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REVIEW

Oral epithelial stem cells in tissue maintenance and disease: the first steps in a long journey

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The identification and characterization of stem cells is a major focus of developmental biology and regenerative medicine. The advent of genetic inducible fate mapping techniques has made it possible to precisely label specific cell populations and to follow their progeny over time. When combined with advanced mathematical and statistical methods, stem cell division dynamics can be studied in new and exciting ways. Despite advances in a number of tissues, relatively little attention has been paid to stem cells in the oral epithelium. This review will focus on current knowledge about adult oral epithelial stem cells, paradigms in other epithelial stem cell systems that could facilitate new discoveries in this area and the potential roles of epithelial stem cells in oral disease.
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INTRODUCTION

In recent years, many labs have focused on the identification and characterization of stem cells in various embryonic and adult tissues. These efforts have led to important discoveries about the nature of stem cells and their roles in tissue maintenance and regeneration. Relatively little work has been done to identify oral epithelial stem cells (OESCs) compared with other tissue systems. This review will focus on the basic biology of the oral mucosa, methods used thus far to identify stem cells (including OESCs), emerging paradigms in other epithelial stem cell systems that may be important for OESC biology and the possible role of OESCs in oral disease.

ORAL MUCOSA DEVELOPMENT AND HISTOLOGY

The epithelium on the inner surface of the lips, floor of the mouth, gingiva, cheeks and hard palate is derived from embryonic ectoderm, whereas the epithelium surrounding the tongue is derived from both endoderm and ectoderm.[1–2] The majority of the connective tissue elements in the head originate from neural crest cells.[3–5] Although outside the scope of this review, considerable efforts have been made to identify and characterize the mesenchymal stem cell populations in the connective tissue, some of which are thought to be derived directly from primitive neural crest cells (see refs. 5-6 for comprehensive reviews).

In mammals, the oral mucosa can be broadly divided into three subtypes: masticatory (hard palate and gingiva), specialized (dorsal surface of the tongue) and lining (buccal mucosa, ventral surface of the tongue, soft palate, intra-oral surfaces of the lips and alveolar mucosa). The oral mucosa consists of an outer, stratified squamous epithelium in direct contact with an underlying, dense connective tissue called the lamina propria, which contains blood vessels, minor salivary glands, structural fibers, nerves, fibroblasts and other cell types (see refs. 1, 7 for comprehensive reviews; Figure 1a). In humans, the masticatory and specialized mucosae are keratinized, whereas the lining mucosa is not; however, the location of keratinized oral tissues can vary depending on the species (Figure 1b).[8]

Histologically, undulations of epithelium, called rete ridges, can be seen protruding downwards into the lamina propria (Figure 1a). This in turn creates corresponding finger-like upward projections of lamina propria, named dermal papillae. The interdigitating rete ridges and dermal papillae provide increased surface area contacts that help prevent separation of the oral epithelium from the lamina propria during mastication.[9]

Similar to the epidermis in the skin, keratinized oral epithelium is stratified and consists of basal, spinous, granular and corneal layers (Figure 1a). Non-keratinized oral epithelium is also stratified, but consists of basal, spinous, intermediate and superficial layers.[7] Cell division in all oral epithelial cells takes place solely in the basal layer. After dividing, the committed cells, similar to epidermal keratinocytes, undergo a differentiation process that leads to the expression of structural keratin proteins and the loss of intracellular organelles as cells move superficially, begin to flatten and are eventually sloughed off the surface.[1,7,10–11] Other than minor salivary glands and occasional ectopic sebaceous glands, the oral mucosa is devoid of secondary structures such as hair follicles and sweat glands.

METHODS USED TO IDENTIFY AND CHARACTERIZE STEM CELLS

Over the last few decades, several techniques have been utilized to identify stem cells. Significant progress has been made recently through the use of genetically modified mouse models, which have

