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Stem cell properties and epithelial malignancies

*Ian C. Mackenzie**

Center for Cutaneous Biology, Institute for Cell and Molecular Science, 4 Newark Street, Whitechapel, London E1 2AT, UK

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ABSTRACT

The growth and repair of normal tissues depends on a small sub-population of cells termed somatic stem cells whose primary characteristic is an ability for indefinite self-renewal. Epithelial stem cells divide to produce cells, termed transient amplifying cells, that undergo a few rounds of more rapid division before they terminally differentiate. Evidence that the growth of tumours, as for normal tissues, is ultimately dependent on a subpopulation of the proliferatively competent cells was first shown for leukaemias by isolation of small sub-populations of phenotypically distinct 'tumour-initiating cells'. Differing cell surface phenotypes also prospectively identify tumour-initiating sub-populations in solid tumours. Even cell lines derived from tumours retain hierarchical stem cell patterns demonstrable as differing clonogenic abilities related to cellular properties such as size, adhesiveness, dye exclusion, and patterns of gene expression. Malignant stem cells appear to form the primary targets of therapy, but how differences between malignant stem and other cells affect therapeutic responses remains unclear. However, transplantation methods exist for their analysis and the in vitro persistence of stem cell patterns may provide systems for developing new therapeutic approaches.

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1. Introduction

Most tissues of the body maintain their structure by patterns of cell proliferation that are associated either with continuous cell renewal or with rapid proliferative responses during cyclic renewal or tissue repair.[1,2] The ability for continuing division with indefinite self-renewal is restricted to a subset of the total proliferative cells, described as 'somatic stem cells', which, in addition to their high capacity for self-renewal, have the ability to generate differentiating cells for tissue function.[3,4] These are therefore the cells ultimately responsible for all tissue renewal. The differentiating cells generated by stem cell divisions may have a limited proliferative potential before they become post-mitotic and express the various phenotypic properties needed for tissue function. Other features of somatic stem cells, such as their numbers, distribution, proliferative patterns, apoptotic responses and ability to generate multiple phenotypic lineages, vary quite markedly from one epithelial tissue to another.[5–9]

Patterns of stem cell behaviour recently revealed in normal tissues and in tumours have generated several questions of both basic and clinical interest concerning the roles of stem cells in malignancy. A range of observations suggests that normal stem cells are usually the functional target of carcinogens and that malignant stem cells, being derived from normal stem cells,[10] may maintain several properties typical of their normal stem cells of origin. The distribution of stem cells in normal tissues is usually related to localised areas, referred to as 'stem cell niches', which appear to have special abilities to support stem cell survival.[11,12] How the loss of normal tissue architecture in malignancy affects the behaviour of stem cells is unclear, but several observations suggest that the basic ability of stem cells to generate hierarchies of proliferative cells is remarkably robust. For example, a substantial degree of autonomy appears to exist in the establishment of

* Tel.: +44 0207 882 7159; fax: +44 0207 882 7171.
E-mail address: i.c.mackenzie@qmul.ac.uk.
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basic epithelial stem cell patterns[13,14] and recent evidence indicates that stem cell hierarchies persist even in cell lines derived from malignancies.[15,16] The growing evidence that tumour growth is essentially dependent on a small sub-population of malignant stem cells indicates that ways must be found to ensure that these cells are part of the cell population responding to therapeutic procedures.[17] This will require methods for their identification and systems to monitor their responses. However, the higher expression of certain molecules in normal and malignant stem cells provides some hope that stem cells can be targeted therapeutically and, if the malignant process is associated with further changes that differentiate malignant stem cells from their normal ancestors, specific targeting of the malignant stem cell population might also be possible.[15,18]

Haematopoietic stem cells have been the focus of over 30 years of investigation and currently represent the most fully characterised type of somatic stem cell.[19] Differential expression of cell surface markers on these cells, and on their progeny as they mature, has allowed identification and selective isolation of cell populations for analysis by various types of in vivo and in vitro assays.[20] Extension of such clonogenic studies to leukaemias led to the first clear demonstration of the existence of stem cells in human malignancies and removed uncertainty about the possible persistence of stem cell hierarchies in tumours.[21] The aim of the present review is to draw together current information about the roles played by stem cells in epithelial malignancies. The amount of information that has been directly derived from studies of epithelial tumours is as yet quite limited, but the general stem cell paradigms derived from studies of normal epithelial stem cells and from studies of normal and malignant haematopoietic and other cells, provide an adequate background for assessing the potential roles of stem cells in epithelial malignancies.

