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Review

Cancer stem cells: Back to Darwin?

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ABSTRACT

Current models of cancer propagation or 'stem' cells pay scant attention to the evolutionary dynamics of cancer or to the underlying genetic, mutational drivers. Recent genetic studies on acute lymphoblastic leukaemia at the single cell level reveal a complex non-linear, branching clonal architecture—with sub-clones having distinctive genetic signatures. Most cancers appropriately interrogated are found to have intra-clonal genetic heterogeneity indicative of divergent clonal evolution. These data further suggest that clonal architecture might be driven by genetic heterogeneity of propagating or 'stem' cells. When assayed for leukaemic regeneration in NOD/SCID/γ mice, genetically diverse 'stem' cells read-out, broadly reflecting the clonal architecture. This has suggested a 'back to Darwin' model for cancer propagation. In this, cells with self-renewal potency or 'stem-ness' provide genetically diverse units of evolutionary selection in cancer progression. The model has significant implications for targeted cancer therapy.

1. Cancer stem cells: now you see them, now you do not

The concept of cancer stem cells (CSCs) has sparked excitement and controversy in equal measure. The arguments touch on fundamental issues of cancer biology but also have potentially critical implications for therapy. The history of the idea has been chronicled elsewhere [1]: suffice to say that the development of the NOD/SCID *in vivo* assay for human leukaemic stem cells by John Dick and colleagues [2,3] resurrected a stalled debate and sparked the current explosion of interest.

The concept itself is deceptively simple, namely that cancer cells in individual patients are phenotypically heterogeneous and only a subset has the competence to propagate long term or to sustain the disease. It follows that these same cells are important targets for therapy [4–6]. The first problem comes from the fact that even if the underlying premise is correct, there are several possible biological bases for segregated propagating ability (Fig. 1). The relevant biological property is self-renewal and strictly speaking, this should be self-renewal coupled with extensive or indefinite replicative potential rather than short term or modest level propagation [7]. Cancer propagating ability has been attributed to:

- a fixed, hierarchically positioned subset of stem cells mirroring normal stem cell hierarchies (in haemopoiesis) [3];
- a non-deterministic or stochastic process with plasticity of 'stem-ness' [8];
- activity of a genetically dominant sub-clone [9–11].

These are often discussed as alternatives but they need not be [12–14]. Normal cells vary in extent of self-renewing potential and expression of stem-ness or self-renewal will be dependent upon context, e.g. niche occupancy, competition, regenerative demand, etc. [15–18] and it is to be expected that cancer propagating cells would be subject to some variation in potency *and* functional expression whatever model is preferred.

Against this background, the controversies have centred on whether CSCs are numerically very rare [3] or very common [19]—to the point where the concept itself is seriously challenged, at least for some cancers [13,20] and whether CSCs have distinctive, fixed phenotypic properties in terms of immunophenotype quiescence/proliferative activity and sensitivity to genotoxic damage [21,22]. As always in biology, much depends upon the assays and here we have another problem. It is akin to the familiar conundrum facing physicists—an uncertainty prevails on the enumeration of stem cells because any functional assay might corrupt, bias or alter the very property of interest. Some authors point to the advantage of syngeneic murine assays for leukaemia stem cells [23]. These have provided examples where a very large fraction of cancer cells appear to have self-renewing or stem cell activity and they clearly indicate that stem-ness need not necessarily be an exclusive character of rare cells. But these models are themselves very contrived or artificial in terms of genetic background, choice of leukaemia genes and uncertain selective pressures *in vivo* or associated with re-transplantation. How such models relate, if at all, to 'real' leukaemia, in patients, is uncertain. By the same

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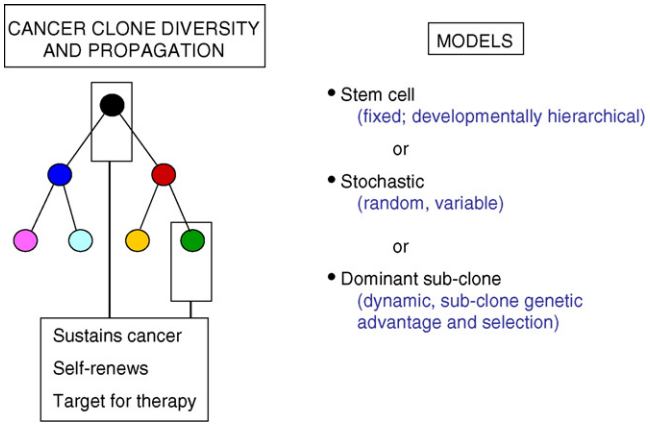

Fig. 1. Alternative models for cancer propagating or 'stem' cells.