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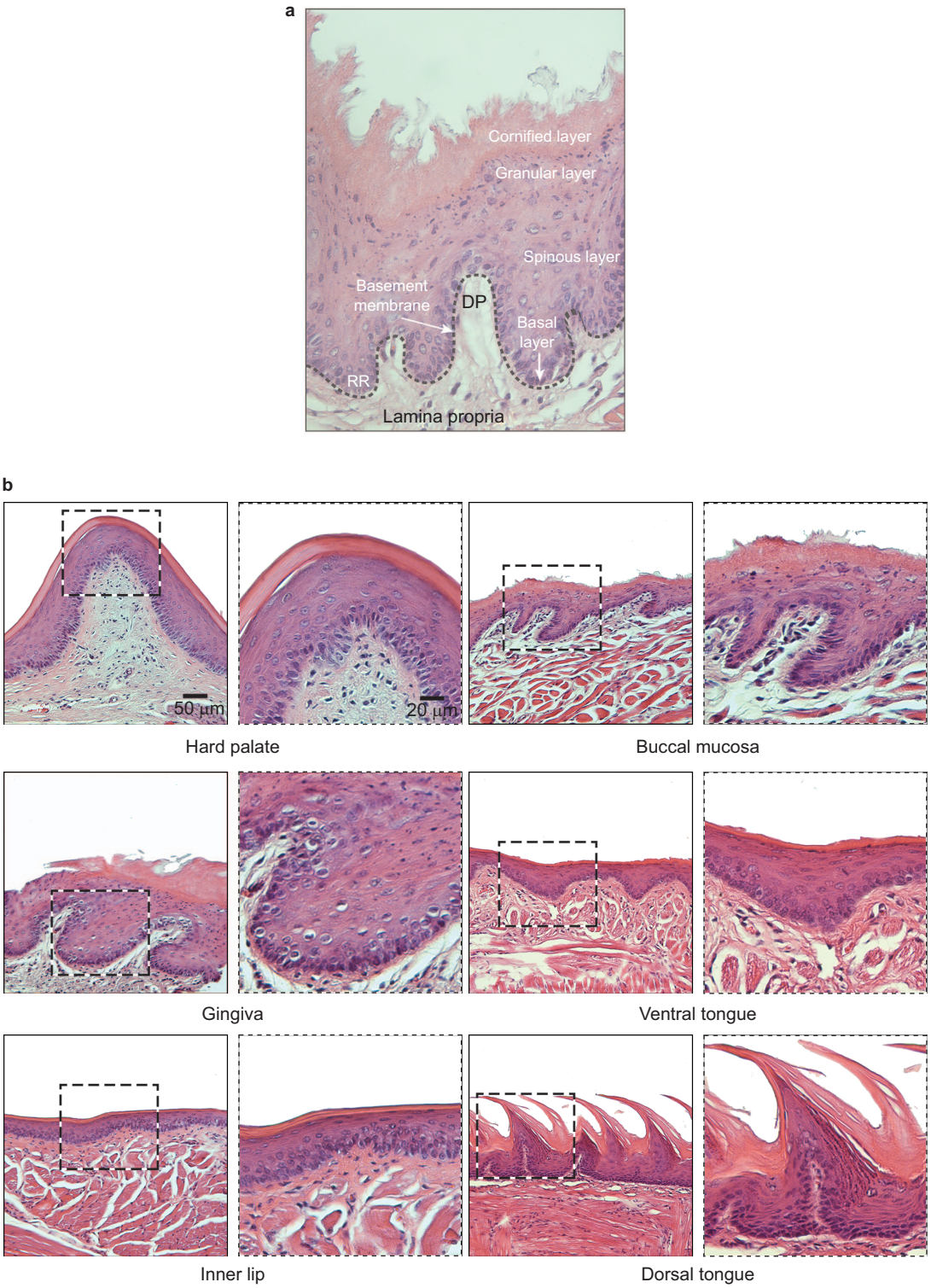

Figure 1 Oral mucosa in *Mus musculus*. (**a**) Diagram of H&E-stained buccal mucosa collected from a 12-week-old C57BL/6 female mouse. In this photo the basal, spinous, granular and cornified layers are all present. Rete ridges and dermal papilla can also be identified. Unlike humans, the buccal mucosa in C57BL/6 mice is keratinized; in general, the location and type of keratinization within the oral cavity differs among mammalian species.[8] (**b**) 7 μm H&E-stained sections from intraoral sites. All surfaces of the oral epithelium in C57BL/6 mice, unlike humans, appear to be keratinized. H&E, hematoxylin and eosin.

been used to build upon the classical techniques that were initially developed to isolate and study stem cells.

A stem cell has the ability to differentiate into some or all of the cell types required to maintain homeostasis within a particular tissue, organ system, or even an entire organism. The developmental stage at which a stem cell is isolated usually determines what types of cells it can differentiate into. For example, embryonic stem cells isolated from the inner cell mass of the blastocyst are pluripotent and can differentiate into any

of the three germ layers (endoderm, mesoderm, and ectoderm). Adult stem cells found in various adult tissues, however, are typically more limited with respect to differentiation and thus are considered multi- or oligopotent.[12] Along with the ability to differentiate into different cell types, stem cells are also able to self-renew, a property that ensures their ability to survive and produce the post-mitotic cells necessary for maintenance of tissue homeostasis.

The gold standards for identification of adult stem cells are genetic inducible fate mapping (GIFM) and transplantation.[13–15] GIFM involves placing a permanent genetic mark on a putative stem cell *in vivo* (usually by genetically activating a fluorescent or colorimetric reporter) such that the cell will be 'labeled' and will pass that label on genetically to all of its progeny, which will pass it on to their progeny, and so on. This technique makes it possible to measure a cell's ability to both self-renew and to produce the various differentiated cells found in a given tissue. Transplantation assays, in contrast, test the ability of a single cell type to fully reform an entire tissue when isolated and transplanted to another animal/location.

