

Viewpoint

Number crunching in the cancer stem cell marketMalcolm R Alison¹, Shahriar Islam¹ and Susan ML Lim²¹Centre for Diabetes and Metabolic Medicine, ICMS, Barts and The London School of Medicine and Dentistry, 4 Newark Street, London E1 2AT, UK²Department of Orthopaedic Surgery, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074, SingaporeCorresponding author: Malcolm R Alison, m.alison@qmul.ac.uk

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Breast Cancer Research 2009, **11**:302 (doi:10.1186/bcr2243)**Abstract**

Like their normal counterparts, many tumours are thought to have a hierarchical organization, albeit a disorganized one. Accordingly, the concept of cancer stem cells has emerged, and that these cells are responsible for perpetuating tumour existence. Operationally, cancer stem cells are regarded as prospectively purified cells that are the most effective at tumour initiation in an *in vivo* assay, usually after xenotransplantation to NOD/SCID mice. The conventional wisdom is that such tumour-initiating cells are rare based upon having to xenotransplant large numbers of human tumour cells into immunodeficient mice to propagate the tumour, but new evidence indicates that perhaps these cells are not so rare, at least in malignant melanoma, if a supportive soil is provided for the transplanted cells along with further restriction of the murine host's immune response.

breast and the colon [4], although the utility in colon cancer has been disputed [5].

In breast cancer cell lines, as few as 500 ALDH⁺ cells can form tumours in NOD/SCID mice [6]. From primary tumours that had cells positive not only for the familiar breast CSC signature (CD44⁺CD24⁻) but also for ALDH, as few as 20 cells were tumorigenic; on the other hand, 50,000 CD44⁺CD24⁻ALDH⁻ cells were nontumorigenic [7]. Breast cancers with cells expressing the CD44⁺CD24⁻ phenotype are most common in basal-like tumours, but not all breast cancers contain a subpopulation with this phenotype [8], and in *Brca1*-deficient mouse mammary tumours there appear to be distinct CD44⁺CD24⁻ and CD133⁺ subpopulations with stem cell properties [9] (see Figure 1). Moreover, within a tumour, cells may acquire stem cell properties through epithelial-mesenchymal transition (see Figure 1). Transformed human mammary epithelial cells with ectopic expression of the transcription factors Twist or Snail undergo epithelial-mesenchymal transition with loss of E-cadherin and gain of vimentin; remarkably, almost all of these cells had the CD44⁺CD24⁻ phenotype [10].

Phenotypic markers of cancer stem cells

The idea that cancers have malignant cancer stem cells (CSCs) is only now gaining widespread acceptance – despite the fact that, using colony formation in soft agar as a surrogate stem cell assay, Hamburger and Salmon found in 1977 that, for many human tumours, only 1 in 1,000 to 1 in 5,000 cells was able to form a macroscopic colony [1]. Selection for many of these putative CSCs is based upon expression levels of either the ABC superfamily of membrane transporters [2] or the aldehyde dehydrogenase (*ALDH*) gene superfamily encoding detoxifying enzymes for many pharmaceuticals and environmental pollutants. As such, these CSCs are typically enriched after conventional treatments to which they are resistant.

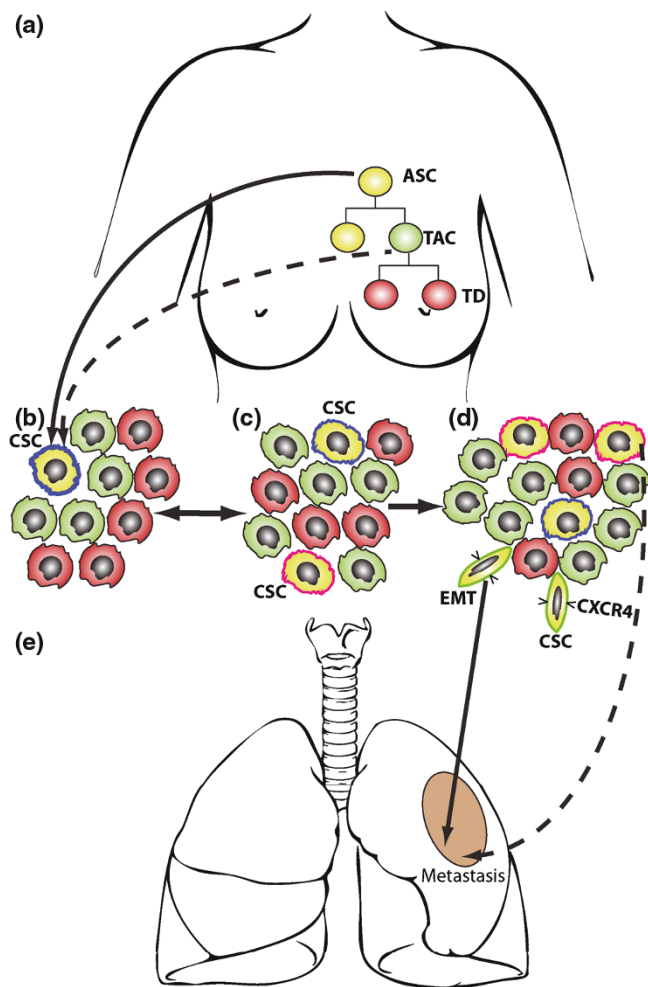
Cancer stem cells: as rare as hen's teeth or as common as muck?

