Cell Stem Cell
Previews

Molecular Targeting of Cancer Stem Cells

Rajeev Gupta,[1] Paresh Vyas,[1] and Tariq Enver[1,*]
[1]MRC Molecular Haematology Unit, The Weatherall Institue of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DS, UK
*Correspondence: tariq.enver@imm.ox.ac.uk
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Cancer stem cells may be important targets for new anticancer drugs. In two recent articles in *Cell Stem Cell*, Jin et al. (2009) and Hoey et al. (2009) provide proof of principle for this idea in experimental models of solid tumors and leukemias, respectively.

The development of disease-specific anticancer drugs is advancing the treatment of many malignancies (Weiner et al., 2009). These new therapeutics have been designed to target proteins expressed by tumor cells and are thought to be equally toxic to all cells within an individual cancer. Their specificity for malignant cells and hence toxicity to normal tissues varies from tumor to tumor.

However, all cells within a cancer may not be functionally equivalent. Evidence for tumor cell heterogeneity emerged in the 1960s (e.g., Bruce and van der Gaag, 1963) and was crystallized in the 1990s by the studies of Dick and others (Dick, 2008). The hypothesis of "tumor-propagating" or "cancer stem" cells (CSCs) suggests that tumor growth is maintained by a subpopulation of cells exhibiting the cardinal stem cell properties of self-renewal and a capacity for differentiation. CSCs are thought to sit at the apex of a cellular hierarchy within a tumor and may be responsible for disease initiation and for relapse. By analogy with normal hematopoietic stem cells, they are predicted to be relatively quiescent and resistant to conventional chemotherapy. Thus, CSCs are the cells to target for the efficient treatment of cancer.

Human CSCs were originally characterized by a combination of their immunophenotype and ability to reconstitute whole tumors after serial xenotransplantation into sublethally irradiated immunodeficient NOD/SCID mice. They were thought to be rare cells, but studies with recipient hosts lacking NK cell activity (e.g., NOD/SCID IL2Rγnull) suggested that cells with CSC potential exist at much higher frequencies in many malignancies, with a range of immunophenotypes (Quintana et al., 2008; LeViseur et al., 2008). Additionally, for leukemias at least, there is variability in the capacities of individual bulk tumors to reconstitute the disease. Although such observations may merely expose the immunological limitations of xenografting, another explanation could be that CSCs are heterogeneous with varying intrinsic capacities for self-renewal and differentiation.

Such experimental complexities and the apparent heterogeneity of cells with CSC properties have hindered the identification of specific markers that reliably identify the CSC subset. Nevertheless, the capacity of CSCs to replicate the heterogeneity of tumors provides an important preclinical model in which new therapies can be tested (Jordan, 2009).

Two recent papers in *Cell Stem Cell* provide important experimental evidence for the merit of CSC targeting. In this issue, Hoey et al. (2009) demonstrate a reduction of CSC function by an inhibitor of notch activity. The authors developed a neutralizing antibody against delta like 4 ligand (DLL4), a membrane-associated notch ligand. The notch pathway has been implicated in intestinal cell homeostasis (Radtke and Clevers, 2005), and many cancers express DLL4. In a NOD/SCID model of human colon cancer, they show that administration of anti-DLL4 reduces tumor growth in recipient mice and reduces tumor engraftment in secondary recipients, implying an impact on CSC self-renewal. The authors also raise the possibility of using their antibody in combination with a widely used chemotherapeutic, irinotecan.

The second paper in *Cell Stem Cell* (in the July issue) by Jin et al. (2009) describes immunotherapy targeting acute myeloid leukemia stem cells (AML-LSCs) isolated from clinical samples. The authors exploit the observation that AML-LSCs (defined by NOD/SCID engraftment) but not normal hematopoietic stem cells exhibit high expression of the IL3 receptor (CD123). Administration of the CD123 antibody 7G3 inhibits the engraftment of AML-LSCs in NOD/SCID mice. The authors find that secondary transplantation of AML is diminished if the primary recipients have also received 7G3, suggesting that the self-renewal potential of AML-LSCs is impacted. They also report that 7G3 has little effect on normal hematopoietic stem cell activity, implying some specificity for AML-LSC self-renewal. In analogous experiments to those of Hoey et al. (2009), the authors observe synergism of 7G3 when used in combination with cytosine arabinoside. Interestingly, the effects of 7G3 are attenuated when the assays are performed in NOD/SCID IL2Rγnull recipients. This observation may reflect heterogeneity in the capacity of cells within the AML-LSC compartment for tumor propagation and self-renewal. Alternatively, or in addition, it may indicate a dependence of the 7G3 effect on extrinsic NK activity in xenograft models.

