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Emerging themes of cancer stem cells: Editorial – overview

Ultimately, all adult tissues are derived by proliferation and differentiation of embryonic precursor stem cells. It is a generally accepted hypothesis that a proportion of these undifferentiated cells may remain, in specific niches, as a small cryptic population in normal adult tissues. In the case of injury they can activate and differentiate into adult phenotypes, even of previously unsuspected and unrelated lineages. There is growing evidence that neoplasias are not derived from equally prolific dividing cells; rather they have a small population of long-lived, low turnover stem cells, while the bulk of the lesion is populated by highly proliferative cells with shorter lifespan. Their properties of self-renewal are in common with those of malignant cells, but whether cancer stem cells arise as a result of mutations in previously dormant stem cells or whether they are phenotypes of mutated cells of other provenance is discussed below. In malignancy, actively proliferating cells may be susceptible to anti-cancer therapies, but potent quiescent cells, if not killed, remain with full capacity to once more populate the tumour. Clearly, treatment to eliminate cancer needs to be designed so that it takes into account resistant tumour stem cells.

Basic studies on asymmetry in cell division and preferential proliferation of one subset of cells over another are easier follow *in vitro* than *in vivo*. Ian Mackenzie describes (see pp. 347–355) the standardized nature of normal epithelial tissues, with exact positioning of progenitor cells and their descendants. *In vitro* how faithful are epithelial cells' properties in their new strange environment? This problem may be overcome with the use of organotypic cultures, in which a more complex and organ-like culture is provided. Yet, descriptions of clones whose heterogeneous progeny have been derived in culture may be valuable. Using oral carcinoma cell lines, expression of a variety of cell adhesion molecules and cell surface markers has shown that specific cell types retain similarity to parent epithelial stem cells and to tumour initiating cells; that is, they have the essential properties of cancer stem cells.

Normal adult bone marrow is one of the easier tissues in which it is possible to demonstrate the relationships between stem cells and differentiated progeny. Here (see Bonnet, pp. 357–361), the natural heterogeneity of haematopoietic stem cells, with reference to derivation of leukaemic stem cells (particularly in acute myeloid leukaemia), has been described. CD34, for example, is a cell surface marker expressed by both normal haematopoetic stem cells and leukaemic stem cells, while CD38, HLA-DR, and CD71 are expressed in neither. Bmi-1 expression is important for self-renewing cells in both normal and leukaemic stem cells, while CD90, Thy.1, c-kit and the IL-3 receptor are differentially expressed in normal and leukaemic tissues. In mouse, haematopoietic cell gene transfer experiments have implied that leukaemic stem cells might be derived from mutations in committed progenitor cells and even from mutations in mature cells.

The prostate gland is the site of one of the most common malignancies in men. Both hypotheses have been tendered, that prostate cancer stem cells originate as normal stem cells (and from transit amplifying cells), and/or from de-differentiated luminal cells, and these possibilities are discussed (see Rizzo *et al.* pp. 363–374). Androgen dependence is one of the first routes of investigation into the description of a prostate cancer stem cell and an androgen receptor-negative cell type has been found that critically, expresses CD133 which, in normal prostate tissue is also expressed by prostate stem cells, which are anatomically basal in this organ. In most prostate tumours the majority of cells are of luminal phenotype expressing cytokeratin K8 and being androgen receptor positive. However, particularly in metastatic prostate carcinoma, there is expression of K5, the basal cell cytokeratin; basal cells of the prostate epithelium express K5 and

CD44. Thus, it seems that the putative prostate cancer stem cell has both basal cell and prostate stem cell characteristics. The authors show their derivation of potential prostate cancer stem cells expressing K5 with the $CD44^{+}/a2b1^{hi}/CD133^{+}$ signature, and additionally transit amplifying cell populations that are $CD44^{+}$.

Normal breast luminal and myoepithelial cells are derived from a common pluripotent stem cell, but only 10–20% of adult breast epithelium is oestrogen/progesterone positive. Like prostate tumours, however, carcinomas of the human breast are predominantly steroid receptor positive. Robert Clarke (see pp. 375–386) has addressed his study of breast cancer stem cells from the starting point of the properties of normal breast stem cells. In contrast to the study on prostate cancer stem cells (Rizzo *et al.*), Clarke proposes a model in which breast cancer stem cells are steroid receptor positive, and he provides a wealth of methodological data appropriate for investigation of the genre. *In vitro* and *in vivo* studies of breast cancer stem cells, however, have yet to yield a definitive answer, although development of side population cell technology will illuminate the link between normal breast cells, normal breast stem cells, breast cancer cells and breast cancer stem cells.

