Stem Cells and Cancer

John Glod, M.D., Ph.D.
The Cancer Institute of New Jersey
Robert Wood Johnson Medical School
The University of Medicine and Dentistry of New Jersey
Diagram of stem cell lineages in bone marrow

Hematopoietic Lineage
Differentiation potential of hematopoietic stem

Mesenchymal Lineage
Differentiation potential of MSC

http://www.isscr.org/public/adultstemcells.htm
What is a “Stem Cell”

• Can differentiate
• Can divide indefinitely (or almost)
• Maybe better to call Cancer Stem Cells, tumor repopulating cells.
Initial inkling of cancer stem cell

- 1963 – Burger and Van Der Gaag show that approximately 1 in 130 cancer cells can regenerate lymphoma in a mouse.
### TABLE IV
Relation of Clinical and Tumor Kinetic Parameters in Patients with Multiple Myeloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>M-Component</th>
<th>Clinical stage at diagnosis*</th>
<th>Status when studied</th>
<th>Bone marrow myeloma cells</th>
<th>Myeloma cell [(^{3}P)Tdr labeling index</th>
<th>Total body myeloma cell number when studied†</th>
<th>M-cfu-c 10^8 myeloma cells§</th>
<th>Plating efficiency</th>
<th>Fraction of M-cfu-c surviving [(^{3}P)Tdr suicide</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. B.</td>
<td>IgG(\lambda)</td>
<td>IIA</td>
<td>Untreated</td>
<td>36</td>
<td>2</td>
<td>2.24 \times 10^{12}</td>
<td>1,772 ± 1.78</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>A. H.</td>
<td>IgG(\kappa)</td>
<td>IIIA</td>
<td>Untreated</td>
<td>36</td>
<td>14</td>
<td>1.81 \times 10^{12}</td>
<td>180 ± 30</td>
<td>0.02</td>
<td>0.33</td>
</tr>
<tr>
<td>L. Mc.</td>
<td>IgA(\kappa)</td>
<td>IIIA</td>
<td>Untreated</td>
<td>38</td>
<td>7</td>
<td>2.60 \times 10^{12}</td>
<td>640 ± 80</td>
<td>0.06</td>
<td>0.57</td>
</tr>
<tr>
<td>J. H.</td>
<td>(\kappa)-BJ</td>
<td>IIIB</td>
<td>Untreated</td>
<td>90</td>
<td>1</td>
<td>7.02 \times 10^{12}</td>
<td>240 ± 20</td>
<td>0.02</td>
<td>1.25</td>
</tr>
<tr>
<td>U. T.</td>
<td>(\lambda)BJ (amyloid)</td>
<td>IB</td>
<td>Remission</td>
<td>4</td>
<td>1</td>
<td>0.98 \times 10^{12}</td>
<td>190 ± 23</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>E. F.</td>
<td>IgG(\lambda)</td>
<td>IIIA</td>
<td>Relapse</td>
<td>25</td>
<td>2</td>
<td>0.36 \times 10^{12}</td>
<td>560 ± 110</td>
<td>0.06</td>
<td>0.36</td>
</tr>
<tr>
<td>V. W.</td>
<td>IgA(\kappa)</td>
<td>IIIA</td>
<td>Relapse</td>
<td>73</td>
<td>1</td>
<td>3.69 \times 10^{12}</td>
<td>300 ± 50</td>
<td>0.03</td>
<td>1.21</td>
</tr>
</tbody>
</table>

* Clinical staging as described in reference 17.
† Calculated from initial cell mass from regression equation from reference 15, and corrected for change in M-component mass with remission or relapse.
§ All cultures were plated at 5 \times 10^8 total nucleated marrow cells per plate, and are normalized to 10^8 with correction for plasma cell percentage. Plating efficiency for M-cfu-c was calculated in relation to the number of myeloma cells plated.
Figure 1 Labeling pattern of leukemic cells in marrow of patient 1