2. Basic proliferative properties of normal somatic stem cells

Haematopoietic stem cells have been shown to divide infrequently and to give rise to hierarchies of proliferative cells that eventually differentiate into the various phenotypic lineages generated in bone marrow.[10,20] The continuous renewal of stratified squamous epithelia is also driven by a hierarchical proliferative system and the primary distinguishing feature of epithelial stem cells is similarly their ability for indefinite self-renewal while producing cells that enter a differentiation pathway for tissue function.[22] However, a stem cell division can have various other outcomes. As illustrated in Fig. 1(Ai), a stem cell can divide symmetrically to produce two cells, both of which enter differentiation pathways with the result that there is loss of all pre-existing stem cell characteristics. The asymmetric stem cell division pattern shown in Fig. 1(Aii) provides the necessary balance for homeostatic tissue renewal. At each stem cell division two cells are produced, one of which retains the characteristics of the parental stem cell and the other enters a differentiation pathway, typically to act as a 'transit amplifying cell' that undergoes a limited series of amplification divisions to augment the number of cells differentiating for tissue function. This results in the generation of three cell compartments: a stem cell compartment which is separated from an amplifying compartment by a transition T1, and a second transition T2 which separates proliferating cells from cells that have entered the post-mitotic phase of differentiation. With increasingly extensive amplification division, the stem cell fraction forms an increasingly smaller proportion of the total cell population. Estimates of the fraction of dividing cells that are stem cells are as high as 25% for dorsal tongue mucosa and as low as 3% for large intestine.[23]

As illustrated in Fig. 1(Aiii), symmetrical stem cell divisions can produce two daughter cells, each of which retains stem cell properties. This sort of pattern can restore stem cell numbers, for example after tissue loss by wounding,[12] and can generate new stem cells during normal or malignant growth. During the normal steady state, as shown in Fig. 1(Aii), cells are generally assumed to move uni-directionally from a stem cell state towards the differentiated state. However, this is not inevitable and Fig. 1(Aiv) illustrates movement in the reverse direction, a pattern expected when stem cell survival is disturbed. Here, as in Fig. 1(Aii), a stem cell divides asymmetrically to renew itself and to produce a cell that initially moves towards the differentiation pathway. However, if there is loss of the daughter cell that had retained stem cell function, further entry of the other daughter cell into differentiation can be blocked resulting in its return to a stem cell state. Evidence for this sort of pattern comes mainly from studies of radiation-induced stem cell apoptosis in the gut, studies which also indicate that marked differences may exist between the responsiveness of stem and differentiating cells to apoptotic stimuli.[24,25]

The type of stem and amplifying cell behaviour illustrated in Fig. 1(Aii) appears to be typical of the renewal of most epithelial tissues. Detailed studies of the renewal of intestinal crypts have identified fixed stem cell positions and hierarchies of amplifying and post-mitotic cells emanating from them with extensive migration of cells and the generation of more than one phenotypic lineage.[8] Renewal of epithelial structures such as hair follicles and glands appear to be similarly associated with hierarchical stem cell patterns, but the structural complexity of such tissues, their intermittent patterns of growth, and their generation of multiple phenotypic lineages complicates detailed analysis of their precise stem cell patterns.[26–28] However, even for stratifying epithelia with uncomplicated structure several uncertainties remain about the validity of the type of pattern illustrated in Fig. 1(Aii). For example, the illustration indicates the transitions from a stem cell to an amplifying cell, and from an amplifying cell to a post-mitotic cell, as sharp transitions but there may be only an increasing probability of loss of stem cell properties and some work suggests that even cells late in the hierarchy can return to a stem cell state if transfected with viral oncogenes or exposed to embryonic mesoderm.[29,30] Whether phenotypic diversity is generated at the stem cell level or later in the cascade is also unclear. For example, the extent to which each separate phenotypic lineage within a tissue requires a separate type of somatic stem cell and whether phenotypic diversity is normally generated during the amplification of cells following stem cell division (as indicated by the change of shading in Fig. 1(Aii)) remains uncertain. Unlike the gut, apoptosis normally occurs infrequently in normal stratified squamous epithelia but can be stimulated by factors such

