Expression of the *APC* Tumor Suppressor Gene is Regulated during Mammary Gland Development and Differentiation

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Background and Purpose: With almost 270,000 new cases of breast cancer and over 40,000 deaths, breast cancer is one of the greatest health concerns facing women. The *APC* tumor suppressor gene has been shown to be inactivated in as many as 40% of sporadic breast cancers, and mice carrying a germline mutaton in *Apc* are predisposed to mammary tumors. Our laboratory has shown previously that APC expression is induced during pregnancy and lactation in the mouse mammary gland, and *Apc*-deficiency results in defective lobulo-alveolar development. The current project tests the hypothesis that APC expression is regulated by lactogenic hormones both *in vitro* and *in vivo*.

Methods: In the *in vitro* study, we exposed EpH4 mouse mammary epithelial cells to 10 nM 17β-estradiol, 10 nM 17β-estradiol with 100 nM progesterone, 50 ng prolactin in saline or vehicle (sesame seed oil with saline) for 4, 24 or 48 h. Total RNA was harvested from the cells. APC and GAPDH (as a normalization control) were amplified using Reverse-transcriptase (RT) real-time PCR to quantify gene expression. For the *in vivo* studies, ovariectomized C57BL/6 (strain) mice (n=7/group) were treated with daily injections of 1cc for 20 days. Mammary tissue was harvested and RNA was isolated. APC and GAPDH gene expression were evaluated by real-time RT-PCR.

Results: EpH4 cells exposed to lactogenic hormone for 4 h demonstrated an increase in APC expression, particularly in the 17β-estradiol and progesterone treated cells, compared to those treated with vehicle. At 24 and 48 h there were no obvious differences in APC expression between the treatment groups. Additionally, we observed that mammary tissues from mice treated with estradiol and progesterone had increased APC mRNA expression compared to those mice treated with vehicle or estrogen alone.

Conclusions: These data suggest that expression of the *APC* tumor suppressor gene is regulated by lactogenic hormones in the mammary gland, and support a model in which APC is an important regulator of mammary gland function during pregnancy and lactation.