Stem Cells in Oral Mucosal Epithelia

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Summary: Oral mucosal epithelia are subject to a wide range of environmental insults that result in abrasions, wounds, burns, bacterial invasion and even malignant change. Functional epithelial structure is normally maintained through rapid tissue renewal associated with patterns of cellular proliferation based on stem and amplifying cell hierarchies. There is now much information available about stem cell properties and the number, pattern of distribution, and mechanisms controlling the proliferation of stem and amplifying cells are becoming better understood. However, uncertainty persists concerning several aspects of stem cell behaviour and two new areas of special basic and clinical interest have recently developed. First, it appears that somatic stem cells have much greater plasticity that previously believed and that this can be manipulated and combined with tissue engineering procedures to generate new tissues for clinical use. Second, it is now apparent that stem cells are the likely target of carcinogens and that epithelial tumours *in vivo*, and also cell lines derived from them, retain proliferative stem cell patterns similar to those of normal epithelia. Asymmetric division is a characteristic property of somatic stem cells and changes occuring during malignancy appear essential for tumour growth and metastasis.

Key words: stem cells, oral mucosa, oral epithelia, cancer

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INTRODUCTION

Many tissues of the body continuously renew their structure, generally by division of immature cells to produce new cells that differentiate into cells with the various phenotypic properties needed for tissue function. It is now apparent that only a subpopulation of the proliferative cells has the ability to divide indefinitely and that tissue renewal depends ultimately on this subset of cells (Potten, 1983). These cells, termed somatic or adult stem cells (SSC), need to be distinguished from other types of stem cells including the embryonic stem cells (ESC) that are derived from the inner cell mass of embryos, and the quiescent stem cells that are present in some adult tissues and are exemplified by mesenchymal stem cells (MSC) (Orkin and Morrison, 2002). Although these various types of stem cells differ in several ways, their common feature is an ability for indefinite self-renewal (Molofsky et al, 2004). Typically, ESC have been thought to have the highest capacity for self-renewal and the greatest plasticity (multipotency) for differentiation into a diversity of phenotypes but recent work suggests that such properties may extend to MSC and SSC to a much greater degree than previously thought (Orkin and Morrison, 2002; Alison et al, 2004). However, despite some shared properties related to self-renewal, even the somatic stem cells of a single type of tissue may differ in several other respects. For example, epithelial stem cells differ in the details of their distribution, behaviour and marker expression depending on whether they are found in skin, oral mucosa, cornea, intestine or glands (Cotsarelis et al, 1999; Lavker and Sun, 2000; Pellegrini et al, 2001; Marshman et al, 2002; Alonso and Fuchs, 2003).

As a result of over 30 years of research into the mechanisms of haematopoiesis, bone marrow stem cells are now quite well characterized and stem cells, as well as cells at different stages of maturation, can be isolated by their differential expression of cell surface markers (Morrison et al, 1995). As individual haematopoeitic stem cells normally generate several phenotypically different cell lineages, multipotentiality is sometimes included among the defining properties of stem cells (Huntly and Gilliland, 2005). In some epithelial structures such as hair follicles and glands, individual stem cells similarly give rise to multiple phenotypic lineages (Oshima et al, 2001; Marshman et al, 2002) but in epithelia such as the stratified squamous epithelia of skin and mucosa stem cells normally generate single lineages (Tudor et al, 2004). However, even these epithelia

contain cells that can generate a wide range of new phenotypes if exposed to embryonic developmental influences (Liang and Bickenbach, 2002). Whether this plasticity is only an experimental phenomenon or is a property normally exploited by somatic stem cells during tissue renewal is unclear because somatic stem cells typically appear to act as if they are committed to generating the limited range of cell types associated with their tissue of origin. For example, cells isolated from different types of epithelia retain some phenotypic differences during growth in culture and are often able to re-express the full basic phenotype of their tissues of origin when returned to an *in vivo* environment (Mackenzie, 1994; Tudor et al, 2004).