Label retaining cells

Several decades ago, pulse-chase experiments were carried out using tritiated-thymidine (^{3}H-TdR), a radio-labeled DNA nucleoside that is incorporated into proliferating cells, to determine cell turnover rates in skin and oral mucosa.[16–17] These experiments showed that in addition to highly proliferative cells that quickly lose their ^{3}H-TdR label, some cells in the basal layer divided much less frequently and retained the label (label retaining cells, or LRCs). Early ^{3}H-TdR studies identified LRCs as long as 240 days post-labeling in mouse palate and buccal mucosa and up to 69 days in hamster tongue.[18–19] More recently, work utilizing 5-bromo-2′-deoxyuridine (BrdU), another labeled DNA nucleoside, showed an increased number of LRCs in the gingiva at 45 days post-labeling compared with the ventral tongue, dorsal tongue, hard palate, buccal mucosa and alveolar mucosa.[20] BrdU was also used to identify LRCs in rat buccal mucosa, tongue and hard palate. After a 10 week chase, LRCs made up about 3%–7% of cells.[21] In all of the ^{3}H-TdR and BrdU experiments, LRCs were restricted to the basal layer. Additionally, in thicker tissues, LRCs were found predominantly at the bases of the rete ridges, whereas in thinner epithelium with few rete ridges (e.g. buccal mucosa), LRCs were found randomly distributed in the basal layer.[20] In the tongue, LRCs were located predominantly at the boundaries of the papillary and interpapillary epithelium near the anterior and posterior columns of the filiform papillae.[19,22]

One important caveat is that none of these studies determined if the LRCs identified were keratinocytes. Melanocytes, Langerhans cells, Merkel cells and inflammatory cells are all known to reside within the oral mucosa.[1] Modern immunohistochemical techniques make it possible to costain LRCs for other markers that can differentiate between these various cell types, and the results of such studies will be important to obtain. A second caveat to LRC studies in general is that for a cell to incorporate a labeled nucleoside, it must go through DNA synthesis, which can make it difficult to label cells that rarely divide. Although one LRC study reported that nearly 100% of all basal cells in the oral epithelium were labeled after a 10-day continuous administration of BrdU, rare populations of slowly dividing cells may still have been missed.[20]

The *K5tTa; tetO-H2B-EGFP* system in mice provides an alternative way to label slowly cycling cells.[23] In this system, all keratin 5 (K5)-positive cells express green fluorescent protein (GFP) beginning in embryogenesis. In the adult mouse, all basal layer cells in the oral epithelium, including presumptive stem cells, continue to express K5.[10] When doxycycline is given to the mice, the cells stop expressing GFP. In rapidly dividing cells, the GFP signal is diluted, while slowly dividing and/or post-mitotic cells remain green. This system has been successfully used in several tissues, including the skin, hair follicle and tooth.[23–25] Because this method initially labels all K5-positive cells in the mouse, including those that cycle very slowly, it could provide a more reliable quantification of LRCs in the oral mucosa.

It is important to note that label retention is not necessarily a characteristic of all stem cells. For example, *Lgr6* marks a primitive epidermal stem cell in the central isthmus of the hair follicle that does not retain any BrdU label.[26] Additionally, epithelial progenitors in the esophagus do not retain any *H2B-EGFP* label.[27]

In vitro morphology and clonogenicity

One of the classical hallmarks of stem cells is their ability to self-renew through proliferation. For this reason, it has been assumed that cells with high *in vitro* growth potential represent stem cells. Several studies have used the *in vitro* morphological and growth characteristics of isolated cell populations to assay for stemness.

In 1985, Barrandon and Green reported that cell size could predict the ability of human keratinocytes to form clones *in vitro*.[28] Smaller cells had, on average, greater clonogenicity (i.e., they can more efficiently form clones in culture). In a subsequent study, they found three different clone morphologies: holoclones (*holo*=entire), meroclones (*mero*=partial) and paraclones (*para*=beyond). Holoclones produced round colonies with smooth edges, while meroclones produced smaller colonies with irregular edges. Fewer than 5% of the colonies formed by holoclones terminally differentiated, whereas paraclones contained cells with very limited *in vitro* lifespans. Meroclones had growth potential intermediate to holoclones and paraclones.[29] Currently, it is generally accepted that holoclones consist primarily of stem cells, meroclones contain slightly more differentiated yet highly proliferative cells called transit-amplifying (TA) cells, and paraclones are comprised of committed, terminally differentiating cells. Several recent studies in the oral mucosa used morphological and clonogenic characteristics to assert that cells isolated using putative stem cell markers were indeed stem cells.[30–33]

It should be noted that *in vitro* clonogenic and morphological experiments mainly provide indirect evidence about stem cell identity. Transplantation assays can be used in conjunction with morphological observations and clonogenic growth assays to demonstrate that the cells in question are able to fully reform the tissue of interest. Other more advanced *in vivo* techniques to identify and study stem cell behavior (described in greater detail below) are becoming the methods of choice over classical *in vitro* techniques.

Stem cell markers

The hematopoietic stem cell system is one of the best characterized stem cell systems in humans.[34] Numerous cell surface receptors and intracellular proteins have been identified that are differentially expressed between hematopoietic stem cells and their differentiated progeny. This has led not only to an increased understanding of hematopoietic stem cell biology, but has also translated into important clinical therapies.[34] In the oral mucosa, some progress has been made in identifying proteins that mark stem cells. Unfortunately, many of the markers identified thus far are also expressed in other basal cells and therefore only allow for the enrichment of stem cells instead of isolation of pure populations.