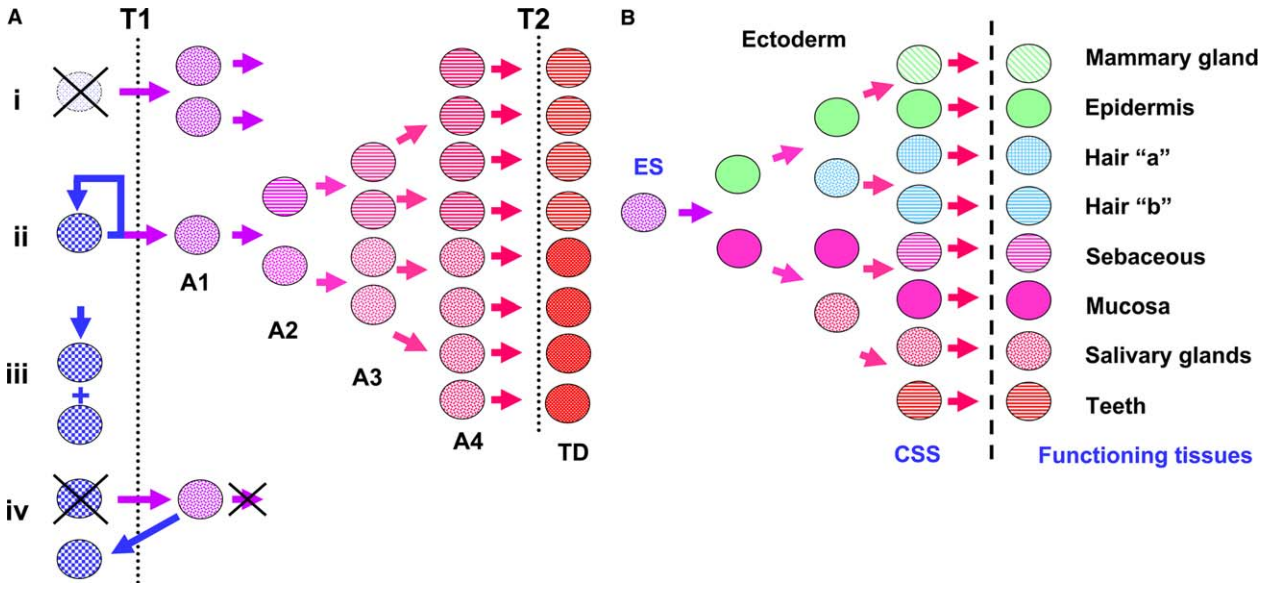

Fig. 1 – Patterns of stem cell division and differentiation. (A) A series of possible outcomes of a stem cell division. (i) Division produces two cells, both of which enter the differentiation pathway with loss of the pre-existing stem cell properties. (ii) A stem cell division results in stem cell maintenance with the production of differentiating cells. (iii) Stem cell division produces two stem cells. (iv) A division as in (ii) initially produces a stem cell and a cell expected to enter a differentiation pathway, but if the stem cell daughter is lost, the differentiating cell returns to the stem cell 'niche'. The type of stem cell system hypothesised to function in continuously renewing epithelia (ii) shows two transitions T1 and T2 (dotted lines) that separate stem, amplifying, and post-mitotic terminally differentiating (TD) cells. Each stem cell division initially results in one stem cell and in one amplifying cell that then undergoes a series of divisions (A1–A4) to produce terminally differentiating cells. In this illustration, one stem division is followed by three amplifying divisions to produce eight terminally differentiated cells. The T1 transition controls stem cell homeostasis: increased transition S → A, as shown in (i) would lead to stem cell loss and atrophy, and lack of transition S → A, as shown in (iii), to accumulation of stem cells. The T2 transition controls the number of differentiated cells produced per stem division: a shift of one tier to the left or right respectively halves or doubles the number of cells produced by each stem cell division and increases or decreases the proportion of stem cells in the total population. Reverse transit of cells from TD → A or from A → S can be induced experimentally, but it is uncertain to what extent this occurs normally. Possibly sharp transitions between compartments do not exist and there is only an increasing probability of differentiation with movement to the right. In some tissues, stem cells generate cells that enter differing phenotypic lineages and it is not always clear in which systems differentially committed stem cells generate independent lineages, and in which phenotypic differences can be generated among the amplifying population (dots versus bars at A2). The presence of a proliferative hierarchy can magnify the effects of apoptotic events: apoptosis at the A1 level would result in the loss of eight cells, but at the A4 level would delete only one cell. (B) Generation of phenotypic diversity in epithelial tissues. Division of a primitive 'ectodermal stem cell' (ES) during development produces cells that become 'committed somatic stem cells' (CSS) under the influence of regional embryonic interactions. These cells then maintain tissues by the generation of specific phenotypic lineages. Various studies show that isolated epithelial cells can maintain regeneration of tissue specific phenotypes, both in vitro and in vivo, indicating the presence of cells functioning as CSS cells. The stability of their commitment is unclear, but the generation of pathologically distinct tumours, for example from each of the tissues listed, suggests that it is stable enough to survive the malignant process and influence the behaviour of the tumours so formed. The pattern of organisation shown here for epithelial stem cells appears to differ functionally from that of haematopoietic stem cells. During development the ectodermal cells shown to the left of the broken line are lost leaving only committed stem cells, each of which generates a relatively narrow range of phenotypes. Haematopoietic stem cells appear to behave more like primitive ectodermal cell and repeatedly generate of a wide range of phenotypic diversity, a difference that may influence various patterns of haematopoietic and epithelial tumour development.

as ultraviolet (UV) radiation. The effect of any particular apoptotic event on cell production will depend on the level within the hierarchy at which it occurs. For example, apoptosis of the cell at position A1 results in the loss of eight cells but apoptosis of a cell in position A4 removes only one cell.