The distribution of epithelial structures, and also their patterns of differentiation and morphology, are controlled by interactions occuring during development between the epithelium and its associated mesenchyme (Mackenzie, 1994). However, such interactions do not cease after birth and remain active in the maintenance of epithelial structures throughout adult, particularly complex structures such as hair and tongue papillae (Mackenzie and Hill, 1984). Adult connective tissues can also be shown, at least experimentally, to retain the capacity to induce new patterns of differentiation in heterotypic epithelia (Cunha et al, 1985; Ferraris et al, 2000; Pearton et al, 2005). Heterotypic cell-cell interactions associated with 'stem cell niches' control the number and distribution of haematopoietic stem cells (Spradling et al, 2001) but it remains to be determined how the behaviour and distribution of adult epithelial stem cells are influenced by similar tissue interactions.

RENEWAL OF STRATIFIED SQUAMOUS EPITHELIA

Long-term self-renewal and the production of differentiating cells are defining stem cell features, but in many continuously renewing tissues, including epithelia, the stem cell pattern of renewal is combined with a mechanism for expansion of the maturing population by further division of stem cell daughter cells before they terminally differentiate (Morrison et al, 1997; Potten, 1981). The general scheme for epidermis and oral mucosa is represented diagramatically in Fig 1. Such renewing tissues consist of a hierarchy of at least three cell types: a) stem cells which typically divide infrequently but retain an extensive self-renewal capacity, b) amplifying cells that have a limited capability for proliferation, and c) post-mitotic differentiating or differentiated cells (Tudor et al, 2004). As discussed below this hierarchical pattern markedly influences the interpretation of kinetic changes occuring in health and disease, and particularly in cancer.

Identification of Epithelial Stem Cells in Vivo

Label-retaining cells. The type of proliferative pattern shown in Fig 1 results in the number of stem cells within the overall population being strongly influenced by the number of amplification divisions undertaken by their daughter cells. If other factors remain equal, each additional tier of amplification division doubles the number of cells arising from each stem cell division and so reduces the proportion of proliferative cells that are stem cells. Also, being at the origin of this proliferative hierarchy, stem cells are expected to cycle more slowly than amplifying cells in order to maintain a steady state. The presence of slowly-cycling cells within epithelia was initially suggested by the lack of cell division in the centre of the 10-12 basal cells beneath the columnar units of structure in murine epidermis (Mackenzie, 1970) and this was confirmed by a method of 'label retention' (Bickenbach, 1981). Assuming that radio-active label incorporated into DNA during cell division halves with each subsequent cell division, slowly-cycling cells can be identified as cells that retain label longer than more rapidly dividing cells. Label-retaining cells (LRCs) have been identified in various rodent epithelia and consistantly

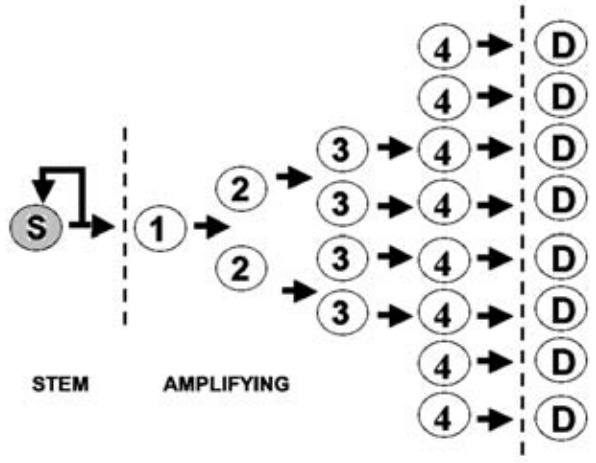

Fig 1 Schematic outline of the stem cell hierarchy in stratified squamous epithelia. The available evidence points to a pattern of of cell generation in which a stem cell **S** divides asymmetrically both to renew itself and to generate a cell that enters the differentiation pathway. The differentiating daughter cell then undergoes a limited number of divisions (one to four) to amplify the cell population before terminally differentiating **D**. In the example shown, daughter cells undergo three amplifying divisions to produce eight terminally differentiated cells. Presumably, decisions concerning cell fate are made at two phases of transition, represented by the broken lines. The first transition determines whether a stem cell daughter will remain a stem cell or differentiate. At the second transition, a decision is made about withdrawal from amplification division cycles. These may not be abrupt changes. Some findings indicate that even cells well advanced on the differentiation pathway can return to re-acquire stem cell characteristics.

show localization to regions that other types of kinetic studies predict to be stem cell zones (Bickenbach, 1981; Bickenbach and Mackenzie, 1984; Cotsarelis et al, 1999).

