A Mouthful of Epithelial–Mesenchymal Interactions

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The oral cavity is a complex environment. It serves as a portal between the outside and the inside of an organism. It is the primary organ regulating what is or is not allowed to enter the gut as food. It performs the first line of food selection (by physical feel and chemical taste) and processing (by mechanical and enzymatic breakdown). It also performs the additional functions of speech and expression. Specialized tissue types are required to accomplish these complex functions. During embryonic development of the face, an invagination of the ectoderm forms the stomodeum and connects to the archenteron, the presumptive gut. The primitive oral cavity is lined with both ectoderm and endoderm (Moore and Schmitt, 1998). The ectoderm gives rise to the anterior two thirds of the tongue and all of the hard palate. The endoderm forms the posterior third of the tongue, the floor of the mouth, the palato-glossal folds, the soft palate, and others. The oral epithelium is composed of stratified squamous epithelium. This oral squamous epithelium keratinizes to various degrees in different regions (Presland and Dale, 2000). Additional complexity is added when parts of the oral epithelia are morphologically transformed into different epithelial appendages in specific locations: teeth are induced, taste buds appear on the tongue, and salivary glands invaginate in the buccal region. Along the muco-cutaneous junction, lips form. Thus, morphogenesis of the oral epithelia in the oral cavity sets up the basis for the diverse functions of the mouth.

How does this tissue variety form during development? The epithelia come from the ectoderm and share the same developmental origin as the skin. Generally the different developmental fates are specified by interactions with the mesenchyme. However, aberrant situations can arise. Hairs can be induced from the oral epithelium in LEF 1 over-expressing transgenic mice (Zhou et al, 1995). Ectopic teeth can form on the skin of the chin[1]. While these are pathological conditions, they give us a clue: the fates of skin or oral epithelium can be switched. Furthermore, different ectodermal dysplasia syndromes frequently have multiple defects in hairs, teeth, glands, etc. due to defects in one gene, as demonstrated in the recently characterized Eda pathway (Wisniewski et al, 2002). These findings support the notion that the epithelial varieties result from epithelial–mesenchymal interactions and are variations superimposed on a common theme (Chuong, 1998). Yet, the molecular basis of the epithelial–mesenchymal interactions underlying oral epithelia phenotype determination remains mostly unknown.

Driven by the desire to tissue engineer the oral epithelium, Costea et al (2003) in this issue developed in vitro organotypic cultures consisting of primary human oral keratinocytes grown on top of a reconstituted collagen matrix with or without oral fibroblasts. It has long been suggested that suboral mesenchyme is essential for epithelial proliferation, but the molecules involved were unknown (Hill and Mackenzie, 1989). Costea et al are able to produce reconstituted oral epithelia in a defined medium, therefore providing an experimental model for determining the growth factors involved. The oral epithelium formed on a collagen matrix was thin and had a dominant basal layer. When KGF, or FGF-7, was added, there was a concentration dependent increase of the epithelial thickness. When fibroblasts were incorporated in the matrix, the reconstituted epithelium was stratified and there was a clear expansion of the spinous cell layer. When both FGF7 and fibroblasts were present, the reconstructed oral epithelium reached optimal growth and differentiation. The thickness of the reconstituted epithelium was not significantly different from that of native oral epithelium. Using a set of carefully designed experiments, they measured cell proliferation, apoptosis, differentiation, and the thickness of each stratified epithelial layer. They concluded that FGF-7 can drive keratinocyte proliferation, but not differentiation. Fibroblasts provided other unknown factors required for epithelial differentiation and could modulate the thickness of the reconstituted oral epithelium by balancing cell division, apoptosis and terminal differentiation.

In histology, we start by teaching that the basic configurations of epithelia are cuboidal, columnar, or squamous, and that the epithelia can be either simple or stratified. Yet, we know very little about these fundamental processes at the cellular and molecular level. The researchers ought to be commended for their success in forming reconstituted stratified squamous oral epithelia using dissociated oral keratinocytes obtained from the superfluous oral tissue after wisdom tooth extraction of a normal person. The differentiation of the re-constructed epithelia could have been assessed more rigorously with more molecular markers to demonstrate intercellular integrity and appropriate cyto-differentiation. The histological appearance is reasonably good, but there are spaces in the supra-basal layer and fewer interpapillary rete pegs at the epithelial–mesenchymal interface, indicating reduced cell interactions. Another significant aspect of this work is the demonstration that underlying fibroblasts are important for this basic histogenetic process, and that the FGF pathway is involved in the initial stratification step.