The outcome of a stem cell division can be related, at least in principle, to the concept of control of stem cell number and patterning by 'niches'.[11] The pattern shown in Fig. 1(Ai), or some other event such as apoptotic deletion of stem cells, would be expected to occur under conditions where there is a loss of suitable niches for stem cell maintenance. Pattern 1(Aii) would be maintained in a balanced homeostatic state, and pattern 1(Aiii) where stem cells need to be generated for recruitment to empty niches. The number of haematopoi-

etic stem cells appears to be related to the number of available supportive osteoblasts, i.e., the number of stem niches,[31] but the nature of the niches associated with epithelial stem cells is less clear. Distinct stem cell positions or 'zones' are identifiable in many epithelial structures and these appear to be dependent primarily on autonomous intra-epithelial mechanisms that are modified by extrinsic mesenchymal influences.[5,14]

3. Behaviour of normal epithelial stem cells in vitro

Despite regionally differing in vivo patterns of stem cell distribution and behaviour, once epithelial cells are isolated and plated in vitro they tend to show patterns of clonogenicity that are largely independent of their tissue of origin. First demonstrated for human epidermal cells, and subsequently for keratinocytes isolated from oral and corneal mucosae, hair follicles, glands, and various other tissues and species, plating epithelial cells in vitro at clonal densities consistently generates a range of differing colony morphologies.[9,13,32,33] Some individual founder cells give rise to compact colonies of small cells, containing a high proportion of cells that can be repeatedly re-passaged. Other cells generate colonies with irregular outline forms and which contain fewer cells that are capable of extensive growth. Yet others form colonies of large flattened cells that show little proliferation after passage. These colony forms, referred to as holoclones, meroclones and paraclones, prospectively identify subsequent behaviour of their constituent cells and are considered to be derived from stem cells and from early and late amplifying cells respectively.[13] Epithelial cells are typically thought to be anchorage-dependent and unable to survive in vitro unless plated on suitable substrates. However, neural cells survive in suspension culture where they form three-dimensional structures, known as neurospheres, containing a high proportion of self-renewing stem cells that are apparently able to maintain themselves because of their establishment of extensive and close cell–cell contacts.[34] Interestingly, mammary epithelial cells have also been shown to form floating structures known as 'mammospheres' and these are similarly enriched for stem cells.[27,35] The repeated regeneration of such cellular heterogeneity as keratinocytes are passaged, under either standard or suspension culture conditions, supports the view that basic stem and amplifying cell hierarchies are largely established by 'epithelial autonomous' mechanisms.

4. Epithelial stem cell patterning in vivo

Despite extensive searches, markers that allow clear and consistent identification of epithelial stem cells have not been identified. Consequently, several uncertainties remain about regional differences in the number and patterns of distribution of epithelial stem cells. However, combinations of methods of marker expression, label-retention, micro-dissection, or lineage analysis indicate that stem cells consistently occupy positions related to patterns of anatomical organisation that are present within tissues.[5] As a result of the type of hierarchical amplification pattern illustrated in Fig. 1(Aii), stem cells normally divide more slowly than amplifying cells and can identified as 'label-retaining cells' (LRCs) and these show patterns of distribution precisely related to the micro-architecture of many epithelial tissues.[36] LRCs isolated from murine epidermis are clonogenic,[37] further supporting their identity as stem cells, and micro-dissection of putative 'stem cell zones' in human hair and cornea (i.e. zones corresponding to LRC-rich zones in mice) demonstrates the presence of highly clonogenic cells at these sites.[9,26] The recent demonstration that some stem cells are able to segregate old and newly copied DNA at the time of cell division raises questions about the extent to which 'label retention' is directly related to a slow cell cycle, but not about the specificity of the stem cell identification achieved if segregation is a stem cell specific property.[38] However, whatever the cause of label retention, a drawback of the method is that the proportion of stem cells initially labelled is uncertain and the total number of stem cells present at a given tissue site therefore cannot be assessed accurately.

