MINI-REVIEW

Branching morphogenesis in the fetal mouse submandibular gland is codependent on growth factors and extracellular matrix

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Abstract : Branching morphogenesis (BrM) is a basic developmental process for the formation of the lung, kidney, and all exocrine glands, including the salivary glands. This process proceeds as follows. An epithelial downgrowth invaginates into underlying mesenchyme, and forms a cleft at its distal end, which is the site of dichotomous branching and elongation ; this process of clefting and elongation is repeated many times at the distal ends of the invading epithelium until the desired final extent of branching is reached. The distal ends of the epithelium differentiate into the secretory endpieces, and the elongated segments become the ducts. This presentation is a brief historical review of studies on BrM during the development of the submandibular gland (SMG). J. Med. Invest. 56 Suppl. : 228-233, December, 2009

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All exocrine glands and several other organs, such as the lung and kidney, develop by a basic developmental process known as branching morphogenesis (BrM) (Fig. 1). In this process, a lining epithelium grows into mesenchyme, which condenses around the epithelium as a capsule. The epithelium elongates to form a stalk that ends in a rounded endpiece. A cleft or groove indents the distal end of the epithelium, marking the site where the epithelium will elongate into two branches, ending in rounded endpieces. The original elongated stalk acquires a lumen to become a duct. A cleft forms at the distal ends of the new endpieces, and the process of branching, lumen formation, and further clefting is repeated over and over again until the desired size of the organ is achieved.

The SMG rudiment arises on the 12th day of fetal life, referred to as E12, as a downgrowth of the oral epithelium, and by E13 the epithelium has branched into 3-5 endpieces surrounded by a capsule of condensed mesenchyme. The study of BrM was greatly facilitated by the work of Elio Borghese, who cultured rudiments of mouse fetal SMGs and showed that BrM could be maintained *in vitro* (1, 2). BrM proceeded well with E14 or older SMGs,

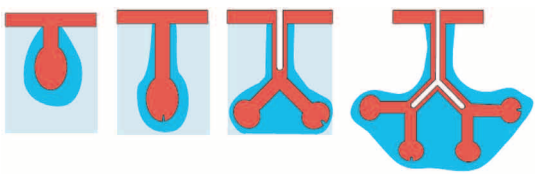

Figure 1. Diagram illustrating steps in branching morphogenesis. Epithelium in red, mesenchyme in blue. See text for explanation.

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but rudiments from mice E13 of younger branched poorly. The extent of BrM was dependent on the amount of mesenchyme taken out with the epithelium, implying that reciprocal interactions between the epithelium and the mesenchyme are needed for BrM. Grobstein then improved culture methods and E13 SMGs also branched *in vitro* (3, 4). Moreover, if the mesenchyme were separated from the epithelium by trypsin, the epithelium failed to branch, but BrM resumed if the epithelium was recombined with mesenchyme (5). When denuded epithelium was cultured across a filter from SMG mesenchyme, abnormal BrM occurred with many long branches ending in a terminal swelling (5). Grobstein proposed that the epithelium required both direct contact with mesenchyme and interaction with diffusible substances of mesenchymal origin for BrM to proceed (6). He then showed that SMG rudiments treated with collagenase lose their branches, but resume BrM if the enzyme is removed, demonstrating the importance of collagen (7). Later on, working with Rutter and Wessells, he concentrated more on the development of the epithelium in the SMG and in other tissues (8).

Wessells trained a number of talented scientists, including Ken Yamada, Brian Spooner and Merton Bernfield (9-11). Bernfield's group focused on the role of the epithelium, and defined the critical role of the basal lamina for BrM to proceed (12-16). By using agents that specifically interfered with the synthesis of collagen or glycosaminoglycans, Spooner's group showed that both of these ECM components are needed for BrM (17-20).

Nogawa and Nakanishi, working independently or in collaboration, intensively studied the role of collagens in BrM of the SMG, and began to introduce specificity into the analysis of the mesenchyme by defining the spatial distribution of different types of collagen, and showed that while collagen I was widely dispersed in the mesenchyme, type III collagen preferentially localized at the points of clefting, and at the constrictions between the stalk and the endpiece (21-27). They also established that BrM could proceed in the absence of cell proliferation (28).