Dick, J. E. Blood 2008;112:4793-4807
Features of human CSCs as assayed in immunodeficient mice.
<table>
<thead>
<tr>
<th>Colon cancer cell source</th>
<th>Cell dose</th>
<th>Number of samples tested</th>
<th>Identification numbers of samples tested</th>
<th>(Number of primary mice with tumours)/(total number injected)</th>
<th>(Number of secondary mice with tumours)/(total number injected)</th>
<th>Total number of mice with tumours (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk</td>
<td>$1 \times 10^4$</td>
<td>8</td>
<td>3-5,10-14</td>
<td>0/4</td>
<td>0/4</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td></td>
<td>$2.5 \times 10^4$</td>
<td>8</td>
<td>4,6,10-14,17</td>
<td>1/6</td>
<td>0/2</td>
<td>1/8 (12.5)</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^4$</td>
<td>10</td>
<td>3-11,15</td>
<td>2/5</td>
<td>2/5</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td></td>
<td>$7.5 \times 10^4$</td>
<td>8</td>
<td>3,5,7-9,11-13</td>
<td>4/8</td>
<td>4/8</td>
<td>4/10 (50)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^5$</td>
<td>10</td>
<td>3-5,7-13</td>
<td>6/6</td>
<td>4/4</td>
<td>10/10 (100)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^6$</td>
<td>17</td>
<td>1-17</td>
<td>17/17</td>
<td>17/17</td>
<td>17/17 (100)</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^6$</td>
<td>8</td>
<td>6-9,12-17</td>
<td>8/8</td>
<td></td>
<td>8/8 (100)</td>
</tr>
<tr>
<td>CD133$^+$</td>
<td>$1 \times 10^3$</td>
<td>4</td>
<td>7,8,14,15</td>
<td>1/4</td>
<td>4/5</td>
<td>5/6 (83.33)</td>
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<tr>
<td></td>
<td>$5 \times 10^3$</td>
<td>6</td>
<td>5,6,11,13,14,17</td>
<td>1/1</td>
<td>7/7</td>
<td>8/8 (100)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^3$</td>
<td>7</td>
<td>5-8,10,12,17</td>
<td>1/1</td>
<td></td>
<td>7/7 (100)</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^3$</td>
<td>8</td>
<td>8-13,15,17</td>
<td>1/1</td>
<td></td>
<td>7/7 (100)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^4$</td>
<td>10</td>
<td>5-7,14,17</td>
<td>9/9</td>
<td>9/9</td>
<td>9/9 (100)</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^4$</td>
<td>9</td>
<td>5-7,9-11,13-15</td>
<td>9/9</td>
<td></td>
<td>9/9 (100)</td>
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<tr>
<td>CD133$^-$</td>
<td>$5 \times 10^3$</td>
<td>5</td>
<td>6,9,11,12,17</td>
<td>0/5</td>
<td></td>
<td>0/5 (0)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^4$</td>
<td>6</td>
<td>8,10,12,14,15,17</td>
<td>0/1</td>
<td>0/5</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td></td>
<td>$2 \times 10^4$</td>
<td>6</td>
<td>5-7,9,10,13</td>
<td>0/1</td>
<td>0/6</td>
<td>0/6 (0)</td>
</tr>
<tr>
<td></td>
<td>$5 \times 10^4$</td>
<td>8</td>
<td>5-7,9,11,12,14,15</td>
<td>0/1</td>
<td>0/7</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td></td>
<td>$1 \times 10^5$</td>
<td>8</td>
<td>6,8,10,12-15</td>
<td>0/1</td>
<td>0/7</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td></td>
<td>$2.5 \times 10^5$</td>
<td>9</td>
<td>5-7-9,11,13-15</td>
<td></td>
<td>1/9</td>
<td>1/9 (11.1)</td>
</tr>
</tbody>
</table>

NOD/SCID mice were transplanted with: bulk ($n = 61$), CD133$^+$ ($n = 49$), and CD133$^-$ ($n = 47$) human colon cancer cells. All doses are displayed with the exception of $2 \times 10^3$ for CD133$^+$ ($n = 5$) and CD133$^-$ ($n = 5$), in which tumour-formation rates were 100% and 0%, respectively. Mice were killed at 6–21 weeks post-injection. Mice were considered negative if no tumour tissue was identified. Only doses that resulted in a mix of positive and negative mice were used to calculate the limiting dilution experiments.
Multiple facets to CSC self-renewal.

Diagram of stem cell lineages in bone marrow

Hematopoietic Lineage
Differentiation potential of hematopoietic stem

Mesenchymal Lineage
Differentiation potential of MSC

http://www.isscr.org/public/adultstemcells.htm
Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized

Elsa Quintana1,2, Mark Shackleton1,2,6, Hannah R. Foster2, Douglas R. Fullen3, Michael S. Sabel4, Timothy M. Johnson5, and Sean J. Morrison2,7
Therapy Targeting Tumor Stem Cells

- Designing the appropriate pre-clinical assays
- New ways of evaluating clinical efficacy
CD133 expression affects survival.