Lineage markers. It can also be seen from Fig 1 that each stem cell generates a clonal lineage of daughter cells and, if these cells remain clustered, epithelia consist of a series of clonal units, each coresponding to a single 'stem cell territory'. Kinetic studies indicate cell production originating from the centre of the columnar units in epidermis and from the base of the anterior and posterior columns of cells that form tongue papillae (Bickenbach, 1981; Hume and Potten, 1976). However, as the proportion of stem cells initially labeled is not known, the label retention method cannot determine the number of stem cells present at these sites. An alternative method is to examine the lineages arising from individual stem cells after they have been transduced with a heritable marker *in vitro* and re-assembled for transplantation back to *in vivo* sites. This demonstrates that the clonal units formed in rodent epidermis correspond to the columnar morphological units present in this tissue and estimate the number of stem cells present within this tissue to be about 8% of the total basal cells (Mackenzie, 1997).

Clonogenic assays. Much of the information available about human epithelial stem cells has been derived from *in vitro* studies of clonogenicity (Barrandon and Green, 1985, 1987). When isolated and grown *in vitro*, some of the *in vivo* characteristics of stem cells are retained and are reflected in the type of colonies that they form. Colonies developed from individual epidermal cells have beeen classified into three types (Barrandon and Green, 1987). Holoclones, which are considered to contain mainly stem cells, have a smooth rounded outline and consist of small closely packed cells that have an extensive growth capacity. When passaged, holoclone cells mainly produce new holoclones but both other colony forms are also generated. Paraclones, which are considered to consist mainly of late amplifying cells, have an irregular outline form and consist of larger flattened cells that have little capacity for growth and produce only paraclones when re-plated. Meroclones have intermediate properties and are considered to contain mainly early amplifying cells. The presence of such clonogenic patterns in cultures of epidermal keratinocytes indicates that at least some stem cell properties are intrinsic and persist after isolation and *in vitro* growth (Tudor et al, 2004). Clonogenicity also provides a method for assaying the proportion of stem cells among cells isolated from other tissues such as tumours (Locke et al, 2005).

Side populations. In many tissues the stem cell population characteristically expresses higher levels of ABC transporters and thus have an enhanced ability to clear molecules such as dyes from their cytoplasm. Consequently, if a mixed population of stem and amplifying cells is exposed to a diffusible fluorescent dye and then incubated in dye-free medium, the presence of a stem cell fraction can be demonstrated as a dye-free 'side population' by fluorescent activated cell sorting (Goodell, 2002). This method has allowed stem cell enriched populations to be isolated from haematopoietic and epithelial tissues, and has also demonstrated maintenance of cells with stem cell characteristics in tumour cell lines (Hirschmann-Jax et al, 2004).

Markers of the epithelial stem cell phenotype. Research with haematopoietic stem cells has been greatly advanced by the discovery of cell surface molecules that are differentially expressed by stem cells and by differentiating cells. The search for molecular markers that can similarly identify epithelial stem cells has been less successful but cells with stem cell properties have been shown to have higher expression of certain molecules such as β1- and α6-integrins, cytokeratins 15 and 19, melanocyte cell surface protein (MCSP), (see Fig 2) and p63 (Jones and Watt, 1993; Michel et al, 1996; Cotsarelis et al, 1999; Pellegrini et al, 2001; Legg et al, 2003). However, expression of these markers is not restricted only to stem cells and there are also variable stem cell expression patterns in different epithelial regions. Attempts to localize and quantify epithelial stem cells have typically compared the distribution of such markers with stem cell positions or properties derived from a range of other methods. For large structures, such as hair follicles and the cornea, there is general agreement between different methods for stem cell localization but the results for interfollicular epidermis remain conflicting (Cotsarelis et al, 1999).

