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CRTC1 expression during normal and abnormal salivary gland development supports a precursor cell origin for mucoepidermoid cancer

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ABSTRACT

Dysregulation of the transcription factor CRTC1 by a t(11;19) chromosomal rearrangement mediates the formation of mucoepidermoid salivary gland carcinoma (MEC). Although the *CRTC1* promoter is consistently active in fusion-positive MEC and low levels of *CRTC1* transcripts have been reported in normal adult salivary glands, the distribution of CRTC1 protein in the normal salivary gland is not known. The aim of this study was to determine if CRTC1, like many known oncogenes, is expressed during early submandibular salivary gland (SMG) development and re-expressed in an experimental tumor model. Our results indicate that CRTC1 protein is expressed in SMG epithelia during early stages of morphogenesis, disappears with differentiation, and reappears in initial tumor-like pathology. This stage-dependent expression pattern suggests that CRTC1 may play a role during embryonic SMG branching morphogenesis but not for pro-acinar/acinar differentiation, supporting a precursor cell origin for MEC tumorigenesis. Moreover, the coincident expression of CRTC1 protein and cell proliferation markers in tumor-like histopathology suggests that CRTC1-mediated cell proliferation may contribute, in part, to initial tumor formation.

1. Results and Discussion

Mucoepidermoid carcinoma (MEC) is the most frequent primary malignancy of salivary glands in both children and adults, representing 5% of all salivary gland tumors and 35% of all malignant forms (Enlund et al., 2004; Behboudi et al., 2006; Kaye, 2009; O'Neill, 2009). MECs, composed of mucous forming goblet, epidermal and intermediate cells, are thought to originate in the excretory and intercalated ducts of the salivary gland (Behboudi et al., 2006). In approximately 40% of the primary salivary gland MEC carcinomas, the fusion protein CRTC1-Maml2 has been detected (see reviews, Stenman, 2005; Kaye, 2009; O'Neill, 2009). Importantly, the CRTC1-Maml2 fusion protein is associated with distinct tumor subtypes and clinicopathologic features (Behboudi et al., 2006; Okabe et al., 2006; Nakayama et al., 2009; Kaye, 2009).

The *CRTC1* gene (CREB-Regulated Trancription Coactivator 1; also known as Mect1 or Torc1) is a coactivator of cyclic AMP (cAMP)/cAMP response-element binding protein (CREB) transcription (Conkright et al., 2003; Iourgenko et al., 2003). CREB regulates genes involved in cell proliferation and differentiation, and abnormal CREB activity is associated with carcinogenesis (Wu et al., 2005). CRTC1 acts by binding CREB and enhancing its transcription, independent of phosphorylation (O'Neill, 2009). The *Maml2* gene (related to the Drosphila gene *Mastermind* and to the mammalian mastermind-like gene *Maml1*) is an essential coactivator for Notch receptor transcriptional activation and signaling for cell proliferation and differentiation (Wu et al., 2005; Tonon et al., 2003). In the t(11;19) chromosomal translocation generated *CRTC1-Maml2* fusion oncogene, the CREB binding domain from *CRTC1* replaces the free intracellular Notch-binding domain from *Maml2* to produce a protein with novel transformational properties (O'Neill, 2009). Since deletions in *CRTC1* abolished transforming activity, and disruption of CREB activity eliminated CRTC1-Maml2-mediated tumorgenicity, indications are that CRTC1 dysregulation is the principal mediator of cell transformation and tumor formation

Abbreviations: AP-1, activator protein-1; E13, embryonic day 13; cAMP, cyclic AMP; CREB, cAMP response-element binding protein; CRTC1, CREB-regulated trancription coactivator 1; CRTC2, CREB-regulated trancription coactivator 2; DAPI, 4,6-diamidino-2-phenylindole; LKB1, serine/threonine liver kinase B1; Maml, mammalian mastermind-like gene; MEC, mucoepidermoid carcinoma; mCMV, mouse cytomegalovirus; PCNA, proliferating cell nuclear antigen; SMG, submandibular salivary gland.

