Keeping Faces - Saving/Maintenance of Oral Mucosa and Salivary Glands

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INTRODUCTION

The oral mucosa is divided into an epithelium and underlying connective tissue. The epithelium is stratified and was originally characterized by its site-specific morphology (Squier and Kremer, 2001; Schroeder, 1981; Sakai et al, 2003). Later, epithelial products such as keratins, carbohydrates and epithelial turnover were included in the characterization of the epithelium (Dabelsteen, 1996; Clausen et al, 1986; Presland and Dale, 2000; Hume and Potten, 1979, 1980; Squier and Kremer, 2001). A few decades ago it was believed that the epithelium was an independent entity and that all basal cells in the epithelium contributed equally to the continued renewal of the epithelium. However, a major change in the understanding of the oral mucosa has occurred. It is now apparent that interaction between epithelial and mesenchymal tissues is not only present during embryonic development but also contributes to the maintenance of normal structure and function in adult life (Sakai et al, 2003; Mackenzie and Hill, 1984). Furthermore, the basal cell appears to be heterogeneous with respect to cell proliferation and there is strong evidence that indicates that the renewal of the epithelium depends on a subpopulation of basal cells known as stem cells (Mackenzie and Bickenbach, 1985; Hume and Potten, 1979; Tudor et al, 2004).

STEM CELLS

When newborn mice are injected with radioactive labelled thymidine most cells in the basal cell layer of the skin and oral mucosa will be labelled due to the uptake of thymidine in cells that actively synthesize DNA. If the mice are left for eight weeks, it appears that single cells in the basal cell layer will still retain the radioactive label (Bickenbach, 1981). When the epithelium was stimulated, the cells divided and the label disappeared due to cell differentiation and desquamation. The label-retaining cells are termed slow-cycling cells and are suggested to represent a subpopulation of basal cells (Mackenzie and Bickenbach, 1985). It is hypothesized that they can divide an indefinite number of times and are therefore regarded as adult stem cells (Mackenzie and Bickenbach, 1985).

When stem cells divide, they typically produce one cell that remains a stem cell and another cell that enters the differentiation pathway. The one to two basal cell layers in the oral mucosa thus appear to consist of three functional different types of cells: 1) A small subpopulation of stem cells, the only basal cells with essentially unlimited cell renewal capabilities; 2) Transit amplifying cells produced by stem cell divisions and able to undergo a few rounds of cell division before they differentiate; 3) Post-mitotic differentiating cells preparing to emmigrate and differentiate into spinous cells (Potten, 2004). At present, we have no markers except for the label-retaining property that can identify epithelial stem cells in the basal cell layer of the oral mucosa. There is, however, some evidence that stem cells in the oral mucosa are located at the tips of epithelial rete ridges (Hume and Potten, 1979, 1980; Tudor et al, 2004). There is also evidence that the localization of the basal stem cells varies from tissue to tissue (Jensen et al, 1999). New research suggests that it is the cells' microenvironment that determines whether the cells behave like stem cells or transit amplifying cells. The microenvironment may include blood vessels, growth factors and support cells (Song et al, 2002). Demonstration of support cells that hold a stem cell in its niche have been demonstrated in the bone where osteoblasts express the receptors for notch that are carried on hematopoetic stem cells. The stimulation of the notch pathway in the hematopoetic stem cells encourages them to retain their dividing capacity instead of differentiation (Calvi et al, 2003). Supporting cells have not yet been defined in the oral mucosa, but they may be found in the connective tissue or may be neighbouring epithelial cells.

One of the critical questions is how stem cell division is regulated. Recent studies suggest that a class of small non-coding RNA species, known as microRNA, is differently expressed in stem cells and that stem cell division – at least in the fly *Drosophila melanogaster* – is regulated by these RNA's (Hatfield et al, 2005).

As dividing cells are more susceptible to carcinogens than non-dividing cells, the presence of slow-dividing stem cells may be part of a protective mechanism in the oral mucosa. It is generally accepted that cancer is not the result of a single gene mutation but requires mutation of several genes - not less than four. Genetic changes may occur in the more frequent dividing cells, the transit amplifying cells. However, as these cells have limited cell cycle capacity, the cells will be lost due to differentiation before the tumor may develop. However, if genetic change occurs in stem cells, a malignant clone may arise, and it is interesting that a recent investigation shows that stem cell hierarchies have been found not only in leukemias but also in oral tumors (Mackenzie, unpublished). Moreover, it is interesting that microRNA that may regulate stem cell division shows a down-regulation in tumors compared to normal tissue (Lu et al, 2005).

