

Expression of C/EBP β Isoforms During Mammary Gland Development

Srinivasan Durairaj, Alexis Drolet, Richard J. Miksicek, Sandra Z. Haslam, and Richard C. Schwartz. Departments of Physiology and Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI 48840

C/EBP β is a critical transcription factor in the regulation of mammary gland proliferation and development. Experiments with C/EBP β knockout mice have demonstrated a requirement for C/EBP β for ductal morphogenesis and alveologenesis. Virgin C/EBP β knockout mice display reduced ductal growth and branching, as well as limited alveolar development in response to estrogen and progesterone. C/EBP β has been observed to occur in three isoforms in mammary and other tissues: C/EBP β p39 (LAP-1) and C/EBP β p36 (LAP-2), both potent transcriptional activators, and C/EBP β p20 (LIP), a truncated form lacking classical transactivation domains and generally reported to be an inhibitor of C/EBP-dependent transcription. Studies of C/EBP β isoform expression in rat and murine mammary development have reported that increased C/EBP β expression during pregnancy is largely attributable to a 100-fold increase in LIP expression. This finding along with a correlation of LIP overexpression with mammary tumor progression have led to the suggestion that LIP overexpression is associated with proliferation. We have performed an immunohistochemical analysis of C/EBP β isoform expression in virgin and pregnant mammary gland tissue using antibodies specific to LAP isoforms, as well as an antibody that detects all C/EBP β isoforms through a carboxyl-terminal epitope. Unexpectedly, both LAP-specific and carboxyl-terminal-specific antibodies detected similar increases in C/EBP β expression in the alveolar structures of pregnant mammary glands compared to virgin mammary glands, indicating that the increase in C/EBP β expression may in fact be attributable to LAP rather than LIP. Western analyses of the same tissues have been performed to confirm this finding. Western analyses of C/EBP β isoform expression in mammary tumor cell lines have also been performed. In order to study the specific function of the various C/EBP β isoforms, expression vectors that individually express LAP-1, LAP-2 and LIP have been generated.