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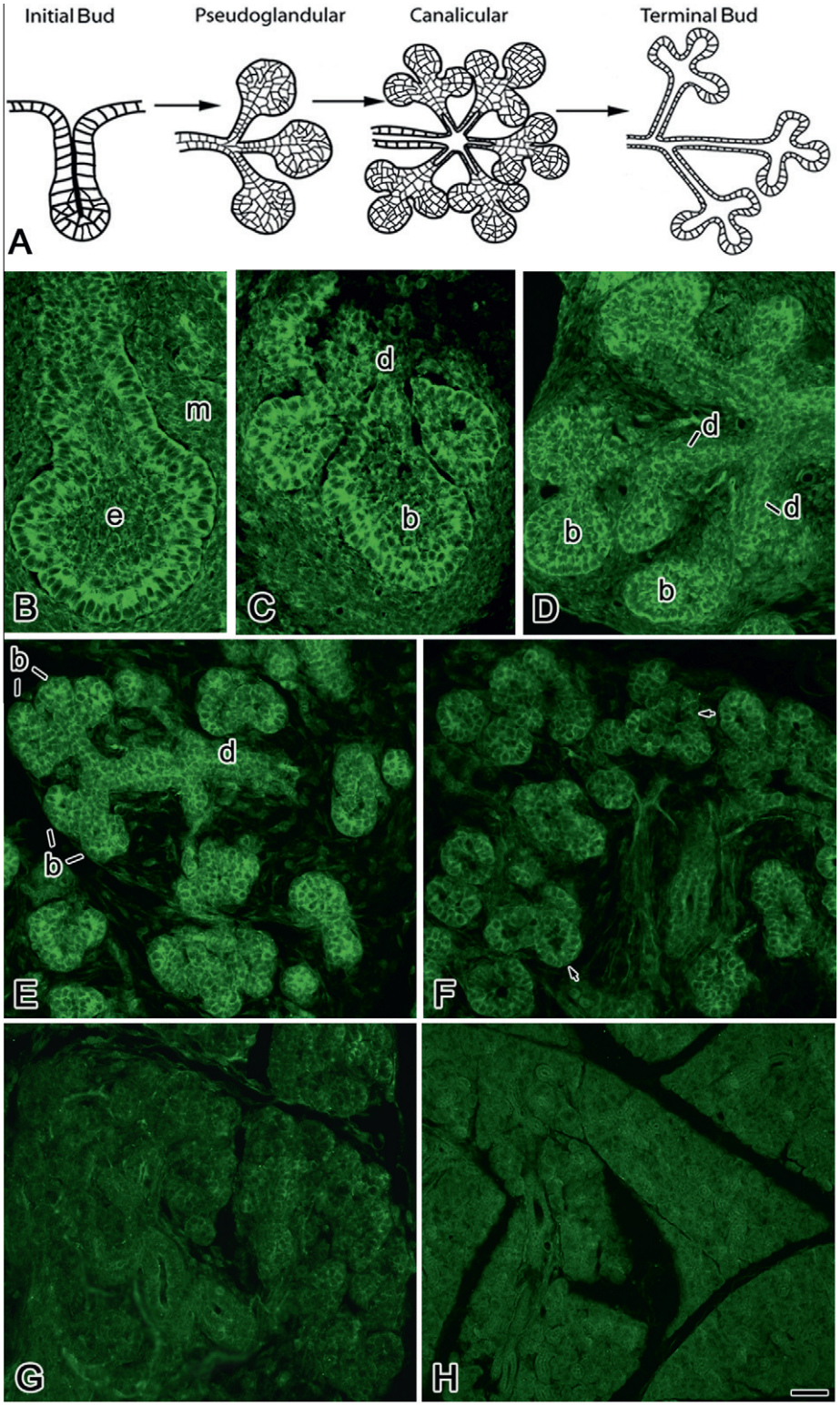

Fig. 1. Cell-specific localization of CRTC1 protein with progressive SMG morphogenesis and differentiation. A. Stages of embryonic SMG development. (B–H) Stage-dependent immunolocalization of CRTC1 protein. (B) Initial Bud stage. (C) Early Pseudoglandular stage. (D) Pseudoglandular stage. (E) Canalicular stage. (F) Terminal Bud stage. (G) Newborn (Late Terminal Bud stage) SMG. (H) 3-week-old SMG. In the Initial Bud stage, intense CRTC1 immunostaining is seen in the epithelial stalk and end bud (e) and is relatively absent from mesenchyme (m). In the Pseudoglandular and Canalicular stages, strong CRTC1 immunostaining is localized to epithelial ducts (d) and buds (b). CRTC1 immunostaining is primarily seen in the cytoplasm and, to a lesser extent, in nuclei, of epithelia. By the Terminal Bud stage, reduced CRTC1 immunostaining is found in epithelial cell surrounding distinct ductal and terminal bud (pro-acinar) lumina (arrows) as compared to that seen in earlier stages. Postnatally, CRTC1 immunostaining is weakly detected in the newborn SMG and is relatively absent in the differentiated 3-week-old SMG. Bar scale: 30 μm.

(Coxon et al., 2005; Wu et al., 2005). Although the precise role of the CRTC1-Maml2 fusion oncogene remains unclear, molecular studies suggest that CRTC1-Maml2 plays a role during initial stages of tumor formation (Kaye, 2006, 2009).