Analysis of lineages arising from individual murine stem cells after transduction with marker genes generally confirms the distribution patterns suggested by label retention in mice. The size of stem cell territories so determined allows estimates to be made of the number of functional stem cells present in a tissue.[39] Immunohistochemical staining of human tissues for molecules that are differentially expressed by stem and differentiating cells provides another method of assessing stem cell positions. Molecules such as integrins α6 and β1, keratins 15 and 19, p63, and several others, are reported to be expressed at higher levels by human stem and early progenitor cells and can therefore be used to identify 'stem cell zones' if not individual stem cells.[5,9,14,40] Staining for such markers indicates a general correspondence of the distribution patterns of stem cells in human cornea and hair follicles with that of the equivalent murine tissues. Surprisingly, however, the stem cell positions in human epidermis indicated by lineage marking,[41] do not correspond to the in vivo distribution of molecules such as β1-integrin, whose expression has been functionally correlated with stem cell properties.[40]

5. Multipotency of somatic stem cells

Haematopoietic stem cells generate several phenotypically different cell lineages and, consequently, definitions of somatic stem cells have sometimes included multi-potentiality as a necessary property.[42] In some epithelial structures, such as hair follicles, intestinal crypts, and glands, individual stem cells have been shown to give rise to multiple phenotypic lineages.[8,43] In some other epithelia, such as the stratified squamous epithelia of skin, oral mucosa and cornea, stem cells appear normally to generate cells of only a single phenotype.[14] However, even these epithelia can be shown to contain cells that are able to generate a wider range of phenotypes if exposed experimentally to developmental influences derived from the blastocyst or from tissues at later embryonic stages.[30,43,44] Redirection of the phenotypes of adult epithelia can be shown after their recombination with heterotypical adult connective tissue.[45,46] It is uncertain whether such

plasticity is normally exploited by somatic stem cells for tissue renewal, but it is apparent that somatic epithelial stem cells typically act as if they are committed to generating only the limited range of cell phenotypes associated with their tissue of origin (Fig. 1(Aii)). For example, cells isolated from different types of epithelia and grown in culture often retain some markers of phenotypic diversity and are able to re-express the basic phenotype of their tissue of origin when they are returned to an in vivo environment.[14,46]

6. Malignant epithelial stem cells

Tumours often show patterns of differentiation which, although more or less abnormal, can be seen as an attempt to generate normal structure and the presence of a subpopulation of malignant stem cells that drives tumour growth is not a new concept.[47] As 'caricatures of normal tissues', it was suggested that tumours retain some form of stem cell pattern similar to their tissues of origin.[48] Until relatively recently, however, technical difficulties, as well as differing expectations about the criteria required to define stem cells, resulted in concepts concerning malignant epithelial stem cells being sources of controversy.[49,50] As indicated by their persistent growth, tumours clearly contain cells with the basic stem cell property of a high capacity for self-renewal. Therefore uncertainty has not been primarily about the existence of 'malignant stem cells' as such, but about the extent to which tumours retain other stem-cell-related properties, such as the generation of hierarchies of proliferative cells with different regenerative abilities.[10,15,49] The prospective identification of small subpopulations of 'tumour-initiating' cells among the larger populations of proliferative cells in leukaemias, breast cancers and brain tumours has now confirmed that only a small fraction of the proliferative cells is capable of maintaining persistent malignant growth.[10,51,52] This is an important concept that indicates that an understanding of the origins of malignant stem cells, and of their responses to therapeutic procedures, is likely to be critical to the therapy of malignancies.[17,53]

7. Stem cells as targets for malignant change

All continuously renewing tissues, whether normal or pathological, are derived from cells capable of indefinite self-renewal and these, by most definitions, correspond to stem cells. Early ideas about cancer origins, largely based on observations made with teratomas, suggested that the development of tumours results from 'maturation arrest' of stem cells, perhaps derived from embryonic rests.[54] However, subsequent experimental studies of cancer indicated that exposure of skin to initiating agents produced cellular changes that are retained within the tissue for extended periods of time.[55,56] The stem and amplifying hierarchy present in epithelia results in somatic stem cells being the only permanent residents of the epidermis and, consequently, the only cells capable of harbouring the experimental damage, or of the accumulating mutations, necessary for malignant transformation. The observations that stem cells retain carcinogens,[56] and have proliferative responses proportional to the promoting abilities of hyperplasiogenic agents to which they are exposed, support the concept of their involvement in the generation of malignancy.[57]

