G0S2: Establishing a Role in Breast Epithelial Differentiation and Cancer

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**ABSTRACT**

G0S2 switch gene 2 (G0S2) is a direct retinoic acid target gene widely expressed in diverse organs and implicated in cancer based on frequent methylation-mediated silencing in diverse solid tumors. We have previously shown G0S2 to be a novel breast cancer tumor suppressor. In addition, there is some evidence that G0S2 is highly expressed in normal breast epithelial tissue and may become downregulated by immortalization and transformation. We found that G0S2 is highly expressed in the mammary gland ductal branching of mice. Further, G0S2 knockout mice have a lactation defect. However, it is not yet clear whether G0S2 is involved in breast cancer initiation and progression. We hypothesize that G0S2 plays a role in normal breast epithelial cell differentiation and that loss of G0S2 may result in aberrant differentiation leading to a more cancer prone state. Our results show that G0S2 expression is increased during lactogenic induced differentiation in immortalized mouse breast epithelial cells. We have also observed that not all mouse breast cancer cell lines have a decrease in G0S2 expression compared to normal mouse mammary derived cell lines. For future studies, we will further elucidate the role of G0S2 in breast epithelial differentiation and cancer. This will aid in the development of future breast cancer therapies.

**BACKGROUND**

- G0S2 is a direct retinoic acid target gene implicated in the regulation of diverse biological processes.
- G0S2 has been defined as a novel tumor suppressor opposing oncogenic transformation by repressing MYC activity and mTOR signalling.
- G0S2 is involved in the control of lipolysis. It inhibits the activity of adipose triglyceride lipase (ATGL) which is the first enzyme in a series of three to break down lipid droplets.
- G0S2 knockout female mice have a lactation defect.
- G0S2 is highly expressed in normal breast epithelial tissue and may become downregulated by immortalization and transformation.
- G0S2 is a direct retinoic acid target gene implicated in breast cancer based on frequent methylation-mediated silencing in diverse solid tumors.

**AIM**

To establish the role of G0S2 in breast epithelial differentiation and cancer using an in vitro model in mouse breast epithelial cells

- **Question 1:** Does G0S2 expression increase with lactogenic differentiation in immortalized mouse breast epithelial cells?
- **Question 2:** Is G0S2 overexpressed in mouse normal breast epithelial cells in comparison to mouse breast cancer cells?

**RESULTS**

- Lactogenic induced hormonal differentiation of HC11
  - G0S2 expression increases with lactation in HC11
  - Not all mouse breast cancer cell line show a decrease in G0S2 transcript

**MATERIALS & METHODS**

**Cell culture.** Cells were cultured either in DMEM or RPMI media with 10% fetal bovine serum supplemented with glutamine and antibiotics.

**Cell Differentiation.** Cells were plated at 100,000 cells per well in 6 well plates. The lactogenic hormones dexamethasone, insulin, and prolactin were added to the cells every 2 days for 8 days to induce lactation in HC11 cells.

**RNA extraction.** RNA was extracted from cultured cells using purelink RNA Isolation Kit - Thermo Fisher Scientific.

**cDNA synthesis.** cDNA was made using the High-Capacity cDNA Reverse Transcription Kit - Thermo Fisher Scientific.

**Real-time PCR.** cDNA with SYBR green was used for real-time PCR and data was analyzed using the ddCT method normalized to GAPDH.

**CONCLUSION**

- Increased milk protein gene expression confirms induction of lactogenic differentiation in HC11 cells.
- Lactogenic induced differentiation in HC11 cells leads to an increase in G0S2 expression.
- Comparison of G0S2 expression levels between immortalized normal mouse mammary epithelial cells and tumorigenic mouse mammary epithelial cells did not show the anticipated decrease of G0S2 in the tumor cell lines.

**FUTURE DIRECTIONS**

- Determine which estrogen receptors are expressed by each of the three cancer cell lines used.
- Test whether G0S2 expression is altered in a time-dependent manner during induced differentiation of HC11 cells.
- Generate HC11 cells with a stable overexpression or a stable knockdown of G0S2 to test whether G0S2 plays a causal role in HC11 cell differentiation.
- Assess if G0S2 knockdown and overexpression alters the proliferative and other pro-cancer properties of HC11 cells including the susceptibility of the cells to be transformed with oncogenes.
- Establish the in vivo role of G0S2 in xenograft and transgenic mouse models of breast cancer.

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