Identification of Progestin-Responsive Genes in Mammary Gland Primary Three-Dimensional Organoid Cultures

Richard J. Miksicek, Susan E. Conrad, Alexis Drolet, Sarah Santos, Richard C. Schwartz, Timothy Zacharewski, and Sandra Z. Haslam, Departments of Physiology, Microbiology & Molecular Genetics, and Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI.

The specific functions of progesterone receptor (PR) isoforms A and B (PRA, PRB) in the normal mammary gland are of interest for understanding the molecular mechanisms of progesterone action during normal development and function as well as in the etiology of mammary cancer. While PRB expression is essential for alveologenesis and differentiation leading to lactation, the function(s) mediated by PRA have not been elucidated. PRA is highly expressed in the pubertal gland, but its expression decreases with maturation in the adult virgin gland. To analyze regulatory events mediated by PRA, primary mammary organoids obtained from pubertal or adult mammary glands were cultured in collagen gels in serum-free medium. These organoids respond to progestin (R5020) treatment by forming cyst-like structures, organized such that a central patent, fluid-filled lumen is surrounded by a layer of luminal epithelial cells which in turn is encircled basally by a single layer of myeopithelial cells. Thus, these organoids exhibit a cellular organization similar to ducts in vivo and display a morphologic response to R5020 mimicking lumen formation, associated with in vivo ductal development. To identify genes and gene networks that are involved in this in vitro response, R5020-induced gene expression was analyzed using 44K whole mouse genome oligonucleotide arrays (Agilent Technologies). Using a 2-fold cutoff, these experiments have defined a set of genes induced by R5020 treatment that play roles in regulation of transcription, signal transduction, cell survival, cell adhesion, innate immunity and cellular transport. A number of genes displayed stage-specific (pubertal vs. adult) regulation, exemplified by the calcitonin/CGRP precursor gene (Calca) which was highly induced in adult, but not pubertal organoids. Novel genes were identified that have not previously been associated with mammary gland development or function. Many but not all genes previously identified to be regulated by progestins in breast cancer cells were also regulated by progestin in the normal mammary organoids. The overall pattern of regulation will be summarized, along with a discussion of individual genes and their potential roles in mammary gland function.