Localization of Epithelial Stem Cells in Vivo

Label retention has localised slowly-cycling cells in rodents to the central region beneath epidermal units, to the deep tips of epithelial rete, to the bulge region of hair follicles, to the limbal region of the cornea, and to other discrete regions in a range of tissues (Bickenbach, 1981; Cotsarelis et al, 1999). Microdissection of human tissues has localized clonogenic cells to the bulge region of hair follicles and to the limbal region of the cornea, positions corresponding to those indicated by label retention in rodents (Oshima et al, 2001; Pellegrini et al, 2001). Human epidermal cells that express higher levels of β1-integrin have been found to be more clonogenic than cells with lower levels of expression, and staining skin sections shows foci of β1-integrin-rich basal cells lying over the tips of the connective tissue papillae (Jones and Watt, 1993; Jensen et al, 1999). This suggests the selective presence of clonogenic stem cells in this region, but cells with high α6-integrin expression are also highly clonogenic, and the pattern of tissue

staining for α6-integrin does not co-incide with that for β1-integrin (Li et al, 1998). Lineage studies of retrovirally-transduced human epidermal stem cells detect small clonal columnar units in reconstructed human skin, and these are similar to the columnar units in rodent epidermis (Tudor et al, 2004). Further, a more recent lineage study, using transplantation of intact human skin to immune-defficient mice followed by *in situ* lenti-viral transduction, similarly shows small columnar lineages unrelated to either rete structure or to integrin staining (Ghazizadeh and Taichman, 2005). Thus the pattern of distribution of stem cells in human interfollicular epidermis remains uncertain, as does the role of 'stem cell niches' in determining this pattern. As individual stem cells are difficult to identify and manipulate, the nature of epithelial stem cell niches has remained largely theoretical (Spradling et al, 2001). However, murine epidermal cells are able to set up clonal columnar units of structure in the absence of regionally specific mesenchymal signals suggesting that patterning for these small units is set up by intraepithelial mechanisms, perhaps involving notch signalling (Lowell et al, 2000).

Stem Cells in Oral Epithelia

The distribution of stem cells in mucosal epithelia has not been extensively studied but the information available indicates that all stratified squamous epithelia have a basic hierarchical proliferative pattern similar to that of the epidermis. When human oral epithelial cells are plated *in vitro* they form a series of colony morphologies essentially similar to those of epidermis, indicating the presence of a hierarchical stem cell pattern of renewal (Gao and Mackenzie, 1992, 1996). Label retention indicates that stem cells in rodent mucosa are localized to the deep tips of the epithelial rete in the palate, are scattered with no discernable pattern in buccal mucosa, and are localized to the base of the anterior and posterior columns of the tongue papillae (Bickenbach, 1981; Bickenbach and Mackenzie, 1984). Staining oesophageal epithelium for β1-integrin indicates the highest expression on basal cells at the deep tips of the epithelial rete, the reverse of the pattern found for human epidermis but similar to that for rodent mucosa. Given uncertainty about the patterns of distribution of stem cells in mucosal epithelia, we examined the localization of stem cells in human palatal epithelium using an extensive panel of antibodies against putative stem cell markers, differentiation markers, and markers of cell proliferation (Tudor et al, 2004). Palate has a marked and consistent rete pattern and the general findings are that the basal cells overlying the tips of the connective tissue papillae show stronger staining for putative stem cell markers such as β1- and α6-integrins and MCSP. However, basal cells at the deep tips of the epithelial rete stain strongly for keratins 19 and 15, molecules reported to be stem cell markers in hair follicles and other epithelia. The data for the expression of putative stem cell markers at this site is clearly conflicting. As lack of maturation is an essential feature of stem cells, tissues were also stained for their expression of keratins K6 and K16, markers of early differentiation, and for K10, a later differentiation marker. The zone of basal cells lying over the tips of the connective tissue papillae, cells that stained for β1- and α6-integrin, also showed strong staining for K6 and K16 and some staining for K10. The expression of differentiation markers by cells in this position appears to rule them out as potential stem cells. The basal cells at the deep tip of the rete, the region staining for K15/K19, showed a small 'cap' of basal cells lacking staining for the K6/K16 differentiation markers, suggesting that that stem cell are located among the basal cells at the deep tip of the epithelial rete (Tudor et al, 2004).