In humans, the gastrointestinal tract is a common site for malignancies (although not equal in all portions), and this may be attributable to pre-malignant mutations being frequently incurred after contact with dietary toxins, combined with molecular-level diminution of control of the naturally high cell proliferation rate in the tissues. Assuming the presence of a normal adult intestinal stem cell in a basal niche, Leedham *et al.* (see pp. 387–405) describe extensive investigations concerning molecular pathways in carcinogenesis. The term ‘cancer stem cells’ here specifically relates to the ability of certain cells (not all), derived from tumours, to be able to generate new tumours when passaged on, then to develop tumour cell clones when cultured *in vitro*. The indication is that such cells are the initiators of all the tumour cell proliferation. Human hereditary gut cancer syndromes such as familial adenomatous polyposis coli are of enormous importance in the availability of human tissue with specific, predictable genetic changes for use in research. Molecules involved in biochemical pathways of stem cell activity here include Mushashi-1, which is related to the activity of Hes-1 protein of neural stem cells.

The two major primary liver tumours are hepatocellular carcinoma and cholangiocarcinoma. In normal liver, unidirectional anatomical flux of proliferating cells to terminally differentiated cells is absent and the site of putative normal liver stem cell niches is not as clear as in the gut. However, as in the gut there is an enormous background of liver tumour research and liver stem cell research to form a basis for investigation into liver cancer stem cells (see Alison and Lovell, pp. 407–421). Hepatocytes have a considerable potential to regenerate and several axes are involved in the response of the liver to damage. To experimentally elicit a histologically evident local stem cell presence, normal hepatocyte proliferation must be annulled. In such cases, a variety of types of proliferative small cells has been described. Hepatocyte differentiation from bone marrow-derived stem cells has been successful as a proof of principal rather than as a standard mechanism by which liver healing can occur. Liver stem cell location and identification, and liver cancer stem cell identity are complex. Yet, hepatocellular carcinomas at least, are largely monoclonal; the initial genetic injury or mutation that evokes hepatocellular carcinoma might be a unicellular event.

There are self-renewing neural stem cells in the central nervous system (CNS) of both children and adults. Cells of malignant tumours of the system are phenotypically heterogenous, and they insidiously invade neighbouring nervous tissues. Geoff Pilkington (see pp. 423–433) describes primary brain tumours, N-ethyl-N-nitrosurea carcinogenesis, neurosphere and tumour spheroid culture, signalling pathways in stem cell renewal and tumorigenesis, stem cell antigenic markers, ganglioside GD3 and the neural/glial2 chondroitin sulphate proteoglycan, and the

neuronal-glial proteoglycan. Of the stem cell antigenic markers, nestin, Mushashi-1, CD44 and CD133 are of particular importance. Nestin, unlike Mushashi-1, CD44 and CD133, is particularly associated with neural tissues. Of added interest, it is of importance in the developing embryonic brain, but not in the adult, and is again present in certain neural proliferating cell populations and neoplasias, including those of childhood. However, the current increase of brain tumours in our already ageing population strongly indicates that these neoplasms are the result of the burden of ever-increasing mutations, in this case to neural stem cells.

The stem cell niche, under normal circumstances is a protective environment, but as people age the inhabitants of this environment unavoidably suffer cumulative numbers of damaging mutations to their DNA, leading them over the brink to neoplasia. Themes here, running through descriptions of potential cancer stem cell populations invoke images of heterogeneous tumour cell populations, and cell populations that resemble early progenitor cells. Expression of mechanisms related to p53 control of cell division, TGFb activity, Notch, Hedgehog and Wnt signalling are commonly mentioned paramenters in cell self-renewal in both normal stem cells and cancer stem cells. The concept of the existence of side population cells (in haematopoietic cancers, neuroblastoma and others), and cells with ABC transporter properties is addressed. Therapeutic agents are well known to be rapidly expulsed by ABC transporter cells. For a novel type of therapy, the biology of cancer stem cells induces workers to investigate the possibility of experimentally adjusting overall asymmetric cell division – the property of stem cells only – to total symmetric division and to cell differentiation in tumours. In one swoop, by the second generation of cell division, there would be no more progenitor cells. This is beguiling, and some progress has been made on the control of such systems. As the existence of specific cancer stem cells becomes accepted, cancer therapies need to take into account that these cells have the properties of resistance to steroid hormone ablation, relative impunity to chemotherapeutic agents, longevity, and low population number. Cancer treatment needs to add a new aspect to its already prodigious scope – mechanisms to kill cancer stem cells.

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