Cancer Stem Cells: From Identification To Eradication

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RATIONALE

A fundamental problem in cancer research is identification of the cells within a tumor that sustain the growth of the neoplastic clone. The concept that only a subpopulation of rare cancer stem cells (CSCs) is responsible for maintenance of the neoplasm emerged nearly 50 years ago; however, conclusive proof for the existence of a CSC was obtained only relatively recently.

As definition, cancer stem cells (CSCs) are a sub-population of cancer cells (found within solid tumors or hematological malignancies) that possess characteristics normally associated with stem cells as high self-renewal potential. These cells are believed to be tumorigenic (tumor-forming) in contrast to the bulk of cancer cells, which are thought to be non-tumorigenic. The first conclusive evidence for CSCs was published in 1997 in Nature Medicine by Bonnet & Dick who isolated a subpopulation of leukemic cells in AML that express a specific surface marker CD34 but lacks the CD38 marker. The authors established that the CD34+/CD38− subpopulation is capable of initiating leukemia in NOD/SCID mice that is histologically similar to the donor [1]. This subpopulation of cells is termed SCID Leukemia-initiating cells (SL-IC).

A theory suggests that such cells act as a reservoir for disease recurrence, are the origin of metastasis and exert resistance towards classical antitumor regimens. This resistance was attributed to a combination of several factors [2], suggesting that conventional antitumor regimens are targeting the bulk of the tumor not the dormant stubborn CSCs.

PURPOSE

Better understanding of the leukemogenic process and the biology of CSCs to define the most applicable procedures for their identification and isolation in order to design specific targeted therapies aiming at reducing disease burden to very low levels up to eradication of the tumor.

IDENTIFICATION OF CSCs

- **Functional relationship between normal stem cells and CSCs** [2]:
  - Self renewal capacity.
  - Hierarchically organized "life-long renewal after division".
  - Development of specialized microenvironment "niche" to support self renewal.
  - Signaling pathways responsible for cell division.

- **Implications of the CSCs Hypothesis.**

Only a very small restricted population of tumor cells sharing cell surface phenotype of normal human hematopoietic stem and progenitor cells has the capacity to engraft in NOD/SCID mice [3,4] and induce malignancy. Populations from haemopoietic malignancies have been identified and summarized in Table (1).

Why do CSCs exert resistance towards Chemotherapeutic Agents?:

There are several criteria attributed to such resistance of CSCs (Fig. 2-A,B):
- Entrance into a long-term dormant state.
- Dormant CSCs are concealed in hypoxic vascular niche.
- CSCs highly express multidrug resistance - ABC transporters.
- CSCs efficiently repair DNA.
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Fig. (1): Cell-surface markers and therapeutic targets of myeloid leukemia stem cells:

Right: Diagram of a normal HSC, which expresses the cell surface markers CD34, CD90 and CD117 [5]. Some of the signaling pathways known to promote expansion and self-renewal of HSCs include Wnt-frizzled-ß-catenin [6], Jagged-Notch [7], Hedgehog-Patched-Smootherned-Gli [8], and HoxA9/HoxB4 [9,10]. The polycomb group protein Bmi-1 promotes HSC maintenance through inhibition of the cell cycle and apoptosis regulators p16 and ARF [11].

Left: Diagram of a hypothetical myeloid LSC, some of which can express the cell surface markers CD33 [12], CD123 [13] and CLL-1 [14]. Potential agents and strategies for the eradication of LSCs are depicted in red and include antibodies and fusion proteins as well as small-molecule inhibitors.