Stem Cells, Plasticity and Tissue Engineering

Kinetic studies have indicated that the renewal of skin and mucosal epithelia is normally dependent on a subpopulation of stem cells and there is now increasing interest in developing mechanisms for stem cell manipulation that may facilitate wound healing and the generation of 'engineered' oral tissues. Present 'stem cell therapies' rely essentially on the ability of somatic stem cells to maintain a stable differentiation pattern during *in vitro* expansion. Therapies involving the isolation and re-transplantation of bone marrow stem cells have shown that such therapies can efficiently regenerate the entire haematopoietic system after cancer therapy. *in vitro* regeneration of a stratified epidermis using epithelial cells isolated from small autologous biopsies has been widely and effectively used for the treatment of burns patients (Gallico et al, 1984) and an important developing clinical application of mucosal stem cells is the use of small biopsies of the stem-cell-rich limbal region of the cornea for the *in vitro* generation of corneal epithelium (Rama et al, 2001). These procedures do not involve the isolation of pure stem cell populations but depend on the successful *in vitro* expansion of the stem cells present in the cell population initially isolated. The *in vitro* generation of mucosal grafts, and their successful transplantation within the oral cavity, has indicated both the feasibility of manipulating oral epithelial cells and the potential value of such techniques in oral reconstruction (De Luca et al, 1990; Langdon et al, 1990). Although some experimental evidence indicates that using isolated epithelial stem cells results in more effective patterns of *in vitro* amplification of stem cells (Dunnwald et al, 2001) suitable methods for isolating stem cells from either skin or mucosa have not yet been developed for therapeutic use.