Many of the initial proteins used to identify and isolate OESCs were first reported as stem cell markers in the hair follicle and interfollicular epidermis (IFE). These include α_6 and β_1 integrins,[35–36] keratins 15 and 19,[37–38] p63 (ref. 39) and melanoma chondroitin sulphate proteoglycan.[40] Along with other putative stem cell markers such as $\alpha_6\beta_4$, oct3/4, CD44H, p75, ATP-binding cassette subfamily G member 2 and K5, the epidermal markers are indeed expressed by the oral epithelium (Table 1).[30–32,41–45]

To test whether the stem cell markers identified in other tissues specifically label OESCs, oral epithelial cells were sorted based on the expression of putative stem cell markers and then studied *in vitro*. One study showed that $\alpha6\beta4^{pos}$ $CD71^{neg}$ gingival keratinocytes not only had a high colony forming efficiency, but also expressed other putative stem cell markers such as p63 and K19. Moreover, these $\alpha6\beta4^{pos}$ $CD71^{neg}$ cells were able to form oral epithelial equivalents (OEE), a fully stratified epithelium derived from isolated oral epithelial cells that is grown *in vitro*.[41]

Tongue epithelial cells sorted for high levels of K5 and β_1 integrin produced more holoclones in culture than the corresponding epithelial cells expressing lower levels of these markers. These cells could also form OEEs in culture.[31] A collagen IV matrix was used in another study to enrich for buccal epithelial stem cells. The most adherent cells had the highest colony forming efficiencies *in vitro*.[33] Finally, cells in the buccal and gingival epithelium expressing high levels of the neurotrophin receptor p75 had greater *in vitro* proliferative capacity, were typically slowly cycling *in vivo*, and could recapitulate OEEs *ex vivo*. These cells were only present in the tips of the dermal papillae and the rete ridges, suggesting that p75 specifically labels OESCs.[32]

Although no *in vitro* system can perfectly mimic the *in vivo* environment, together these studies suggested that the basal layer of the oral epithelium consists of a heterogeneous mixture of cells with varying proliferative capacities. The observation that only certain cells have

Table 1 Candidate OESC markers in the oral mucosa, the specific oral sites in which they have been studied, their molecular function and their importance in stem cell biology

Candidate OESC markers in normal tissues	Oral site(s) studied	Function	Importance in stem cell biology
β_1 integrin[32,35,43]	Human-derived buccal/ gingival cultured cells	A component of integrin complexes; binds to molecules expressed by the BM	Functionally downregulated in epithelial cells leaving the basal layer that have committed to differentiation; expressed in basal keratinocytes where OESCs reside
$\alpha_6\beta_4$ integrin[30,41–42,82]	Human gingiva and hard palate	Cell adhesion receptor; part of hemidesmosome complex that binds to laminin 5 in BM	Expressed exclusively on surface of basal keratinocytes where OESCs reside
Collagen IV[33,35]	Rabbit buccal mucosa	Found predominantly in BM	Basal layer stem cells are thought to be more adherent to BM; shown to enrich for keratinocyte stem cells
CD44H (refs. 41,83)	Human gingiva	Type 1 transmembrane glycoprotein involved in cell–cell interactions, cell adhesion, and migration	Cell adhesion molecule associated with stem cells; used to identify mesenchymal stem cells
CD71 (refs. 30,84)	Human gingiva	Transferrin receptor	Highly expressed in actively cycling cells; expressed at low levels in slower cycling keratinocyte stem cells
CD117 aka (c-kit) (not expressed)[41,85]	Human gingiva	Cytokine stem cell growth factor receptor	Expressed in hematopoietic stem and progenitor cells
MCSP (refs. 40,42)	Human hard palate	Cell surface proteoglycan involved in spreading, migration and invasion of melanoma cells	May contribute to stem cell clustering by promoting cell–cell adhesion
p75 (refs. 32,43,86)	Human gingiva and buccal mucosa	Low affinity neurotrophic receptor that binds NGF	May protect stem cells from apoptosis and affect cell growth
Keratin 5[23,31]	Mouse tongue	Structural intermediate filament protein expressed by all basal epithelial cells in body	Expressed in basal keratinocytes where OESCs reside
Keratin 14[52–53,87]	Mouse tongue and buccal mucosa	Intermediate filament protein	Expressed in basal keratinocytes where OESCs reside; shown to mark OESCs and epidermal stem cells
Keratin 15[42,88]	Human hard palate	Intermediate filament protein	Expressed in hair follicle bulge stem cells and less differentiated keratinocytes in neonatal mice
Keratin 19[37,41–42]	Human gingiva and hard palate	Intermediate filament protein; smallest acidic keratin	Expressed in glabrous (non-hairy) skin stem cells
Nestin (not expressed)[41,89]	Human gingiva	Class VI intermediate filament	Expressed in developing neuroepithelial stem cells
p63 (refs. 39,41,43–44)	Human gingiva and buccal mucosa; rat palate	Transcription factor involved in morphogenesis, esp. in stratified epithelia	Expressed in epidermal stem cells
Oct 3/4 (refs. 41,90)	Human gingiva	Homeodomain transcription factor	Oct 3/4 levels influence self-renewal of embryonic stem cells
Nanog (not expressed)[41,91–92]	Human gingiva	Homeodomain transcription factor	One of the critical transcription factors needed for self-renewal in embryonic stem cells and iPS cells
Sox2 (refs. 51,53)	Mouse tongue	Transcription factor containing HMG domains	Maintains self-renewal in embryonic stem cells; expressed in several adult stem cells
ABCG2 (refs. 43,45,93)	Human derived buccal cultured cells	Transporter that can pump a wide variety of compounds out of cells	Expressed by stem cells from several different tissues

OESC, oral epithelial stem cell; AGCG2, ATP-binding cassette sub-family G member 2; BM, basement membrane; HMG, high mobility group; iPS, induced pluripotent stem cells; MCSP, melanoma chondroitin sulphate proteoglycan; NGF, nerve growth factor.