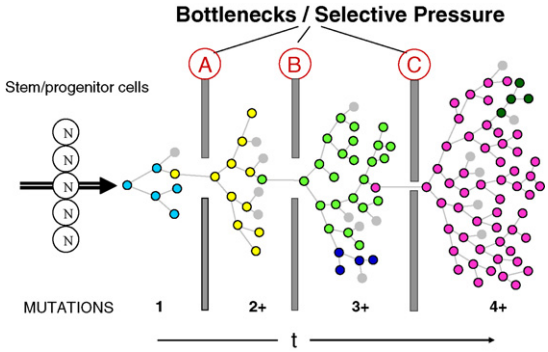

Fig. 2. Clonal evolution of a cancer. N: normal cells; A and B: intracellular and intercellular/microenvironmental constraints; C: decimation by therapy (equivalent to catastrophic environmental change in speciation); t: time. Different coloured cells: distinctive mutant genotypes. Modified after Nowell [25].

token, the NOD/SCID or NOD/SCID/γ assays for human cancer are likely to underestimate stem cells. They are nevertheless assays for stem-ness in clinical samples and at present are the best we have available. Further modification of the *in vivo* assay and use of melanoma cells rather than leukaemia produces what appears to be a very different result—namely a very high fraction of propagating cells [19]. But this does not necessarily contradict the AML data [12]; it is perfectly possible, and indeed likely, that the preponderance of propagating or stem cells in any clinical sample varies markedly with cancer subtype and stage of disease [14,24].

2. The missing link?

A particular anomaly in the cancer stem cell debate is that much of the underlying genetics of cancer tends to be ignored. Cancer development is fundamentally a dynamic, Darwinian process of mutational diversification and clonal selection [25–28]. In this context, mutant cells with self-renewal or 'stem' cells could well be the crucial units of selection. But, in this context, they simply cannot be a fixed entity. They can be anticipated to differ in frequency and in phenotypic properties in concert with progression of disease and cancer subtype. The underlying mutational landscape of any cancer will determine frequency and properties of CSC but, rather extraordinarily, cancer genetics is rarely considered in the CSC debate. It may well be that different oncogenes or different sets of mutations can convert different normal stem or progenitor cells to varying degrees of long term self-renewal competence [29,30]. But the genetic perspective that is missing from the debate is something else: *intra-clonal genetic heterogeneity*.

Intra-clonal genetic diversity is a fundamental and hallmark feature of cancer and provides the substrate for Darwinian selection of sub-clones through major bottlenecks and progressive evolution of disease [25–28] (Fig. 2). This has been recognised for a long time though the timing and sequence of these events have only more recently been illuminated.

Once this basic perspective on cancer biology is grasped, two predictions highly relevant to the CSC debate suggest themselves. First, that the CSCs in the earliest stages of leukaemia or cancer are highly likely to be very different in genotype, number and phenotype from those in advanced disease. This should be generally true of cancer and several authors have speculated that CSCs are highly likely to evolve in concert with disease progression [14,31–34]. We have previously provided evidence for this in childhood acute lymphoblastic leukaemia (ALL) with *ETV6–RUNX1* fusion, exploiting both the unusual situation of ALL in monozygotic twins and modelling with human cord blood cells [35]. These data indicated that the *ETV6–RUNX1* driven 'pre-leukaemic' stem cell was distinct from the overt leukaemic stem cell in genotype (IgH rearrangement status), frequency and phenotype (Fig. 3). Second, that as Darwinian selection through bottlenecks requires genetic diver-

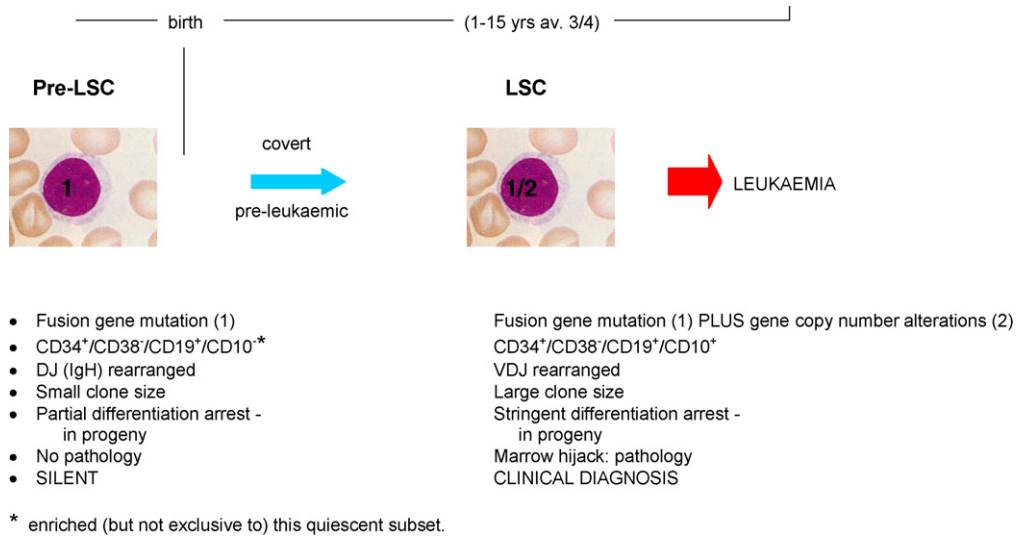

Fig. 3. Sequential, evolutionary development of stem cells in *ETV6–RUNX1*+ acute lymphoblastic leukaemia. Data taken from Hong et al. [35]. *LSC enriched in (but not exclusive to) this quiescent subset.