Using the NOD/SCID assay, malignant melanoma initiating cells have been found to be rare amongst the unsorted tumour population (~1 in 10⁶) – but they can be enriched to about 1 in 158,000 when cells are sorted for the likely melanoma chemoresistance mediator ABCB5, a member of the ABC transporter family [11]. Optimization of the assay, however, can drastically improve the tumorigenicity of the cells down to a level where even single cells can initiate tumour growth [12]. Using the standard NOD/SCID assay, limiting dilution assays – whereby between 10⁷ down to 10² unsorted cells were transplanted – suggested that, as observed before, about 1 in 837,000 cells was a tumour-

The most popular marker of putative stem cells is seemingly Proliferating Cell Nuclear Antigen (PCNA), the first identified member of the rapidly growing proliferating cell nuclear antigen family of pentaspan membrane proteins [3], with expression restricted to plasma membrane protrusions, such as epithelial microvilli. CD133 has been used to enrich for cells with tumour-initiating ability from a variety of human solid tumours, including the brain, the prostate, the liver, the

ALDH = aldehyde dehydrogenase; CSC = cancer stem cell; TIC = tumour-initiating cell.

Figure 1



Current concepts regarding stem cells and tumour evolution in tissues with ordered structure. **(a)** In normal tissue, adult stem cells (ASC) (yellow) self-renew and give rise to transit amplifying cells (TAC) (green) that divide several times before undergoing terminal differentiation (TD) (red). Many lines of evidence, including direct lineage tracing from genetically marked ASC, indicate that tumours arise from ASC, although an origin from TAC is also possible. **(b)** Tumours also have a hierarchical structure, albeit a relatively disorganized one. The cancer stem cells (CSCs) (yellow, blue border) may have a single phenotype and be rare or relatively common. **(c)** Genetic or epigenetic events may result in new clones driven by phenotypically diverse populations of CSCs. **(d)** Further genetic or epigenetic changes may result in some cells undergoing epithelial–mesenchymal transition (EMT), equipping them with CSC properties. **(e)** Metastasis may be caused by migrating CSCs detaching from the tumour mass; in particular, these may be the CSCs formed through EMT that may respond to chemotactic gradients by virtue of expression of chemokine receptors such as CXCR4.

initiating cell (TIC). Palpable tumours appeared earlier and grew faster when the transplanted cells were supported by Matrigel; when similarly prepared cells from primary tumours were transplanted to NOD/SCID *Il2r γ ^{-/-}* mice, one in four cells was estimated to be a TIC. These recipient mice clearly

represent a more permissive immune microenvironment, in particular lacking natural killer cell activity. Likewise, 254 single-cell transplantations derived from four patients yielded 69 tumours (a take rate of 27%), again demonstrating a fantastic improvement in tumorigenicity with this new model.

Refining the cancer stem cell hypothesis: not one cap fits all

So is the CSC hypothesis dead or merely in need of modification? We would argue for the latter. Particularly in epithelial tissues with ordered structure, such as the epidermis, there is no doubt that many cells are undergoing terminal differentiation – in fact, all of the suprabasal cells. Likewise, in epidermal squamous cell carcinomas – particularly well-differentiated ones – many cells are reproductively sterile; for example, the cells comprising the pearls of keratinized squames – clearly less than one in four of these tumour cells are TICs.

So are malignant melanomas unrepresentative of the majority of tumours? Perhaps in some respects they are; for example, metastases typically occur very early in tumour development, melanomas are one of the few tumours where the isolated cells are highly resistant to apoptosis (anoikis) [13], and, with respect to epithelial mesenchymal transition, melanocytes developmentally upregulate transcription factors such as Slug during migration from the neural crest. On the other hand, melanocytes can divide and are renewed from the hair follicle bulge region, and are responsive to differentiating influences [14].

Despite the caveats regarding malignant melanoma, this new study alerts us to the fact that the NOD/SCID assay merely identifies cells able to form tumours in a hostile murine environment, and that many factors conspire to vastly underestimate the frequency of TICs. These include the absence of a niche for the transplanted cells (partially mimicked by Matrigel), and the recipient's residual immune system. These assertions are underscored by the fact that as few as 10 mouse lymphoma or acute myeloid leukaemia cells can regularly propagate tumours when transplanted into histocompatible mice [15]. So are all cells in these tumours possible TICs?

The rarity of cancer stem cells has also been questioned in mouse mammary cancer. Using up to a dozen murine mammary cancer cell lines, cell colonies could be regularly generated from randomly selected single cells; and when 2×10^5 cells from these clonally derived colonies were allografted into histocompatible mice, tumours were consistently produced – suggesting that perhaps the TICs do not have a unique surface marker signature [16]. On the other hand, cell sorting of heterogeneous mammary tumour cells from p53-null Balb/c mice has identified a distinct subset of CD29^{high}CD24^{high}Lin⁻ cells that were highly enriched for TICs when transplanted into the cleared fat pads of syngeneic wild-type Balb/c mice [17].

Limiting dilution transplantation experiments certainly supported the CSC hypothesis, and the small numbers of cells transplanted (100 cells) to obtain heterogeneous tumours indicated that perhaps the TICs were at least bipotential. The question of whether tumour heterogeneity is due to distinct clones from different CSCs or whether CSCs, like their normal counterparts, are multipotential is of fundamental importance. This question has been answered in colorectal cancer; clonal populations derived from a colorectal cancer cell line and from primary colorectal cancer can subsequently recapitulate the heterogeneity of the original tumours when transplanted in nude mice, exhibiting enterocytic, neuroendocrine and goblet cell differentiation – all from a single cell [18,19].

Conclusions

The CSC hypothesis is not dead, but it needs refining to accommodate the likelihood that the frequency of CSCs may vary from tumour to tumour and is likely to change during tumour progression by both epithelial–mesenchymal transition as well as by the symmetric cell division of CSCs themselves.

Competing interests

The authors declare that they have no competing interests.

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