Cancer Stem Cells:
Getting to the root of Cancer

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Overview

• Evidence for the existence of Cancer Stem Cells in Acute Myeloid Leukaemia (AML).

• Current implications of Leukemic Stem Cell (LSC) for therapy

• Considerations for targeting LSCs

• Future directions
The “Hematopoietic” (Blood) System

Stem cell assay?

CD34  CD38

HSC

CMP

CLP

ELTC-IC

LTC-IC

CFC

Megakaryocytes

Monocytes

Neutrophils

Eosinophils

Basophils

Erythrocytes

Granulocytes

T cells  NK B cells

Lymphocytes
Gold Standard for Hematopoietic Stem Cells

Hematopoietic stem cells are assayed \textit{in vivo} by their ability to repopulate the entire blood system

- Capable of extensive proliferation and multilineage differentiation
- Capable of self-renewal
Background of Acute Myeloid Leukemia (AML)

- Heterogeneous disease characterized by the overproduction of leukemic blasts.

- Leukemic blasts have a short life-time and thus needed to be replenish by a subset of self-renewing cells.

- No good *in-vitro* model to study AML progenitor / stem cells.
MAJOR ISSUES

✓ Which leukemic cells is capable of initiating and maintaining the disease?
  - need functional quantitative assay

✓ Which normal cell(s) does LSC originate from?
  - need to purify LSC and functionally and phenotypically compared Normal and LSCs
NOD/SCID model for AML

Peripheral Blood or BM of AML patients

375 cGy irradiation

Tail vein injection into NOD/SCID mouse

6-12 weeks ± GF injections

Unpurified mononuclear cells or Purified cells expressing primitive surface phenotype

Ficoll Gradient

Southern Blotting to quantify percentage of human cells in mouse BM

FACS analysis to phenotype Human cell present in mouse BM

• Model that faithfully reproduces the AML disease in mice
• Enables characterisation of engrafted cells
Confirmation of AML: Morphology

Original AML-M2

NOD/SCID Marrow after 6 weeks

Sorted as Human CD45+

“Auer Rod”
NOD/SCID model for leukemia

- AML shares similar morphology, phenotype and FISH abnormalities as original sample

- Similar profile of gene expression before and after engraftment

LIMITATION: Only 60-70% AMLs engraft in NOD/SCID
Engraftment in NOD/SCID assay correlates with karyotypically defined prognostic group

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>Engrafter</th>
<th>Non-engrafter</th>
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<tbody>
<tr>
<td>Good risk</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>13</td>
<td>14</td>
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<tr>
<td>Poor risk</td>
<td>8</td>
<td>0</td>
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Overall survival of AML patients with intermediate risk cytogenetics (<60 years) with respect to engraftment observed in NOD/SCID mice

\[ \chi^2_1 = 4.71 \]

\[ P = 0.0300 \]

*Censored at allograft
Model of Tumor Cell Proliferation

Stochastic model

Cancer Stem Cell model

Tumor growth

No Tumor

Tumor growth

Tumor growth

Tumor growth

Tumor growth
Identification of Leukemic Stem Cells

Leukemia formation

Leukemia

No Leukemia
Leukemia is arranged as a hierarchy similar to normal hematopoiesis.

Leukemia events lead to the formation of bulk leukemia cells, which block terminal differentiation.

Dominique Bonnet and John Dick
Nat Med. 1997
What is the significance of AML-ICs?
What is the significance of LSC?

Despite years of research and therapeutic advancement, the majority of patients succumb to the disease.

Elimination of the LSC is crucial to obtain cure.

Nevertheless, most of the new therapeutic drugs are being develop against the bulk blast population not against the leukemic stem cells.
Considerations in targeting LSC

• Effectively target AML-ICs while selectively sparing normal HSC function.

• Target potential biological differences between LSC and normal HSC:
  - Surface phenotype
  - Self-renewal mechanisms.
  - Interfered with the LSC microenvironment
Antigen expression on LSC in AML

- **Normal HSC phenotype** –
  - CD34+
  - CD38- 
  - CD123- 
  - HLADR- 
  - CD33+ 
  - CLL-1- ? 
  - CD90+ 
  - CD96- ? 

- **AML HSC phenotype** –
  - CD34+ 
  - CD38- 
  - CD123+ ? 
  - HLADR- 
  - CD33+ 
  - CLL-1+ ? 
  - CD90 - 
  - CD33 + ? 
  - CD96 + ?
Immunotherapy of AML LSC

Normal progeny

Leukemic progeny

HSC

LSC
Immunotherapy of AML LSC

HSC

Normal progeny

Leukemic progeny

LSC
Immunotherapy of AML LSC

HSC

Normal progeny

LSC

Leukemic progeny
Immunotherapy of AML LSC

HSC

Eradication of disease

Normal progeny
Mechanisms of self-renewal in AML-IC

- Proposed mechanisms of self-renewal in AML-ICs.
  - Wnt/β-Catenin
  - Notch
  - BMI-1
  - Shh
  - HOX genes

- **ALL** ALSO IMPLICATED IN NORMAL STEM CELL SELF-RENEWAL

Need to Identify self-renewal pathways preferentially utilized by LSC
The stem cell niche model

Bone marrow

Sinusoids

Bone

Endosteum

Reticular cells

(SDF-1 rich)

Pericytes

Megakaryocytes

Hematopoietic Stem Cell
HSC

Osteoblastic niche

Vascular niche

ECM

SDF-1

G₀

Ca++

ECM

SDF-1

G₂/M

O₂
Mechanisms that regulate LSCs?
Conclusions

• The leukemia stem cell (LSC) is the critical target in AML therapy.

• A further understanding of the biology of both the LSC and the normal HSC is required.

• Target pathways preferentially utilized by LSC.

• Growing body of evidence that differences in biology between LSC and HSC may be exploited for therapeutic benefit.
Impact of our work

Generalisation of the concept of “Cancer Stem Cell” to other tumours

– Brain tumours (Singh et al, 2003)
– Colon Cancer (O’Brien et al. , Ricci-Vitiani et al. 2007)
– Prostate Cancer ( Li C et al., Collins A et al. 2007)
– Head and Neck Cancer (Dalerba et al., 2007)

Our work on LSCs may have some impact on other cancers