Table (1): Molecular Markers of Normal and Malignant Hematopoietic Cells.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Description</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell surface markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34</td>
<td>Sialomucin transmembrane protein</td>
<td>Unknown cell adhesion molecule</td>
</tr>
<tr>
<td>CD38</td>
<td>Transmembrane protein with cyclic ADP ribose hydrolase ectoenzyme activity</td>
<td>Positive and negative regulator of cell proliferation; cell adhesion molecule</td>
</tr>
<tr>
<td>CD44</td>
<td>Receptor for hyaluronan and osteopontin; increased on BCR-ABL LSC</td>
<td>Required for homing/engraftment of CML LSC; negative regulator of AML LSC self renewal</td>
</tr>
<tr>
<td>CD123</td>
<td>Interleukin -3 receptor α-chain</td>
<td>Survival and proliferation signaling in normal cells; no known role in LSC</td>
</tr>
<tr>
<td><strong>Intracellular markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bmi-1</td>
<td>Polycomb group transcription factor</td>
<td>Regulates self renewal of HSC and LSC through repression of p16</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>Ligand for Patched Gli, a transmembrane receptor</td>
<td>Proliferation signaling in normal HSC</td>
</tr>
<tr>
<td>HoxA9</td>
<td>Homeodomain transcription factor</td>
<td>Proliferation of HSC; over expression in AML.</td>
</tr>
<tr>
<td>HoxB4</td>
<td>Homeodomain transcription factor</td>
<td>Proliferation of HSC</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa β; transcription factor activated by inflammation and lymphoid development</td>
<td>Active in LSC but not HSC; role in LSC unknown but survival factor</td>
</tr>
<tr>
<td>Notch</td>
<td>Transmembrane receptor/transcription factor activated by proteolysis following ligand (jagged) binding</td>
<td>Proliferation signaling in normal HSC and many T-cell Leukemias</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and Tension homolog; lipid phosphatase</td>
<td>Antagonist of PI3K; regulates HSC self-renewal; leukemia suppressor</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol 3Kinase; lipid kinase</td>
<td>Activated in AML LSC; proliferation and survival signaling</td>
</tr>
<tr>
<td>Wnt</td>
<td>Ligand for Frizzled receptor, mediates stabilization and activation of ß-catenin</td>
<td>Proliferation signaling in normal HSC</td>
</tr>
</tbody>
</table>
Procedures should be performed after an informed written consent from the patient and approval from the Institutional Review Board.

**Hematological malignancies:**

Peripheral blood or bone marrow → **LSCs**

- CD34+, 38–
- isolated by magnetic beads

→ **Culture in methyl cellulose**

- + RPMI
- + Penicillin, Streptomycin
- + 10% FCS
- + L-glutamine
- + 1% BSA
- + EPO
- + GM - CSF
- + IL3
- + SCF

**Solid tumors:**

Tumor tissue → **CSCs**

- CD44+, 24–
- isolated by magnetic beads

→ **Culture in growth factor cocktail**

- + EGF
- + bFGF
- + FBS
- + LIF

**What technologies could we apply now and what technologies need further development?:**

**NCI workshop 2006 for detection and characterization of CSCs** [15]:

1. The magnetic activated cell sorting system (MACS) developed by Miltenyi, et al. [16] uses biodegradable beads. Griwatz, et al. [17] used MACS to enrich PB for breast carcinoma cell line cells in an experimental model using monoclonal mouse anti-cytokeratin. Combining MACS followed by RT-PCR proved to give encouraging results to detect multiple tumor types [18,19].
2- Immunocytochemistry (ICC) following ficoll-hypaque density centrifugation [20] in most of the studies on epithelial cancers for the detection of CTCs “circulating tumor cells”. Since some studies have suggested that the CTCs will not always be found in the mononuclear cell fraction after ficoll-hypaque density centrifugation, this needs to be taken into consideration when CTC detection methods are being developed.

3- Detection of tumor cells by FACS has been extensively used to detect CTCs in epithelial cancer such as breast cancer [20-22], but less sensitive than standard ICC [23,24].

4- Immunomagnetic beads and other bead-based detection methods for CTC in PB and DTC in the BM [25]. Recently-developed enrichment techniques enable the use of larger sample sizes, exceeding 100 million cells, thereby increasing the sensitivity and number of detected tumor cells. Different types of enrichment methods have been used [26,27], such as immunomagnetic beads as an enrichment procedure for both hematopoietic progenitor cells and tumor cells [20,28]. The beads coated, with monoclonal antibody, bind to the cells of interest. By the use of a magnet, the cells can be selected (direct method) or cells of non-interest removed (indirect method). When compared to ICC, immunomagnetic bead enrichment can provide a multi-fold increase in detected tumor cells. Oftentimes, enrichment has been followed by ICC.

5- Newly developed epithelial immunospot (Epispot) technique has been used successfully to identify secreted products of CTC/DTC from breast and prostate cancer patients [29,30].

Development of specific therapies targeting CSCs (Box 1) [31]:

- Promotion of their cell cycle using cytokines. Cytokines are known to activate normal stem cells. This activation has been achieved by imatinib treatment of intermittently G-CSF activated CML-CSCs in vitro resulting in enhanced efficacy of CML-CSCs elimination compared to treatment with imatinib alone [2,32] (Fig. 2).

- Inactivation of the hypoxia inducible factor 1 α (HIF-1α).

  Given that hypoxia seems to be important in maintaining CSCs this provides an opportunity for tumor – selective therapy (Fig. 2) [33,34].