Stem cells are ultimately responsible for all tissue regeneration in clinical techniques that transplant cells for regeneration. These techniques are only successful if they include stem cells in their transplants (Rama et al, 2001). It is attractive to be able to use tissue generated from stem cells in tissue repair. In this respect, methods for identifying and isolating stem cells from adult tissue are strongly needed for future therapy.

EPITHELIAL-MESENCHYMAL INTERACTIONS

It is well established that interactions between mesenchymal and epithelial cells play an important role in regulation of tissue development. Furthermore considerable amount of work suggests that epithelial-mesenchymal interactions also play an important role in regulation of tissue homeostasis and repair in adult life (Maas-Szabowski et al, 2001; El et al, 2005; Bissell and Radisky, 2001; Cunha et al, 1992). Experimental studies have thus shown that regional specific differentiation patterns of the oral mucosa is maintained through the interaction between epithelial and regional specific connective tissue (Mackenzie and Dabelsteen, 1987; Hill and Mackenzie, 1984). With the use of the classical experimental methods in which tissue samples from different regions of the oral mucosa is dissociated into the epithelial and connective tissue components and then heterotypically recombined, it has been demonstrated that connective tissue has a strong influence on the direction of the differentiation pattern in the overlying epithelium (Mackenzie and Hill, 1984). Not only does epithelial morphology change in accordance with the influence from the connective tissue, but also the expression of cytokeratins and cell surface carbohydrate markers are influenced by the nature of the connective tissue (Hill and Mackenzie, 1989; Mackenzie and Hill, 1984). The influence of connective tissue on epithelial differentiation is, however, restricted. This appears, for example, from studies where epidermis retains its differentiation pattern when recombined with oral mucosal connective tissue (Mackenzie and Hill, 1984).

Three-dimensional *in vitro* systems in which epithelial cells are cultured on collagen gel with fibroblasts are proven to be useful for analysis of the epithelial differentiation process and its regulation (Smola et al, 1993; Stark et al, 1999). By use of this system a network of cytokines and growth factors that control epithelial proliferation and differentiation has been identified (Florin et al, 2005; Maas-Szabowski et al, 2000; Maas-Szabowski et al, 2003; Bissell et al, 2002). It also appears that the growth and differentiation of epithelium are not solely based on growth factors produced by connective tissue fibroblasts, but represent an active interplay between epithelial cells and fibroblasts and maybe other connective tissue components in a double paracrine mechanism (Smola et al, 1993; Stark et al, 1999; Florin et al, 2005; Maas-Szabowski et al, 2000). Keratinocytes may release interleukin-1 which induces enhanced expression of growth factors in stromal cells such as keratinocyte growth factor, granulocyte macrophage colony stimulating factor and hepatocyte growth factor (HGF). The crucial role of AP-1 transcription factor subunit C-JUN in this trans-regulatory control of keratinocyte proliferation has recently been demonstrated (Florin et al, 2005).

Besides diffusible factors, tissue differentiation and homeostasis are coordinantly regulated by cell-cell and cell-matrix interactions (Bissell et al, 2002). Studies with normal breast tissue have shown that breast epithelial cells grown in 3-D collagen gels retain their metabolic functions and retain functional differentiation as evidenced by *de novo* synthesis of milk proteins (Lee et al, 1985; 1987). It has further been demonstrated that a requirement for the organized glandular structures in 3-D gels is the presence of laminin 1, which in the normal glandular epithelium is produced by myoepithelial cells (Gudjonsson et al, 2002). By studying tumor cells it has been demonstrated that there is a complex interaction between cell surface receptors, and extracellular matrix components in the microenvironment of the tumor. By inhibition of overexpression of β1 integrin and the EGF receptor, malignant mammary gland tumor cells could be successfully reverted to a normal phenotype when cultured in cell-free laminin 1 containing 3-D gels (Wang et al, 2002). These elegant studies demonstrate that at least in certain tissues the interaction between epithelium and connective tissue is not only limited to normal development and homeostasis but is also a major factor in tumor development. *In vitro* studies of oral carcinoma cells lines have shown that many of these do not invade collagen gels unless the gels are populated with fibroblasts (Matsumoto et al, 1989; Hasina et al, 1999). It has been suggested that this is due to the synthesis of hepa-

tocyte growth factor that, when secreted by the fibroblasts, facilitates the local invasion of the epithelial cancer cells. Studies have shown that the HGF receptor is only weakly expressed in normal mucosa, moderately expressed in epithelial dysplasia, and strongly expressed in carcinomas indicating that also an *in vivo* altered interaction between connective tissue and epithelium takes place and is an important step in tumor development (Murai et al, 2004).

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