Several other indirect arguments also support a derivation of cancer stem cells from their normal tissue counterparts. Normal and malignant stem cells share the unusual property of indefinite self-renewal and also have common patterns of gene expression that may be related to mechanisms that control stem cell proliferation and differentiation.[18] It therefore seems more likely that, rather than developing new self-renewal pathways, newly arising cancer stem cells just appropriate the existing self-renewal machinery of normal stem cells.[10] If this is so, various protective mechanisms would be expected to operate to reduce the risk of normal stem cells undergoing malignant change. Some of the properties of normal stem cells can be interpreted as acting to reduce either the risk of mutational changes or the consequences of their occurrence. For example, the basic stem and amplifying pattern itself, as illustrated in Fig. 1(Aii), has two interesting outcomes. One is that cell division, during which cells are at greatest risk of acquiring mutations, occurs most frequently in amplifying cells that are committed to maturation and death. The other is that a slower rate of stem cell division provides an extended intermitotic time for enhanced repair of any newly acquired genetic errors before re-entry to division.[58]

Stem cells may also protect themselves from accumulating genetic damage by selective segregation of DNA with allocation of the newly synthesised DNA strands to the daughter cells expected to leave the stem cell pool. Support for this 'immortal strand' concept is provided by the non-random patterns of distribution of DNA label in mitotic cells in stem cell zones of the gut and breast.[38,59] An interesting consequence of this mechanism is that the risk of generating stem cells with genetic damage would be a higher during symmetrical divisions; for example, during growth, healing and premalignant expansion of stem cell populations. The rate of tumour formation is accelerated by inherited genetic errors[60] and somatic stem cells that have acquired mutations are similarly expected to be at higher risk of further progression towards malignancy. Expansion of the total population of altered stem cells at risk, by mutations affecting either the control of asymmetric division or stem cell survival, would heighten the probability of further malignant change. Exposure to ultraviolet (UV) light expands clones of epidermal stem cells carrying alterations in p53[61] and similar displacement of normal stem cells by altered cells perhaps underlies the development of some types of clonal 'field cancerisation'.[62] Such expansion could be prevented by early detection of genetically damaged stem cells followed by their apoptotic elimination; the higher apoptotic sensitivity of some normal stem cells to genetic damage, resulting in their replacement by a recently formed daughter cells, appears to provide such a mechanism.[24]

Haematopoietic stem cells express higher levels of ABC transporters and consequently can be isolated by fluorescence-activated cell sorting techniques due to their ability to exclude certain dyes.[63] Higher transporter expression is also a property of various other normal stem cell populations, including epithelial stem cells.[64] Rapid exportation of noxious substances from the cytosol may enhance stem cell resistance to the effects of carcinogens.

8. Persistence of stem cell patterns in epithelial tumours in vivo

The maintenance of stem cell patterns in epithelial malignancies generates a tissue containing cells with a range of differing self-renewal and proliferative abilities and there is now considerable evidence for such hierarchical patterns in a range of tumour types. Murine models indicate the presence of stem cells in epithelial malignancies; the pattern of regrowth of irradiated murine epithelial tumours suggests the presence of stem cell hierarchies and radio-resistant stem cells[50] and a stem cell precursor for murine lung cancer has recently been identified.[65] However, most of the evidence for malignant stem cell patterns comes from studies of human tumours. The ability to re-initiate the growth of leukaemias after transplantation to immune deficient mice is found only in a small subpopulation of tumour-initiating cells that is prospectively identifiable by its $CD34^+$, $CD38^-$ phenotype.[10,21] Several reports have described similar stem cell subpopulations in tumours of the central nervous system and have associated malignant neural stem cells with a $CD133^+$ phenotype.[52,66,67] The prospective identification of tumour-initiating cells in breast cancers by their $CD44^+$, ESA^+, $CD24^{-/low}$, $lineage^-$ phenotype has provided definitive evidence for hierarchical stem cell patterns in at least one type of human epithelial tumour.[51] The identification of individual stem cells in intact tumours is hindered, as for normal epithelia, by lack of markers allowing stem cell identification. It therefore remains to be determined how the behaviour, numbers and distribution of malignant stem cells in tumours reflects the stem cell patterns present in their tissues of origin. However, as well as indicating the presence of sub-populations of malignant stem cells within tumours, in vivo clonogenic techniques also provide some indication of the proportion of the total population that may be tumour-initiating cells (see Fig. 2).