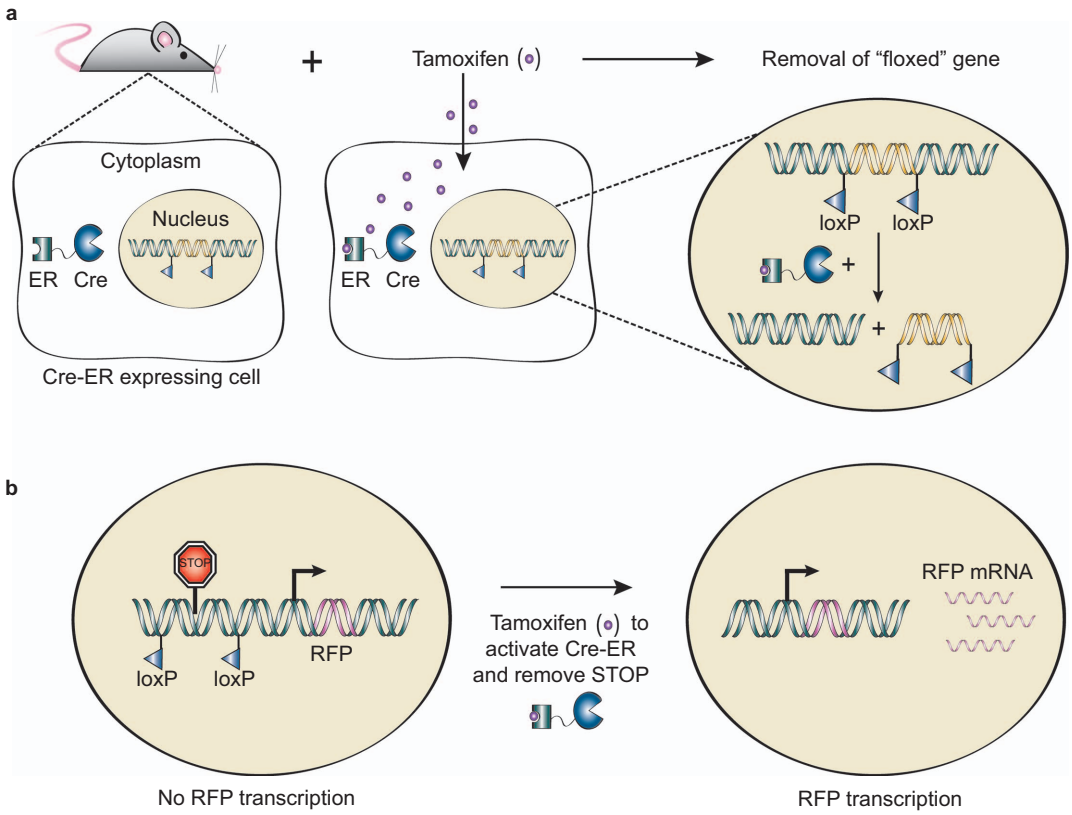

Figure 2 CRE recombinase technology. The CRE recombinase enzyme was identified in the P1 bacteriophage, where it recognizes and recombines 34 base-pair DNA sequences called *loxP* sites.[94–95] *LoxP* sites consist of two 13 base-pair palindromic DNA sequences separated by an eight-base spacer region. When two loxP sites are oriented in the same direction on a strand of DNA, the CRE recombinase can recombine them such that the intervening DNA will be removed from the genome. Transgenic mice have been developed that harbor genes flanked by loxP sites ('floxed' genes). When bred with mice that express a tissue specific CRE recombinase (i.e., a CRE whose expression is controlled by a specific promoter that is only active in a particular tissue), floxed gene expression can be completely abrogated in very specific cell populations. Recently, newer mouse models have been created that allow for temporal control of *Cre* expression. CRE recombinases fused to mutant ERs have been developed that no longer bind endogenous estrogens at physiologic levels, but instead are only activated by binding tamoxifen or its active metabolite 4-hydroxy-tamoxifen.[87] In the absence of tamoxifen, the *Cre-ER* construct is sequestered in the cytoplasm (**a**). When tamoxifen binds the ER domain of the fusion protein, the CRE recombinase translocates to the nucleus, where it removes floxed genes from the genome. Some transgenic fluorescent reporters are constructed such that they are inhibited from being transcribed by floxed transcriptional STOP elements (aka lox-stop-lox or LSL elements). When the *Cre-ER* construct is activated and enters the nucleus, it can remove this STOP sequence, which will allow the fluorescent reporter to be expressed, which in this example is RFP (**b**). ER, estrogen receptor; RFP, red fluorescent protein.

the ability to form OEEs in culture is probably the most convincing evidence that only a subset of the basal cells are true stem cells. *In vivo* methods, such as GIFM, will be needed to confirm the results of these studies and to allow for the *in vivo* characterization of these cells.

