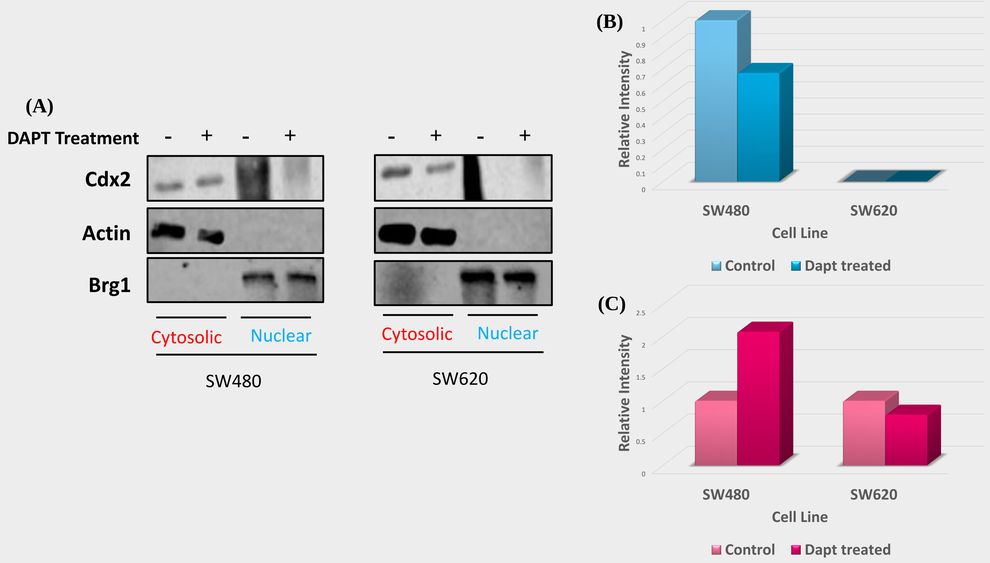

Figure 4. Second trial of Notch inhibition with 48 hour DAPT treatment of SW480 and SW620 cells. (A) Western blots of Cdx2, actin and Brg1 in nuclear and cytosolic fractions of SW480 and SW620 cell lines. Actin and Brg1 were the cytosolic and nuclear loading controls, respectively. Cells were either incubated with DMSO (-) (control) or 100μM DAPT (+) for 48 hours. No Cdx2 bands were obtained for the SW620 nuclear fractions due to loading error. **(B)** Quantification of relative nuclear Cdx2 intensity of SW480 (left) untreated versus treated and SW620 (right) untreated vs treated. **(C)** Quantification of relative cytosolic Cdx2 intensity of SW480 (left) untreated versus treated with DAPT and SW620 (right) untreated vs treated with DAPT.

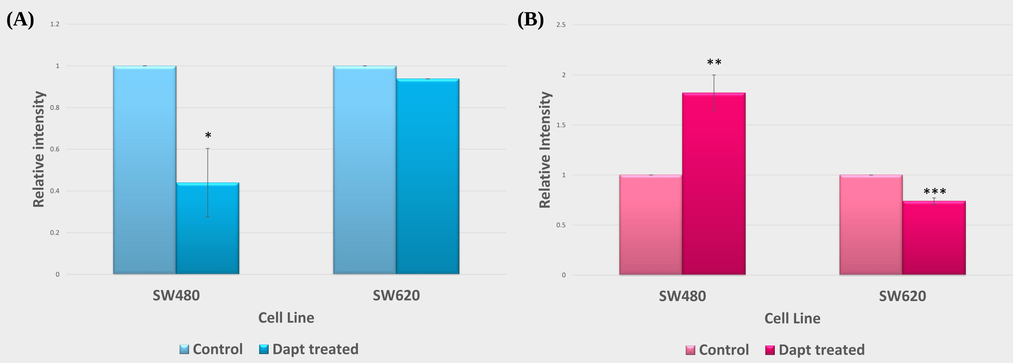

Figure 5. Quantification of western blots. (A) Relative nuclear Cdx2 expression of SW480 and SW620 cells with and without DAPT treatment. **(B)** Relative cytosolic Cdx2 expression in SW480 and SW620 cell lines, with and without DAPT treatment For **(A)** data for SW480 is shown as ± SE of *n*=2 biological replicates; and for **(B),** data are shown as ± SE of *n*=2 biological replicates. Difference in means were assessed by two tailed Student t-tests. * $P<0.15$, ** $P<0.1$, *** $P< 0.05$.

Analysis

The data from **Figure 5** presents the following:

- After 48 hour incubation with DAPT (γ-secretase inhibition), there was a decrease in nuclear Cdx2 and an increase in cytosolic Cdx2 in SW480 cells relative to controls.
- After 48 hour DAPT incubation, SW620 cells did not exhibit an obvious change in subcellular localization for Cdx2.

The difference in SW480 versus SW620 cells was interesting. The pre-metastatic colon cancer tumor cells (SW480) Cdx2 expression was affected by γ-secretase inhibition while the metastatic cells (SW620) were not seemingly affected. The data indicate that DAPT alters the localization of Cdx2 in some cell types but not others. Mechanistic interference of protein shuttling or transporter protein may have caused this shift. The DAPT may also be directly affecting Cdx2 and its ability to enter the nucleus in SW480, but not SW620, cells. Underlying mechanisms causing this change in subcellular localization will require further exploration

Conclusion and Future Directives

Notch inhibition using DAPT affects Cdx2 subcellular location within colorectal cancer cells. Different effects were observed between pre- and post-metastatic colorectal cancer cells. In pre-metastatic tumor cells, Cdx2 accumulated in the cytosol and decreased in the nucleus. There was no such trend for metastatic cells. This opens further experiments to see how Cdx2 subcellular distribution may be related to metastasis as there is a difference in Cdx2 behavior between cell types.

Further work could also employ animal models to see if DAPT causes similar effects *in vivo*. To understand the mechanism behind Cdx2 shuttling from the cytosol to the nucleus or vice versa, different experiments can be done. BioID can be used to see different protein-protein interactions with Cdx2 to understand if there is an increase or decrease between certain proteins and Cdx2 when the Notch signaling pathway is inhibited.

References

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