Expression of C/EBPβ Isoforms During Mammary Gland Development

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C/EBPβ is a critical transcription factor in the regulation of mammary gland proliferation and development. Experiments with C/EBPβ knockout mice have demonstrated a requirement for C/EBPβ for ductal morphogenesis and alveologenesis. Virgin C/EBPβ knockout mice display reduced ductal growth and branching, as well as limited alveolar development in response to estrogen and progesterone. C/EBPβ has been observed to occur in three isoforms in mammary and other tissues: C/EBPβ p39 (LAP-1) and C/EBPβ p36 (LAP-2), both potent transcriptional activators, and C/EBPβ p20 (LIP), a truncated form lacking classical transactivation domains and generally reported to be an inhibitor of C/EBP-dependent transcription. Studies of C/EBPβ isoform expression in rat and murine mammary development have reported that the hallmark of increased C/EBPβ expression during pregnancy is a 100-fold increase in LIP expression. These findings 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/EBPβ isoform expression in virgin and pregnant mammary gland tissue using antibodies specific to LAP isoforms, as well as an antibody that detects all C/EBPβ isoforms through a carboxyl-terminal epitope. Unexpectedly, both LAP-specific and carboxyl-terminal-specific antibodies detected similar increases in C/EBPβ expression in the alveolar structures of pregnant mammary glands compared to virgin mammary glands, indicating that the increase in C/EBPβ expression may in fact be attributable to LAP rather than LIP. Western analyses of the same tissues confirm this finding. Furthermore, immunofluorescence studies suggest that C/EBPβ and progesterone receptor A (PRA) expression are mutually exclusive. Prior findings associating PRA expression with non-proliferating cells suggest that C/EBPβ expression may correlate with proliferation. Western analyses of C/EBPβ isoform expression in mammary tumor cell lines have found cell lines that display progesterone-induced LAP expression and progesterone-induced LIP expression. In order to study the specific function of the various C/EBPβ isoforms, retroviral expression vectors that individually express LAP-1, LAP-2 or LIP have been generated, introduced into mammary gland organoids derived from C/EBPβ-deficient animals, and their ability to complement the C/EBPβ-deficiency lesion assessed in a 3-D culture system.