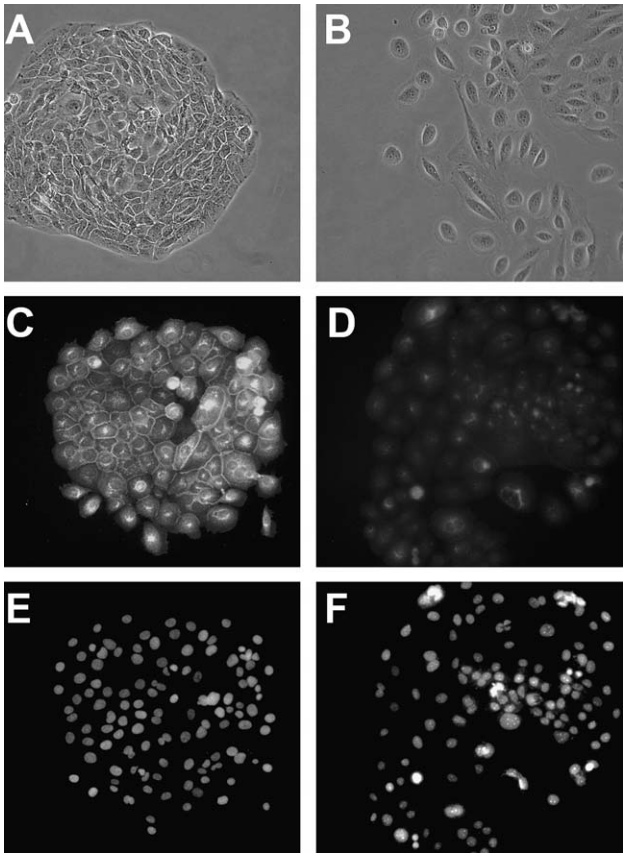

Fig. 2 – Colony morphologies produced by malignant cell lines. (A) and (B) show phase microscopic images of colonies formed by plating cells of a carcinoma-derived cell line at low density. The colony in (A) has a smooth circular outline and consists of small densely packed cells, features typical of holoclone colonies. The colony in (B) consists of larger scattered cells with irregular and often spindle-like morphologies, features typical of paraclone colonies. (C) and (D) show, respectively, a late holoclone and a paraclone after immunofluorescent staining for the epidermal growth factor receptor. The holoclone shows bright membrane staining which is lacking from paraclone cells. (E) and (F) show Hoechst staining of the nuclei of the colonies shown in (C) and (D). The holoclone has small uniform nuclei, but the paraclone shows variation in nuclear size and spacing.

9. Stem cell patterns in cell lines derived from epithelial malignancies

In vitro methods for detecting differential sensitivities of malignant stem cell populations to therapeutic procedures would clearly be of value[47] and in vitro systems that retain stem cell patterns would also be useful for the investigation of key stem cell properties such as asymmetric division.[68] It has long been known that only a small fraction of the cells directly isolated from human tumours is clonogenic under in vivo or in vitro conditions[47] but whether this should be attributed to pre-existing hierarchies of cells with differing cellular properties or results from a uniform but low probability of growth of all cells has been unclear.[10] Clonal heterogeneity is also a typical property of malignant cell lines[69] and even in 'organotypic' culture conditions, which provide a more in vivo-like environment than standard cultures, few of the cells in cell lines derived from oral carcinomas are clonogenic.[70] The disordered structure of malignant tissues suggests that stem cell patterns are altered, even in tumours growing in vivo. The generation of cell lines from such tissues can be expected to lead to further cellular changes as a result of selection during adaptation to in vitro growth. However, the isolation of sub-populations of cells with differing proliferative capabilities from the MCF7 breast cell line by density sedimentation indicates that some intrinsic cellular differences persist.[71] Cell lines derived from a range of tumours also contain 'side-populations' of cells with stem cell characteristics.[66,72,73] These findings point to the persistence of some form of stem cell patterns in malignant cell lines, but the normality of such patterns, and the value of information that might be derived from them, has been questioned.[74] However, closer in vitro study of malignant epithelial cell lines indicates that the proliferative hierarchies that can be detected in them appear in many ways similar to those generated by normal keratinocytes.

When normal epithelial cells are plated at low densities in vitro they generate colonies whose differing morphologies predict their content of stem and amplifying cells.[13] Holoclones, colonies containing a high proportion of stem cells, take the form of compact round colonies of small cells. Paraclones, which contain few or no self-renewing cells, form loose irregular colonies of larger cells. Meroclones are an intermediate form. The colony patterns generated by cell lines derived from oral carcinomas, from prostate and from breast tumours, are essentially similar to those of normal keratinocytes. The cells of malignant holoclones, and of holoclones formed by normal epithelia, are small, rapidly adhesive and highly clonogenic.[15] Normal epithelial stem cells are characterised by their stronger expression of molecules such as β1-integrin, β-catenin, e-cadherin, epidermal growth factor receptor (EGFR) and cytokeratin 15[5,14] and these molecules are also expressed at higher levels by malignant holoclones.[15] Malignant paraclones, like normal paraclones, show expression of some markers of early differentiation. Interestingly, in most cell lines, malignant holoclones show stronger staining than paraclones for CD44, a molecule expressed by haematopoietic stem cells and the major distinguishing marker for the tumour-initiating cells in breast cancers.[51] The cells of malignant holoclones thus share several aspects of their behaviour and patterns of marker expression with both normal epithelial stem cells and with tumour-initiating cells.[15] Surprisingly, normal neural stem cells can be expanded when grown in suspension culture as neurospheres and this method has been extended to the culture of malignant neural stem cells isolated from brain tumours.[52,67] Suspension methods also allow the growth of normal and malignant epithelial populations from breast tumours.[35] We have found that cell lines from other epithelial malignancies can also form expanding populations of tumour spheres in suspension culture and it thus appears that malignant stem cells can be maintained, identified and isolated using a range of in vitro methods. This ability should facilitate the investigation of molecular changes that lead to their altered growth and survival, and the stable and readily quantifiable populations of stem and amplifying cells present in cell lines provides simple in vitro models for initial tests of the activities of therapeutic agents on malignant stem cells.