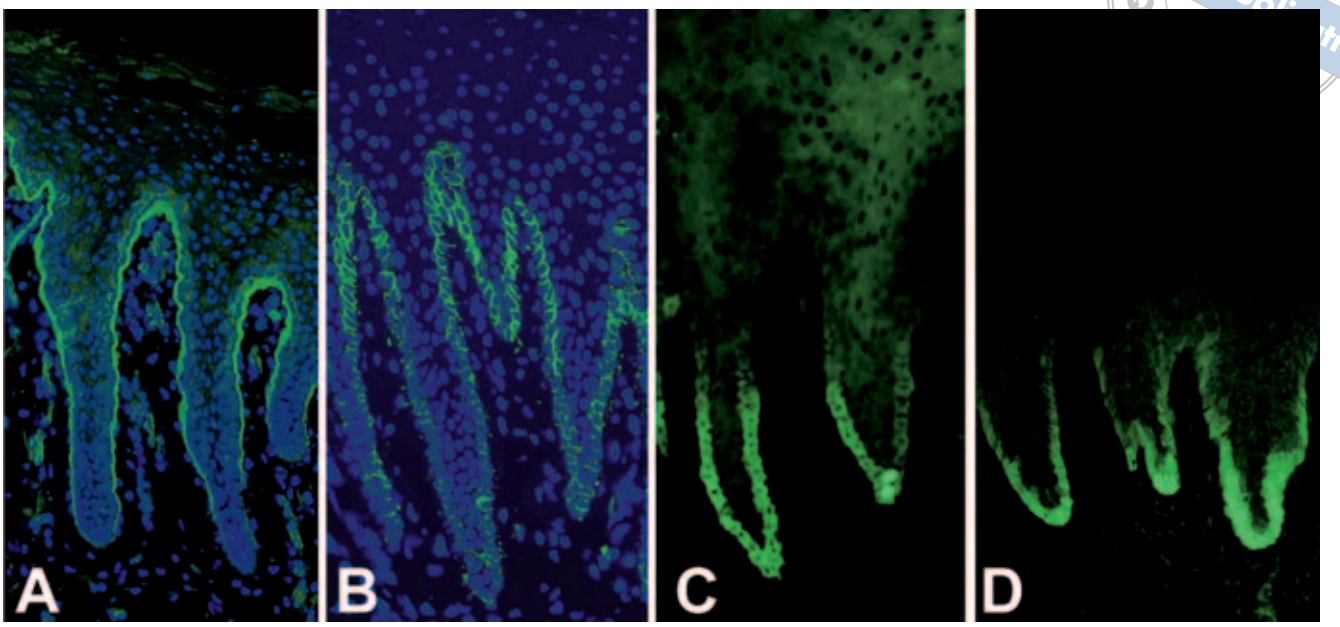

Fig 2 Expression of putative stem cell markers in relation to the rete of palatal epithelium. The panels show frozen sections of human mucosa from the anterior palate stained using an indirect immunofluorescent method to display binding of antibodies against a range of markers reported to be more strongly expressed on epithelial stem cells: **(a)** α6-integrin, **(b)** MCSP, **(c)** K19, and **(d)** K15. Staining for both α6-integrin and MCSP is stronger on basal cells of the upper part of the epithelial rete and is largely absent from the deep tips of the epithelial papillae, the region that shows basal cell expression of K15 /19 and lack of expression of differerentiation markers (not shown). Such conflicting localization of putative stem cell markers is reflective of regional differences in stem cell properties.

It appears likely that the plasticity of foreign stem cells can be manipulated to provide an additional source of cells for oral reconstruction. The effectiveness of such procedures remains to be established but the reverse process, using mucosal cells to generate tissues for transplatation to occular and genital sites has been clinically employed. Stem cells isolated from rodent epithelia can transdifferentiate into a wide range of phenotypes (Liang and Bickenbach, 2002; Ferraris et al, 2000) and it is clear that even adult epithelial cells have the ability to express new phenotypic characteristics in response to new mesenchymal signals (Ferraris et al, 2000; Cunha et al, 1985; Mackenzie, 1994). Possibly, therefore, cell plasticity can be manipulated to provide autologous cells re-differentiated for clinical use into new tissue types (Fang et al, 2004). However, despite successful clinical use of SSC and their potential for redifferentiation (Alison et al, 2004), much of the attention of many tissue engineering laboratories currently focusses on the potential value of ESC as the source of new tissues (Orkin and Morrison, 2002). The ability of ESC to generate many differentiated phenotypic lineages is of great interest but, as for other non-autologous materials, such applications face major challenges associated with evasion of immune rejection (Goessler et al, 2005).

Although the above discussion points to the essential role of local SSC in normal tissue renewal, some recent work has raised questions about the validity of this concept, at least during times of forced renewal, chronic damage or other pathological change (Alison et al, 2004). A striking clinical observation is the presence of Y-chromosomes in fully-differentiated oral keratinocytes of the buccal mucosa of women who have received male bone marrow grafts (Tran et al, 2003). It is unclear whether these cells enter the epithelium individually, rather than being part of a clone of locally renewing cells, but their presence appears to indicate an ongoing contribution of foreign stem cells to epithelial renewal. The extent to which this occurs is uncertain but its occurrence is supported by observations in mice, where transdifferentiation of bone-marrow-derived stem cells can apparently contribute to the clonal renewal of epidermis as well as endothelium (Brittan et al, 2005).

Stem Cells and Cancer

The concept that the growth of tumours, like the growth of normal tissues, depends on a sub-population of stem cells was proposed several years ago (Hamburger and Salmon, 1977). Although controversies concerning the roles of stem cells in cancer (Kummermehr, 2001) have not yet been fully resolved, it has become apparent that stem cells play important roles in the initiation, progression and spread of cancers. It has been argued, with credibility, that stem cells are

the effective targets of carcinogens and that most tumours, including squamous cell carcinomas, arise from stem cells (Reya et al, 2001). Carcinogenesis is a multistep process requiring several mutations (Braakhuis et al, 2004) and in a renewing tissue only stem cells are likely to be present long enough to undergo the necessary sequence of genetic changes for malignant alteration. Further, both normal stem cells and cancer stem cells share patterns of continuous self-renewal that suggest the origin of malignant stem cells from normal stem cells rather than their acquisition of new metabolic pathways (Reya et al, 2001). Stem cells also appear to have acquired mechanisms that protect them from genetic alterations. These include a slow cell cycle that allows time for repair of damaged DNA, patterns of DNA segregation that reduce copying errors, differential sensitivities to apoptosis inducing events for removal of damaged cells, and high levels of transporters to allow rapid export of noxious molecules (Potten et al, 1978; Potten, 1981, 2001; Goodell, 2002). Potential influences of the presence of stem cells in oral epithelial on mucosal pathology has been discussed previously (Hume and Potten, 1979).