These studies establish the principle that the self-renewal capacities inherent in CSCs can be targeted with therapeutic intent by monoclonal antibodies. They also demonstrate that such antibodies synergize with standard drugs. Although it is to be hoped that these exciting findings make their way to the bedside, important questions remain unanswered.

Just what the CSC hypothesis means for cancer medicine is unclear. The field is in its infancy and correlations between CSC activity and clinical outcome are the focus of much interest. The main clinical problem is disease relapse after initial responses to treatment. Most CSCs have been isolated from untreated clinical samples. Perhaps the pertinent therapeutic targets are those cells that

remain after (or have been selected by) treatment.

A prevailing view of carcinogenesis is of a stepwise accumulation of transforming genetic changes that arise in the progeny of an initially normal cell that has been exposed to some mutagenizing event. This "linear progression" of sequentially more mutated progeny is thought to culminate in a cell capable of sustaining the cancer (the CSC).

However, the variability in tumor-initiating capacities observed when the various cellular compartments of pretreatment cancer tissues are tested in different mouse recipients could imply that cells capable of sustaining cancer are heterogeneous and that multiple subclones capable of tumor initiation arise in parallel (as opposed to a strict linear evolution) and compete for dominance in a Darwinian fashion. Molecular genetic evidence for such heterogeneity has come from the study of lymphoid malignancies where global sequencing exposes molecular variability within individual tumors, suggesting that they are comprised of interrelated subclones derived from common ancestors (Campbell et al., 2008). Those clones that win the initial battle of selection during carcinogenesis may not be the ones that survive/are selected by chemotherapy and which drive relapse.

Circumstantial evidence for the emergence of relapse propagating clones (RPCs) has come from single-nucleotide polymorphism studies of paired diagnostic and relapse samples from patients with childhood leukemia (Mullighan et al., 2008). Here, the genetic abnormalities that were dominant at the time of disease relapse often differed from those detected at presentation, when they represented a minor component of the disease.

The functional and molecular characterization of RPCs would be subject to the same limitations as for CSCs and would additionally require diligent archiving of matched diagnostic, remission, and relapse material.

This notion serves to underline the fact that patients represent the best "test tubes" for such work and may point the way forward for this fascinating field.

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Unraveling the Human Embryonic Stem Cell Phosphoproteome

Andrew P. Hutchins[1] and Paul Robson[1,2,*]
[1]Stem Cell & Developmental Biology, Genome Institute of Singapore, Singapore 138672, Singapore
[2]Department of Biological Sciences, National University of Singapore, Singapore 117543, Singapore
*Correspondence: robsonp@gis.a-star.edu.sg
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The human embryonic stem cell (hESC) transcriptome is well described, but minimal proteome characterization is available. In this issue of *Cell Stem Cell*, Brill et al. (2009) and Van Hoof et al. (2009) describe the hESC phosphoproteome and its changes upon differentiation.

Human embryonic stem cells (hESCs) are capable of differentiation into all lineages of the body, but directed differentiation to pure populations of cells has proven difficult to accomplish. Ideally, hESCs could be coerced to a particular lineage by making a series of changes to their culture environment. These changes could be mediated via the application of growth factors, small molecules, and other effectors of cellular response, resulting ultimately in a signaling cascade(s) that directs differentiation along a particular path. Major transducers of these environmental stimuli are the protein kinases, which transfer information by adding phosphate groups to Ser, Thr, and Tyr residues of proteins to create differences in the biochemical properties of their targets and thus their binding affinities (Pawson and Nash, 2003).