Many questions remain unanswered. What are the other factors produced by fibroblasts? What are the effects of other types of fibroblasts? What molecular pathways are involved? The establishment of this in vitro organ culture model with defined medium opens doors for testing many candidate molecules. A complementary approach is to use an in vivo model and genetics. One line of exciting work showed that p63, a molecular homolog of p53, is essential for epithelial stratification. Mice lacking p63 showed persistent simple epithelia and missing epithelial appendages that require epithelial – mesenchymal interactions including hairs, teeth, mammary glands, etc. (Mills et al, 1999).

Obviously, oral epithelial appendages involve more complicated epithelial – mesenchymal interactions than oral epithelia. For example, some regions are induced to form teeth while others are not. For example, mice have no canine teeth. Taken to the

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[1]RJ Gorlin, personal communication.

extreme case, one may ask why chickens do not form teeth? Is the tooth forming process blocked in the epithelium or the mesenchyme? Can we awaken the tooth forming potential? This was first approached by Kollar and Fisher (1980) by recombining the mouse dental mesenchyme with chicken oral mucosa which showed that tooth-like structures are induced that express an "enamel" matrix. A recent interesting paper addresses this issue by transplanting a segment of mouse neural cephalic crest to the corresponding regions in developing chicken embryos. Tooth-like appendages were induced in the chimeric embryo, suggesting that the avian oral epithelia still retains the ability to respond to odontogenic signals (Mitsiadis *et al*, 2003).

This is consistent with earlier work that used a recombined tissue explant model. The recombination of chicken oral epithelium and chicken dorsal skin mesenchyme produced many tooth-like follicular structures arranged in feather patterns. Furthermore, FGF and BMP could mimic this effect to a lesser degree (Chen *et al*, 2000). These works imply that the inability of the oral epithelium to be transformed into tooth-like structures is due to the imbalance of epithelial – mesenchymal interactions with the neural crest derived mesenchyme in the localized tooth field.

The importance of mesenchyme in setting the specificity of the mouth is further demonstrated in another interesting chimeric study. The cephalic crest of quails and ducks were swapped, and the beak morphology was in accord to the origin of the crest derived mesenchyme (Schneider and Helms, 2003). What molecules could have been involved in mediating the specificity of mesenchyma in the oral region? A study of mice showed that a double knockout of homeobox genes *Dlx 5* and *Dlx 6* (normally expressed in the distal mandibular arch) led to mice with double upper jaws. The homeotic transformation includes both skeletal and integumentary structures (e.g., vibrissa pad) (Depew *et al*, 2002). If we search further for the origin of the vertebrate mouth, there was a heterotopic shift of epithelial – mesenchymal interactions, involving FGF and Dlx, which led to the making of the mouth in gnathostomes (Shigetani *et al*, 2002).

The regulation of epithelial–mesenchymal interactions can go awry as seen in tumors, congenital malformations, ulcerations, metaplasias, and various pathological conditions. We are beginning a new phase of exploration into the basic science and medical applications of these epithelial – mesenchymal interactions. How does an epithelial stem cell develop into multiple tissue types? How does the mesenchyme confer regional specificity? Simple keratinocytes are organized into complex oral epithelial topology through a complex pathway involving cellular and molecular interactions. From recent works, we learned that major

signaling pathways like FGF, BMP, MSx, Wnt, EDA, Notch, SHH, etc. are involved (Thesleff and Mikkola, 2002), but we do not know how to put them together. The epigenetic rules leading these molecules to be present at the right locations at the right time remain to be learned. Just like a chef who can make a culinary delicacy by magically mixing basic ingredients in the right proportions and with the right timing, one day when nature's recipes are known, we may be able to apply lessons learned from the pathological situations described above into useful medical applications through the process of tissue engineering.

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