Kadoya and Yamashina very thoroughly characterized the fine structure of the basal lamina of the SMG, and studied the epithelial synthesis of two of its components, laminin and collagen IV (29, 30). They then demonstrated the presence of the alpha-6 subunit of the integrin receptor for laminin on the basal surface of the fetal SMG epithelium (31). Kadoya and his coworkers then showed that laminins and alpha-6 and beta-1 integrin subunits are needed for BrM, and defined the roles of specific laminin chains, and even specific domains in these laminins that are required for BrM (32-35). This and much more of his work is summarized in their excellent review article (36).

In 1991 Nogawa and Takahashi revolutionized the research on BrM in the SMG by showing that clefting and endpiece formation take place in epithelium stripped of its mesenchyme, and then covered with the basal lamina equivalent, Matrigel, and EGF. If either of these were left out, the epithelium formed a rounded cyst and did not branch (37-38). Nakanishi and coworkers noted that the epithelium formed endpieces, but not elongated stalks that would form the ducts (39). Morita and Nogawa then showed that elongation and stalk formation are driven by the FGF system (40).

Kashimata and Gresik showed that EGF is actually physiologically important, since the mRNAs for EGF and the EGF receptor are expressed during fetal development of the SMG, and that EGF promotes the synthesis of the alpha-6 integrin subunit (41). They also showed that the receptor is localized mainly in the epithelium (42). Hieda and coworkers then established that other ligands related to EGF and other ErbB receptors are also important for BrM, and not only EGF, TGF-alpha and EGFR (ErbB1), namely HB-EGF, Neuregulins, and ErbB2 and 3 (43-44). They have also studied the first signs of cytodifferentiation by defining the roles of junctional proteins in lumen formation (45-47).

Akamatsu and Hosoi and coworkers characterized cytodifferentiation of the rat SMG by following the expression of aquaporin-5 in the epithelium (48), and of the proprotein convertase PACE4, which they showed is also needed for BrM to proceed (49).

Kashimata and Koyama then demonstrated that EGF regulates BrM by activating intracellular signaling cascades involving tyrosine phosphorylation of the EGFR itself, the MAPK Erk-1/2, PLC-gamma1, and PI3K (Akt), and showed that the pattern of activation of these signaling pathways varies with age (50-51).

Larsen and Sakai and Yamada confirmed the role of PI3K (52) and have extensively studied the interaction of specific mesenchymal components and integrins important for BrM, such as fibronectin and the alpha-5 integrin subunit (53-55). By laser capture microdissection Sakai demonstrated that fibronectin mRNA is localized in the epithelium, that

fibronectin is deposited at the cleft site and in the deepening cleft whene it interacts with it receptor on the epithelial cells, alpha-5-beta-1 integrin. Antibodies against either of these two components interfere with BrM. Many others have contributed importantly to further progress in this field, but space does not allow me to give them proper consideration.

Hoffman and his coworkers at the NIH have extensively documented the critical and wide ranging influence of the FGF signaling system for SMG development and BrM (56-60).

Melnick and Jaskoll have demonstrated that FGF and several other signaling systems are involved in SMG BrM and have emphasized that regulation of development of this gland is not linear, but rather results from multiple interactions ; their extensive and important work is summarized in two excellent reviews (61-62).

Recently Nogawa and his coworkers showed that in E12 SMGs FGF induces the epithelium to be able to respond to EGF (63). Mesenchyme-free E12 epithelium exposed first to FGF, and then to EGF, forms endpieces. If it is exposed to FGF and then only to more FGF, it forms elongated stalks, as expected. If it is not exposed to FGF and then exposed to EGF, it does not form endpieces. Koyama and coworkers recently showed that although EGF induces strong phosphorylation of Erk-1/2, FGFs are weak inducers, highlighting the complexity of these interactions (64).

There is a great deal of interest in the study of BrM in the salivary glands and in other organs (e.g. mammary gland, kidney, lung, etc). This knowledge will help not only to elucidate mechanisms of normal development, but will also lay a foundation for understanding abnormal growth, including tumor formation, allowing for new therapeuric approaches. Moreover, the emerging world of tissue engineering would benefit from increased understanding of the factors driving cytodifferentiation and the establishment of tissue architecture.

Given the constraints of space, we apologize to those scientists whose work was not cited.

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