- Depletion of blood vessels by ANTI-VEGF therapy.

  The combination of antiangiogenic drugs and conventional therapies could be highly cooperative, a hypothesis that they affect their vascular niche (Fig. 2) [35].

- Anti-CD 44 monoclonal antibody therapy might separate CSCs from their microenvironment.

  CD44 is expressed by both normal and leukemic stem cells. It mediates adhesive interactions with the endostal bone marrow niche by binding to various ligands including osteoponin and hyaluronic acid [36]. It effectively eliminated AML stem cells from some but not all patients [37,38].

- Inhibition of ABC TRANSPORTER activity.

  The multiple drug resistance (MDR) genes belong to a family of approximately 50 human ABC (ATP Binding Cassette) transporters [39]. The MDR proteins employ ATP hydrolysis to actively efflux drugs from cells, thereby protecting them from cytotoxic agents [39,40]. The four principal MDR genes identified in tumors are ABCB1 (or p-glycoprotein), ABCG2 (breast cancer resistance protein 1, BCRP1), ABCC1 and ABCA3. A number of inhibitors specific for ABCB1 have been identified including Verapamil, Cyclosporine and the most potent compounds PSC833 and VX710 [40,41]. Unfortunately their use in clinical trials has yielded largely negative results, one reason could be that other transporters particularly ABCG2 also need to be efficiently inhibited to achieve significant
effect in the clinic. Third generation inhibitors such as Tariquidar effective against ABCB1 and ABCG2 are currently tested but some phase III trials were terminated early owing to an increased incidence of side effects [42].

- Pharmacological inhibition of cell cycle checkpoints that allow DNA repair:
  
  Inhibition of Chk1/Chk2 kinase renders the gliomas less resistant to radiation [43].

SUMMARY AND FUTURE DIRECTIONS

BOX (1) [31]:

Gemtuzumab,ozogamicin (Mylotarg1).
A Diptheria toxin-IL-3 fusion protein.
Anti-VLA-4 antibodies.
Anti-CLL-1 antibodies.
Anti-CD44 monoclonal antibodies (clones H90 and A3D8).
G-Secretase Inhibitor.
The Proteasome - Inhibitor MG-132 Bortezomib.
Inhibition of IκB kinase (IKK) Parthenolide.
P3K-Akt (m-TOR) pathway.
ATRA or Arsenic Trioxide.
Imatinib, Dasatinib, Nilotinib.

- The era of targeted therapy for leukemia and solid tumors has arrived. The targeted eradication of CSCs which might be essential for permanent cure is a much more challenging task. Owing to the efforts of many different laboratories, we are beginning to understand the properties of CSCs and how they differ from normal HSCs.

- Going forward, we should expect biochemical and genetic studies of CSCs derived from human samples and mouse models to contribute additional rational targets for specific therapy towards their elimination.

- We should appreciate that the CSCs from different cancers, leukemia and solid tumors, might be different in their phenotypic properties and self-renewal pathways and these will need to be defined for each disease. Because of the close similarities between normal stem cells and CSCs we should not expect too many of these "magic bullets" and when CSC-specific therapy finds its way to the clinic, it is probable that a careful balance of targeted and cytotoxic therapies will be necessary to maximize LSC killing and minimize toxicity.

- This, in turn, will require robust methods to monitor CSCs frequency and MRD in clinical samples with the identification of leukemic progenitors as an important step forward.

BOX (2): Abbreviations.

Acute myeloid leukemia "AML"
All-Trans Retinoic Acid "ATRA"
B Fibroblastic Growth Factor "bFGF"
Cancer stem cells "CSCs"
Chronic myeloid leukemia "CML"
Epithelial Growth Factor "EGF"
Erythropoietin "EPO"
Extracellular matrix "ECM"
Fetal Bovine Serum "FBS"
Granulocyte monocytic colony stimulating factor "GM-CSF"
Hematopoietic stem cell "HSC"
Hypoxia-inducible factor 1 alfa "HIF1α"
Immunocytochemistry "ICC"
Interleukin-3 "IL-3"
Leukemia Inhibitory Factor "LIF"
Leukemic stem cells "LSC"
Magnetic activated cell sorting system "MACS"
Mammalian Target of Rapamy cin "m-TOR"
Minimal residual disease "MRD"
Multiple drug resistance "MDR"
Non-obese diabetic/severe combined immunodeficiency "NOD/SCID"
Reactive oxygen species "ROS"
Stem cell factor "SCF"

REFERENCES


