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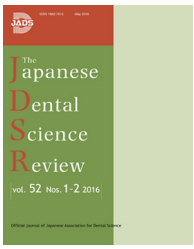

Review Article

Regulatory mechanisms of branching morphogenesis in mouse submandibular gland rudiments

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Summary Branching morphogenesis is an important developmental process for many organs, including the salivary glands. Whereas epithelial—mesenchymal interactions, which are cell-to-cell communications, are known to drive branching morphogenesis, the molecular mechanisms responsible for those inductive interactions are still largely unknown. Cell growth factors and integrins are known to be regulators of branching morphogenesis of salivary glands. In addition, functional microRNAs (miRNAs) have recently been reported to be present in the developing submandibular gland. In this review, the authors describe the roles of various cell growth factors, integrins and miRNAs in branching morphogenesis of developmental mouse submandibular glands.

Contents

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1. Introduction

Branching morphogenesis is an essential developmental process for the formation of various organs, such as the kidney, lung, pancreas, prostate and all exocrine glands, including the salivary glands [1]. Branching morphogenesis is one of the important biological events that are driven by epithelial—mesenchymal interactions [2]. However, the molecular mechanisms responsible for these inductive interactions remain largely unknown. The fundamental processes of epithelial—mesenchymal interactions depend on both the extracellular matrix (ECM) and integrins, as well as on a variety of growth factors and their receptors [1—5]. Cell growth factor receptors and integrins are located on the plasma membrane of cells, which is the point of interface between the environment immediately outside the cell and the cell interior. When receptors bind with ligands, multiple complex signaling cascades are activated, and major cellular events such as cell differentiation, proliferation, migration and formation of organ structures are elicited [6—8]. Protein expression for the signaling systems of these receptors is regulated as part of genetically controlled organ development [9—11].

The fetal mouse submandibular gland (SMG) is a well-characterized model system for *in vivo* and *in vitro* studies of the epithelial—mesenchymal interactions involved in fetal organogenesis [12—17] (Fig. 1). Over 60 years ago, a culture system for SMG rudiments was first reported as a new method for study of organ development by Borghese [18,19]. Since then, numerous reports have generated new information regarding SMG development. In particular, a study of salivary gland development identified cell growth factors and cell adhesion molecules that are involved in cell proliferation, differentiation, migration and movement [20].

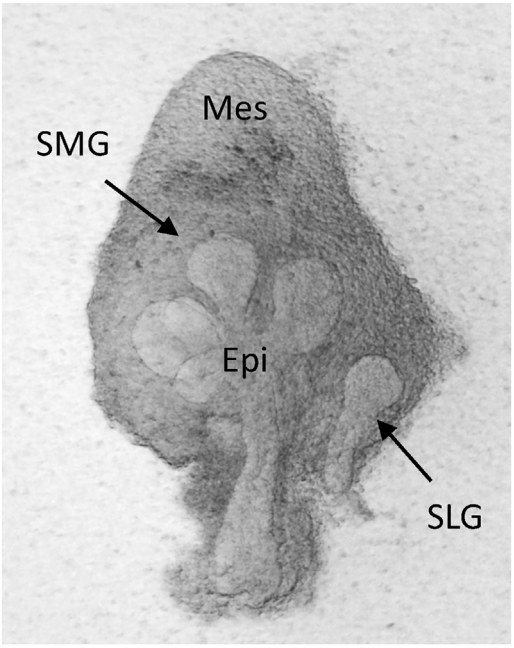

Figure 1 Submandibular gland and sublingual gland rudiments of fetal mouse at embryonic day 13. Mes = mesenchymal tissue. Epi = epithelial tissue. SMG = submandibular gland. SLG = sublingual gland.

2. Regulation by cell growth factors

In 1991, Nogawa and Takahashi [21] showed that epidermal growth factor (EGF) and transforming growth factor-α (TGFα) stimulated branching morphogenesis of cultured epithelium of submandibular gland rudiments, and those growth factors were found to substitute for the mesenchyme of the rudiments. The same research group also showed that EGF supports branching morphogenesis of SMG rudiments, especially cleft formation, and that fibroblast growth factor 7 (FGF7) is needed for stalk elongation of SMG epithelium [22]. The EGF system, including members of its ligand family such as EGF, TGF-α, HB-EGF and neureglin-1 (NRG1), and members of its receptor family such as EGFR (ErbB1), ErbB2 and ErbB3 [21—28], is one of the important regulators of branching morphogenesis of SMG rudiments. Moreover, the ligand family members activate and phosphorylate the ErbB receptor family proteins in the plasma membrane. Tyrphostin (RG 50864), a specific inhibitor of ErbB receptor tyrosine kinase, strongly inhibited branching morphogenesis of cultured SMG rudiments. Therefore, SMG endogenously expresses EGF ligands that regulate branching morphogenesis [23—28]. In addition to FGF7, FGF10 has been reported to induce morphological changes specific to stalk elongation of endpieces of SMG epithelium [29,30]. Although the EGF system is undeveloped in SMG rudiments at embryonic day 12 (E12), it becomes primed on E13 by the FGF system and plays an important role in induction of branching morphogenesis [31]. The cell growth factor systems play individual roles and stage-specific roles in regulation of branching morphogenesis in developing fetal SMG.

Intracellular signaling cascades are known to be activated by receptor tyrosine kinases, such as mitogen-activated protein kinases (MAPKs). [32] Ligand binding by growth factor receptors results in autophosphorylation of tyrosine residues in the cytoplasmic domain of the receptor molecules, and then adaptor proteins are recruited into the plasma membrane, where they link the receptors to Ras in the plasma membrane [33]. Ras in turn activates Raf, resulting in activation of MAPK kinase1/2 (MEK1/2), which phosphorylates and activates extracellular signal-regulated kinase1/2 (ERK1/2), a member of the MAPK family. Binding of complexes of growth factor receptor-bound protein 2 (GRB2) and GRB2-associated binder protein 1 (GAB1) to phosphorylated growth—factor receptor dimers leads to formation of active PI3K complexes, conversion of PIP2 into PIP3 [34] and activation of AKT signaling. Phospholipase Cγ1 (PLCγ1) can also be recruited directly through phosphotyrosine residues of growth factor receptors that serve as PLCγ1 docking sites [35], leading to PLCγ1 phosphorylation by EGFR and activation of DAG and IP3 signaling (Fig. 2).

To investigate the relationships between the cell signaling pathways and the elicited biological changes in branching morphogenesis, we compared the signaling activated in fetal mouse SMGs by EGF, FGF7 or FGF10, and correlated the findings with the specific events of branching morphogenesis [36]. Western blotting showed that EGF strongly stimulated phosphorylation of ERK1/2 [36,37] and