The persistent growth of tumours indicates that they contain cells with a high capacity for self-renewal, a basic stem cell property. The question of interest is not therefore whether stem cells are present but whether malignant stem cells maintain other stem cell properties such as generation of hierachies of proliferative cells with different regenerative abilities (Denekamp, 1994; Reya et al, 2001; Locke et al, 2005). Such information is critical to understanding how stem cells behave in tumours and how therapy may be influenced by differences between the responses of malignant stem and amplifying cells. Demonstration that differential expression of cell surface markers allows isolation of small 'tumor initiating' subpopulations in leukaemias and breast cancers has confirmed that tumours do retain hierarchical proliferative patterns (Bonnet and Dick, 1997; Al Hajj et al, 2003). Malignant stem cells thus form only a small fraction of a tumour and are the only cells capable of maintaining persistent tumour growth and these findings are of clinical interest for several reasons. First, malignant tissues, like normal tissues, consist of distinct stem and amplifying populations and it has been shown that there are marked differences in the metabolism and behaviour of stem and amplifying cells in normal tissues. Persistance of similar differences in malignancy would be likely to result in differential cellular responses to therapeutic procedures (Al Hajj et al, 2004; Jones et al, 2004; Tsai, 2004). Such differences may protect malignant stem cells from the actions of particular therapeutic agents, but it is also possible that they can be exploited to develop therapeutic methods that ensure stem cell targeting.

Within a tumour, only malignant stem cells maintain the asymmetric division pattern characteristic of normal stem cells and a fundamental malignant change is disturbance of the normal, homeostatic pattern of stem cell self-renewal (Reya et al, 2001; Al Hajj and Clarke, 2004; Molofsky et al, 2004; Tsai, 2004; Tudor et al, 2004). It is uncertain whether common molecular mechanisms govern the self-renewal and differentiation of different types of stem cells but several signal-transduction pathways have been implicated as essential in the self-renewal of some types of stem cells (Molofsky et al, 2004; Tsai, 2004). Some information is currently emerging about the molecular characteristics of stem cells and the ability to identify and isolate sub-populations of malignant stem cells should now facilitate studies of the changes that are responsible for the altered growth control of malignant stem cells. As investigation of stem cell systems *in vivo* is extremely difficult, and good experimental model systems are scarce, it is of interest that asymmetric division and hierarchical stem cell patterns appear to be well maintained even in malignant cell lines. Using 'organotypic' *in vitro* assays, cell lines derived from head and neck squamous cell carcinoma show only small sub-populations of clonogenic cells (Mackenzie, 2003) and standard clonogenic assays have demonstrated that malignant cell lines develop clonal patterns (Fig 3) essentially similar to those of normal epithelial cells (Locke et al, 2005). The persistence of stem cell patterns in malignant cell lines should facilitate the development new therapeutic strategies for carcinoma in several ways. Firstly, the consistent asymmetric division pattern provides an experimental model for analyses of the molecular mechanisms associated with the self-renewal of malignant epithelial stem cells. Secondly, identification of differences between holoclone and paraclone cells provides potential molecular targets for the prospective identification and targeting of malignant stem cells. Thirdly, the stable and readily quantifiable stem and amplifying populations present in malignant cell lines provide a simple *in vitro* system for testing potential therapeutic manipulations for their selective activity on malignant stem cells.

CONCLUSIONS

It is now apparent that the continuous renewal of stratified squamous epithelia results from a hierarchical proliferative system that is maintained by a small sup-population of stem cells. The primary distinguishing feature of these cells is their ability to self-renew indefinitely while producing cells that enter the differentiation pathway for tissue function. This is achieved by a unique asymmetric division pattern which normally maintains a constant stem cell number but can respond to damage by producing new stem cells. Despite a large amount of