10. Cancer stem cells as targets of therapy

The degree to which normal and malignant stem cells share common properties remains to be determined, but if malignant stem cells are derived from normal stem cells some aspects of their behaviour may be predicted from what is known about their normal counterparts. In normal tissues, stem and amplifying cells differ in their patterns of division, apoptotic sensitivities, patterns of segregation of DNA, and in their levels of expression of a large range of genes, including those for multi-drug resistance transporters.[15,18,75] Persistence of such differences in tumours is likely to result in differential patterns of survival of malignant stem and amplifying cells under therapeutic conditions.[17,18,74] Characterisation of such differential properties is therefore required both to monitor the effects that current therapeutic interventions may have on stem cells and also possibly for the development of therapies that more effectively target the stem cell population itself.

The differential expression of a wide range of genes in malignant holoclone and paraclone cells suggests that it may be possible to develop better methods for malignant stem cell identification and that there may be stem-cell-related molecules or control pathways suitable for selective therapeutic targeting.[15] However, although in vitro models can be used for such investigations, potential limitations concerning their relevance to the in vivo condition need to be considered.[4] For example, the in vitro growth conditions themselves significantly alter cell behaviour, even of normal cells, and further questions arise about the extent to which cells in cell lines are selected for genetic changes that adapt them to in vitro growth. Despite the latter possibility, the clonogenic patterns of cell lines indicate that they maintain hierarchical proliferative patterns similar to those of normal keratinocytes. Interestingly, much of the extensive information currently available about normal cell-cycle control mechanisms has been generated by in vitro studies of cell lines. Presumably cell lines that have hierarchical stem cell patterns would generate data relating to the majority amplifying population and, consequently, the relevance of these data to the smaller, and more important, stem cell population, needs to be re-assessed.

Most therapeutic procedures depend on the production of cell death by apoptosis or necrosis but an alternative concept of 'directed differentiation' has been suggested as a way of producing tumour atrophy through enhanced transit of proliferative cells into pathways of differentiation.[48] The new awareness of the ultimate dependence of tumour growth on malignant stem cells indicates that critical events able to induce tumour atrophy are likely to occur at the time of stem cell division.[14,17,18,75] Mechanisms controlling stem cell self-renewal in lower organisms are beginning to be understood[76] and signal-transduction pathways have been identified that appear to be essential to the self-renewal of some types of stem cells.[4,18] Molecules such as p63 and c-Myc appear to be central to the maintenance of stem cell properties[77,78] and stem cell proliferation in mammalian malignancies has been linked to altered Notch, hedgehog or Wnt signal-transduction pathways.[4,18] Although the similarity of the molecular mechanisms governing the self-renewal of different types of stem cells is still uncertain, expression of embryonic stem cell markers, such as Oct-4,[79] in both somatic and malignant stem cells suggests the existence of common self-maintenance pathways. Molecular analysis of malignant stem cell self-renewal may be facilitated if the asymmetric division patterns occurring in vitro are found to be controlled by mechanisms similar to those functioning in vivo.

Malignancy is associated with fundamental disturbances in the homeostatic balance associated with normal stem cell self-renewal. The production of differentiating cells in tumours indicates that some degree of asymmetric division persists, but the accompanying malignant stem cell expansion indicates that the probability of stem cell self-renewal is increased. During growth and healing stem cell self-renewal appears to be enhanced by reversible physiological mechanisms[12] and self-renewal can be modulated in vitro by mechanisms related to p53.[68] This suggests that pharmacological

manipulation of normal stem cell self-renewal is feasible and if malignant tissues could be shifted away from self-renewal, stem cell loss and tumour atrophy would effectively follow. Further focus on understanding the mechanisms controlling the asymmetric division of malignant stem cells can therefore be expected to assist the development of new strategies for their therapeutic manipulation.

Conflict of interest statement

None declared.

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