

Gene Regulation in Primary Mammary Epithelial Cells Treated with HGF or R5020

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The steroid hormone estrogen (E) is necessary for proliferation and ductal growth of mammary epithelial cells *in vivo*. Substantial evidence suggests that progestins also play a role in proliferation and differentiation of the adult breast. Knockout animal studies have shown that both Cyclin D1 and Stat5a are important regulators of the proliferative and morphogenic changes in the breast during pregnancy, a period of high estrogen and progestin levels. We hypothesized that the RNA expression of Stat5a, Cyclin D1, and possibly other cell cycle regulators is altered by exposure to estrogen and/or progestin. To investigate this, primary mammary epithelial cells were grown as organoids in a 3-dimensional collagen gel culture system. It has previously been shown that the cultured cells do not respond to E treatment. However, hepatocyte growth factor (HGF), which is normally produced by E-treated mammary fibroblasts, mediates a proliferative and tubulogenic response. Also, organoids treated with progestin (R5020) exhibit a morphological response of lumen formation. Real time PCR was used to measure changes in the RNA levels of Stat5a and Cyclin D1 as well as several other G1 and S phase cell cycle regulators in cultured organoids. Treatment with HGF resulted in a two-fold increase in both Cyclins D1 and A2. Stat5a expression was increased two-fold in response to R5020 treatment. These findings were similar in cells from both mature (16 wk) and pubertal (7 wk) animals. Thus, HGF may be inducing epithelial cell proliferation at least in part by increasing levels of the positive cell cycle regulators, cyclins D1 and A2, but not by downregulating cdk inhibitor expression. In addition, the morphological response to R5020 may be mediated through the upregulation of Stat5a, but not through changes in cell cycle regulator expression.

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