In vivo lineage tracing

The *Cre-ER-loxP* system in mice is the principle type of GIFM used for lineage tracing, and it has significantly increased our understanding of the identity and behaviors of stem cells in numerous tissues (Figure 2).[25,27,46–47] Use of this system has enabled researchers to specifically label cells that express a gene of interest to determine if that gene is a bona fide stem cell marker. Although cell surface markers have been used in the past to isolate and then grow putative stem cells *in vitro*, *in vivo* labeling and lineage tracing allows cells to be studied in their native environments and avoids the artificial nature of *in vitro* culture systems. Several epithelial stem cell markers have been identified in this way, including *Lgr5*, *Lgr6*, *Blimp1*, *Lrig1*, *Sox2* and *Bmi1*.[26,46,48–51]

To date, few studies have utilized *in vivo* lineage tracing to study adult OESCs. In one study that utilized a *K14-CreER; Rosa26-LSL-LacZ* mouse model, columns of labeled blue cells could be found on the dorsal tongue and in the buccal mucosa after a one month chase.[52] A subsequent study used the same mouse model and provided evidence that $K14^{+}Trp63^{+}Sox2^{+}K5^{+}$ cells adjacent to the taste bud represent progenitor cells that generated both taste bud receptor cells and keratinized pore cells. These researchers also posited that $K14^{+}Trp63^{+}Sox2^{lo}K5^{+}$ cells represent the long term progenitor cells of the filiform papillae and are located in the basal cell layer.[53] Finally, a *Sox2-Cre-ER*; *Rosa26-LSL-EYFP* mouse model showed that *Sox2* is expressed by basal layer stem cells for at least 10 months after labeling in the dorsal tongue.[51] Numerous Cre-ER mouse constructs are currently available for several genes shown to mark stem cell populations in other epithelial tissues such as the hair follicle, IFE and intestinal crypt. Using these mouse models will enable the identification and characterization of novel OESCs.

EMERGING PARADIGMS

Widely accepted hypotheses regarding basic epithelial stem cell biology over the last 30 years have recently been revisited as more sophisticated tools and mathematical modeling have become available. This has led to the emergence of new paradigms for stem cell biology, as discussed below.

The epidermal proliferative unit model

In 1974, Potten proposed the epidermal proliferative unit (EPU) hypothesis to describe the organization of cells in the epidermis.[54] Based on ^{3}H-TdR data and morphological evidence, the EPU model hypothesizes that groups of approximately 11 cells in the basal layer of the skin are responsible for the production of discrete, hexagonal columns of terminally differentiating cells.[54] Additionally, the ^{3}H-TdR labeling studies suggested that a central, slowly dividing stem cell within each EPU gave rise to peripheral, more rapidly dividing TA cells.[55] Supporting this hypothesis, labeling of cells in the epidermis with retroviruses or mutagens resulted in labeled columns of cells emanating from the basal layer all the way to the cornified layer.[56–59]

The EPU hypothesis proposes that when stem cells within the basal layer of the epidermis divide, they do so asymmetrically and give rise to a stem cell and a TA cell (Figure 3a–3d).[54] In this model, TA cells are thought to be responsible for the majority of cell divisions within the epidermis. The TA cells give rise to both additional TA cells as well as to the post-mitotic differentiated cells within the epithelium. After multiple divisions, TA cells senesce and terminally differentiate. In this way, it is thought that stem cells protect themselves from the