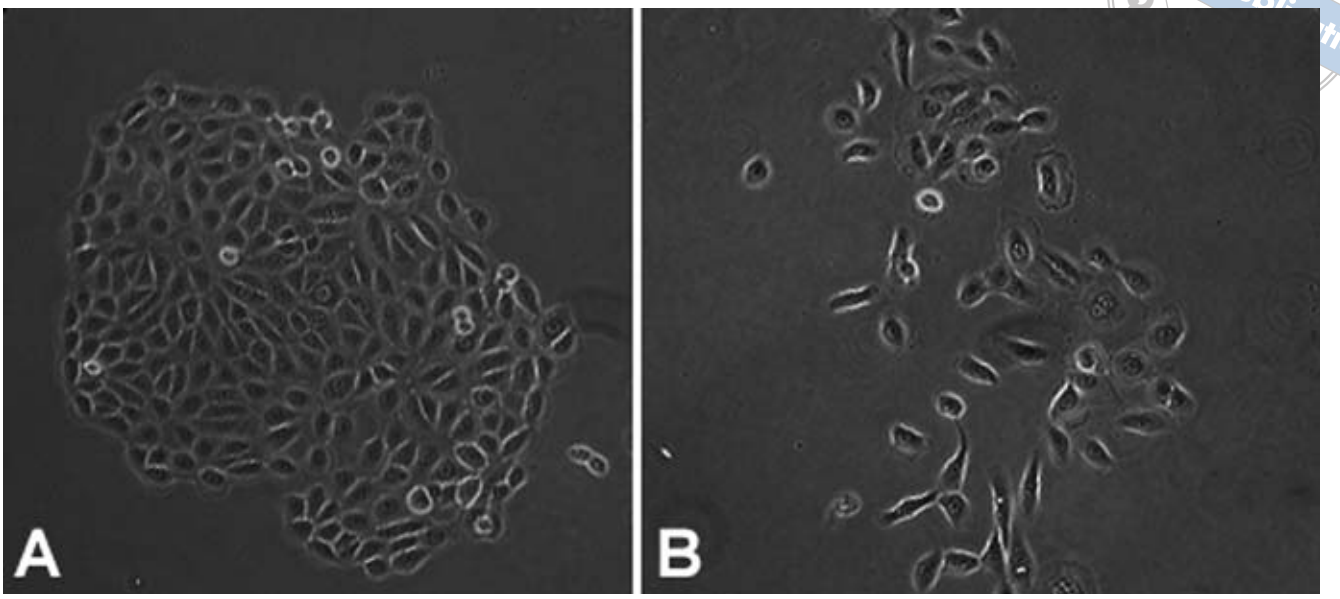

Fig 3 Patterns of colony formation by cells of an immortal cell line derived from oral squamous cell carcinoma. Cells were plated at low density in a basal medium without feeder cells and colonies derived from individual cells were monitored to determine the types of colonies they formed. Panel **A** shows a typical holoclone which has a smooth peripheral outline and consists of small tightly packed cells. Panel **B** shows a typical paraclone consisting of larger scattered cells with an elongated form. These marked morphological differences enable ready distinction of these colony forms for examination of their differential staing patterns and molecular characteristics (for details see Locke et al, 2005).

work, good universal markers of epithelial stem cells have not been identified and there remain uncertainties about regional variations in the number and distribution of epithelial stem cells. However, in many epithelial tissues stem cell positions are clearly related to units of anatomical structure. A surprising finding is the apparent degree of developmental plasticity retained by some somatic stem cells. It had been assumed that once cells had been developmentally determined to produce cells for a particular tissue they were largely committed to a limited phenotypic repetoire. Somatic stem cells normally act as if this were so but there is now increasing evidence that they can also be directed into new phenotypic pathways. This raises many questions about how populations of somatic stem cells can be expanded *in vitro*, how they can be committed to the production of new phenotypic lineages, and about the stability of the newly formed cell lineages. However, there is much interest in determining the clinical value of somatic stem cells for tissue engineering procedures designed to generate new tissues. Recent work also points to the role of stem cells as targets for carcinogens and indicates that hierarchical proliferative stem cell patterns are retained in tumours. These are important findings and may be of explanatory value concerning the mechanisms of spread of tumours and the unpredictable outcomes of some therapies. The robustness of stem cell patterns is indicated by their maintenance in malignant cell lines and these may provide interesting models for further investigation of the basic properties of oral and other stem cells.

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