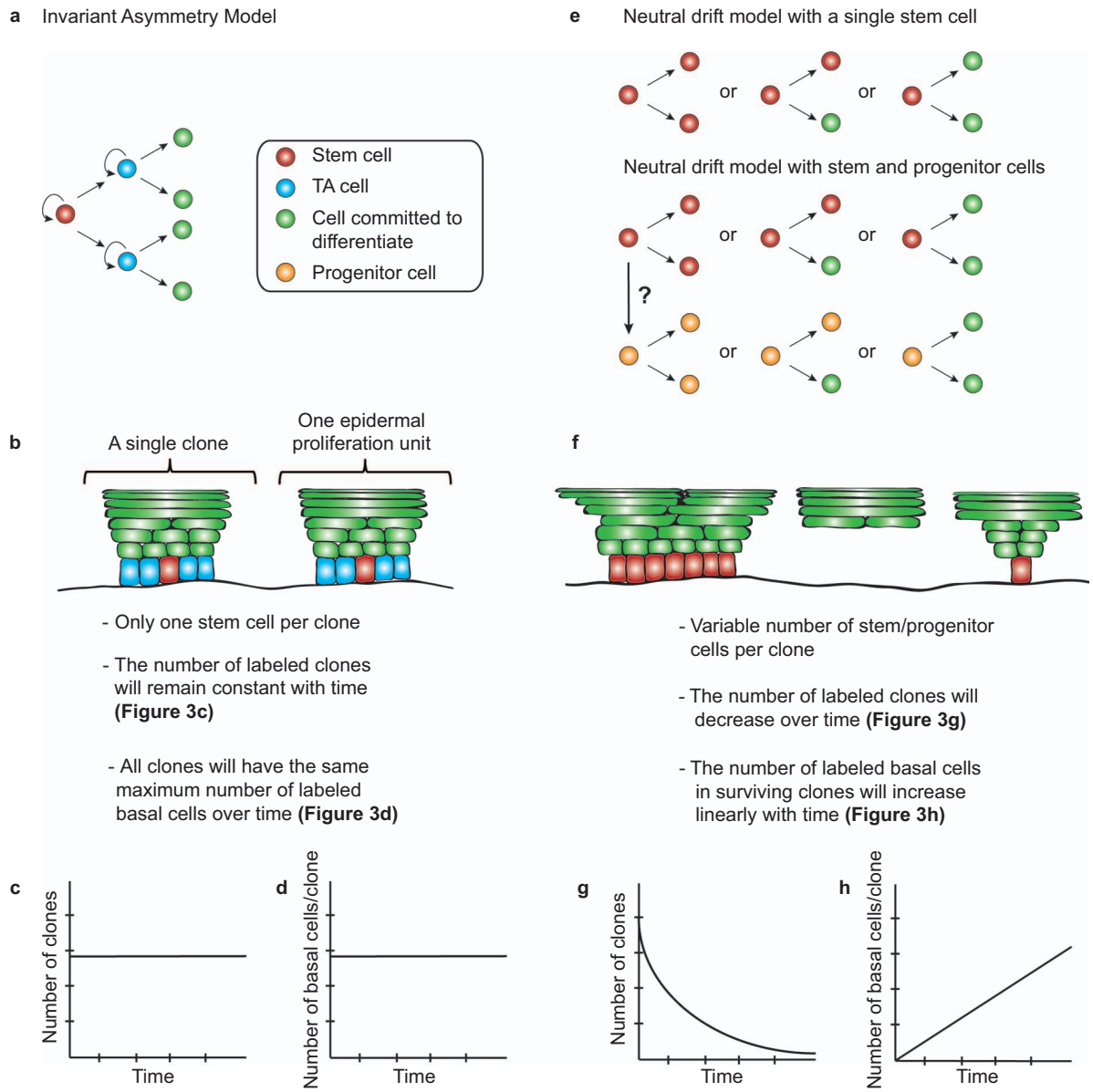

Figure 3 The invariant asymmetry and neutral drift models. (**a**) The invariant asymmetry model in the interfollicular epidermis proposes that a self-renewing stem cell gives rise to transit amplifying cells, which then give rise to differentiating keratinocytes in discrete cellular territories called epidermal proliferation units. If stem cells in this model are labeled using GIFM, then the overall number of clones (groups of labeled cells (**b**)) along with the number of basal cells per clone would be expected to reach a maximum size over time (**c**, **d**). (**e**) In the neutral drift model, one or more stem/progenitor cell populations may be present and cell division results in one of three outcomes: two additional stem/progenitor cells, a stem/progenitor cell and a differentiating keratinocyte, or two differentiating keratinocytes. These divisions occur in a stochastic (random) manner, and thus if these stem/progenitor cells are labeled using GIFM, then clones of various sizes will result (**f**). However, with time, the overall number of clones will decrease due to random chance whereas the number of basal cells in surviving clones will increase linearly with time (**g**, **h**). Figure modified from Klein *et al*.[63] GIFM, genetic inducible fate mapping.

accumulation of random mutations that would otherwise be incurred *via* multiple rounds of DNA replication, thus ensuring long-term integrity of the epidermis. The EPU hypothesis is now known more broadly as the invariant asymmetry model. Since the stem cell in this model always retains its 'stemness' with each division while its daughter does not (i.e. the daughter becomes a TA cell), these divisions are considered invariant and asymmetric. The model implies that there is a hierarchy of cells within the basal layer that creates a heterogeneous mixture of stem cells, TA cells and differentiated cells.

The neutral drift model

Several studies within the last few years have called into question the invariant asymmetry hypothesis; these have used the mouse testes, epidermis, intestinal crypt and esophagus as model systems. These studies provide strong evidence that stem cells in these tissues follow cycling dynamics best explained by the population asymmetry model, also known as the neutral drift model (Figure 3e–3h).[27,47,60–62] Using an *Ah-Cre-ER; Rosa26-LSL-EYFP* mouse, investigators found that the distribution of labeled basal cells in distinct clones exhibited the characteristic scaling behavior predicted by the neutral drift model (reviewed in ref. 63). This model posits that all basal layer cells in the epidermis are equipotent stem cells that can divide randomly in one of three ways: into two stem cells, into a stem cell and a cell marked for differentiation, or into two cells marked for differentiation (Figure 3e). Although this process is stochastic, each basal cell has the potential to remain a stem cell or to terminally differentiate. Thus, at the individual cell level, stem cells can divide symmetrically; however, at the population level, these divisions are asymmetric in that they are balanced such that homeostasis is maintained within the tissue. How this balance is maintained is not yet clear.

To follow up on these experiments, two mouse models (*K14-Cre-ER; Rosa26-LSL-YFP* and *Inv-Cre-ER; Rosa26-LSL-YFP*) were employed for similar lineage tracing and clonal analyses in the IFE as those described above. The authors used a wounding assay to determine whether either of these cell populations contributed differently to wound healing. Whereas *K14-Cre-ER* labeled long-lived, slowly cycling stem cells that contributed greatly to healing, *Inv-Cre-ER* targeted a more differentiated progenitor cell population that did not respond to tissue damage in the same way. In this case, progenitor cells did not have the same proliferative and/or differentiation potential as stem cells, but they could still give rise to differentiated progeny under normal homeostatic conditions. Interestingly, although the *K14-Cre-ER* labeled cells exhibited more canonical stem cell characteristics than the *Inv-Cre-ER* cells, both populations followed neutral drift dynamics.[24] Thus, even though stochastic processes seem to govern stem cell fates in the IFE, a stem cell hierarchy also appears to be present. This differs from the initial hypothesis that all basal layer cells in the IFE behave as equipotent stem cells.

Clonal analysis has not yet been used in the oral epithelium to determine whether basal layer stem cell division follows the invariant asymmetry or neutral drift models. It is unclear whether OESCs behave similarly to the IFE, since both of these tissues are ectodermally derived, or if perhaps OESCs behave more like the endodermally derived esophageal epithelium. Unlike the IFE, there are no epithelial LRCs present in the esophagus.[27] Understanding how homeostasis in the oral epithelium is maintained as well as how it responds to perturbations, such as tissue damage, will be important for understanding how to prevent and treat oral hyper-/hypoproliferative diseases and conditions. Since few lineage-tracing experiments have been performed in the oral mucosa to identify genes that label stem or progenitor cells, for now it is difficult to conduct clonal analyses to study stem cell division dynamics. Further research will be needed to identify specific genes that label stem and/or progenitor cells in the oral epithelium so that comprehensive clonal analyses may be carried out.

STEM CELLS IN ORAL DISEASE

The role that stem cells play in oral disease is of much interest. Most of the work to date has focused on identifying cancer stem cells (CSC) in pre-malignant oral lesions and oral squamous cell carcinomas (OSCC). Although somewhat controversial,[64] CSCs are thought to make up variable portions of solid tumors, are believed to be responsible for maintaining a tumor's growth, and have been shown to be more resistant to radio- and chemotherapies than more differentiated cells within the tumor (reviewed in refs. 65-67). For these reasons, some have attributed tumor recurrence and metastasis to surviving CSC clones.[65] Because many head and neck cancers are resistant to standard radio- and chemotherapies, identifying CSCs and then creating therapies that specifically eliminate them could lead to significantly improved outcomes for patients.

CSCs are defined by their ability to form secondary tumors when isolated from a primary tumor and xenografted to immunocompromised mice. CSCs can then be re-isolated from the secondary tumor and serially transplanted to other immunocompromised mice.[65] Like normal stem cells, CSCs are able to self-renew and differentiate into various cell types; however, due to deleterious mutations, they give rise to tumors instead of normal tissues. Markers such as CD44, CD133 and aldehyde dehydrogenase have been found to mark OSCC cells that can be serially transplanted and reproduce differentiated, heterogeneous tumors in immunocompromised mice.[68–70]

Some of the same markers used to identify OESCs have also been used to identify and/or better characterize CSCs in pre-neoplastic lesions and OSCC. Putative OESC markers such as αvβ6, CD44, Oct-4, Nanog, CD117, ATP-binding cassette subfamily G member 2 and CK19 have all been reported to be expressed at higher levels in putative CSC populations compared to non-transformed cells.[71–74] One study showed that the labeling index of p75 was similar among normal and oral leukoplakia samples, whereas increased p75 expression correlated with a worse OSCC tumor grade.[75] Another group showed that K15 was down-regulated in oral lichen planus, while α6-integrin, β1-integrin and melanoma chondroitin sulphate proteoglycan were upregulated in both oral lichen planus and hyperkeratotic oral lesions.[76] Others also reported that oral dysplasias had decreased expression of p63 (ref. 77). Additionally, several studies have attempted to correlate putative CSC markers with patient prognosis and survival, showing that tumors expressing higher levels of certain CSC markers tend to have poorer outcomes.[72,75,78–81] One caveat is that unless serial transplantation assays have shown that the stem cell markers being studied actually label CSCs, it is difficult to draw firm conclusions about the identity and function of positively staining cells within OSCCs. This is true even for stem cell markers that have already been shown to label normal OESCs, since these cells may not perform similar functions within tumors.

Key questions remain to be answered in order to better understand the relationship between OESCs and oral disease. First, identification of *bona fide* OESC markers is paramount. If specific markers can be identified, then the role of OESCs in various oral disease states can be directly studied using *in vivo* models. Second, elucidating the stem cell hierarchy and cycling dynamics of OESCs will enable a better understanding of how these processes change during disease, which could

aid in the development of therapies that correct these changes. It is possible that many hyperproliferative disorders (e.g., leukoplakia, erythroleukoplakia and OSCC) as well as hypoproliferative conditions (e.g., oral mucositis due to radio-/chemotherapies) are directly caused by pathologic changes in OESCs. Given that the oral mucosa communicates directly with the external environment and is easily accessible, it is conceivable that treatments could be developed that specifically target OESCs *in situ*. A basic understanding of normal OESC biology will be needed to fully appreciate the role that stem cells play in oral disease.

CONCLUSION

Some progress has been made in identifying and characterizing OESCs, but significant questions remain: are there specific genetic markers that are only expressed by OESCs? Do OESCs follow the invariant asymmetry or neutral drift model? Does a stem cell niche exist in the oral epithelium? If so, what are its effects on OESCs and what are the molecular signals that drive these effects? What role do OESCs play in oral disease? The technical and methodological advances that are now available will help to answer these and other questions as work in this area moves forward. A clearer understanding of OESC biology will hopefully lead to novel therapies for oral diseases that will significantly improve patients' lives.

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