The Tumor Microenvironment

Littlepage et al Cancer Cell 2005
Multipotent Mesenchymal Stromal Cells

- Nonhematopoietic cells of mesenchymal origin found in the bone marrow.
- Friedenstein described the isolation and characterization of MSCs in 1980.
- In vitro adherent cells derived from long-term bone marrow cultures.
Human MSCs -- Mesenchymal Differentiation in vitro

Culture expanded human MSCs

osteogenesis

adipogenesis

chondrogenesis
Localization of MSCs

- Bone Marrow
- Solid Tumors
- Sites of Injury (brain, limb ischemia, heart)
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MSC
What do the tumor cells produce to incite MSC chemotaxis?
In vitro migration assay

- Use a transwell migration chamber
- conditioned media from tumor cells or bone marrow or tumor cells in media at bottom
- place $2 \times 10^5$ stromal cells on top
- for control: use plain medium with serum
- 3h, 6h and 24h migration
- stain cells migrating to bottom of transwell
MSCs migrate towards C85 colon tumor cells as well as to conditioned medium from these cells.
In Vitro Migration of MSCs to Tumor Cell Lines

Plate tumor cell line or other stimulus → ON → Plate MSCs in inserts → 18 HR → Stain cells with Crystal Violet

Menon, Picinich et al. *Stem cells.* Feb 2007
Rationale for the cDNA Microarray Analysis

- MSCs migrate to tumor CM comparably to tumor cells
- Exposure of MSCs to tumor CM would allow for the study of genes involved in migration to tumors
- MSCs also migrate to bone marrow
- Therefore, examination of the gene expression profile of MSCs exposed to tumor CM compared to bone marrow CM would provide information on genes that are differentially expressed when exposed to different stimuli
MSCs were exposed to C85 CM or RPMI for 24 h. RNA was isolated and processed for microarray analysis. The Affymetrix cDNA GeneChip® Rat Genome 230 2.0 Array was used. Of the 104 genes upregulated in MSCs exposed to C85 CM as compared to control, SDF-1, CXCL-2, superoxide dismutase 2, and CINC-2 were the top scorers.
A small set of genes are upregulated in MSCs exposed to tumor CM compared to bone marrow CM.

SDF-1 is one gene of interest that is upregulated upon exposure to tumor CM.

Menon, Picinich et al. Stem cells. Feb 2007
### Gene Expression Profile of MSCs

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene Name</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1388920_at</td>
<td>Bone morphogenetic protein 6</td>
<td>5.12</td>
</tr>
<tr>
<td>1388920_at</td>
<td>Chemokine(C-X-C motif) ligand 12, SDF-1</td>
<td>4.25</td>
</tr>
<tr>
<td>1376845_at</td>
<td>Putative ISG12 (b) protein</td>
<td>2.00</td>
</tr>
<tr>
<td>1367774_at</td>
<td>Glutathione S transferase, alpha 1</td>
<td>1.65</td>
</tr>
</tbody>
</table>

**RT-PCR Validation**

Lane 1-3: MSCs exposed to tumor conditioned media  
Lane 4-6: MSCs exposed to media control  

Increased Secretion of SDF-1 by MSCs after Exposure to Tumor CM

1. Control Medium
2. Tumor CM
3. Bone Marrow CM
4. MSCs exposed to control Medium
5. MSCs exposed to tumor CM

* P-value = 0.0049

SDF-1

- Stromal derived factor 1 or CXCL12
- 8-kDa CXC secreted chemokine
- SDF-1 receptor = CXCR4, specific to the SDF-1 ligand
- Plays a role in cellular trafficking and in directing migration of many cell types including neural stem cells (Imitola et al. *PNAS* 2004) and hematopoietic progenitor cells (Wang et al. *Blood* 2000)
- Important for homing of MSCs to bone marrow (Wynn et al. *Blood* 2004)

Does SDF-1 play a critical role in the signaling and trafficking of MSCs to tumor cells?
Exposure to tumor cell CM leads to increased secretion of SDF-1 by rMSCs confirming the microarray data

Tumor cell conditioned medium (bar 1) and RPMI medium (bar 2) have barely detectable levels of SDF-1. Exposure of MSCs to RPMI+10% FBS for 16h (bar 3) and to tumor cell CM for 16 h (bar 4) leads to a significant increase in SDF-1 levels in secreted medium of MSCs in agreement with the cDNA microarray results. The difference between SDF-1 levels induced by RPMI+10%FBS and CM from tumor cells is statistically significant (p<0.005, unpaired t test).
A Role for SDF-1 in MSC Chemotaxis

MSCs with reduced expression of SDF-1 by siRNA knockdown have impaired chemotaxis in response to tumor conditioned media compared to control MSCs, while chemotaxis to bone marrow conditioned media is unaffected.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control siRNA</th>
<th>SDF-1 siRNA</th>
<th>Tumor conditioned media</th>
<th>Bone marrow conditioned media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SDF-1 siRNA</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tumor conditioned media</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bone marrow conditioned media</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* P-value < 0.0008 compared to control siRNA

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36

Days

0 25 50 75 100 125 150 175 200 225 250 275 300 325

Tumor volume (mm³)

- MDAMB231 in Matrigel
- MDAMB231+TCM Activated hMSC (30days)
- MDAMB231+Aza treated hMSC
- MDAMB231+Naïve hMSC
- MDAMB231 Alone
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MIP-1a, IL-8, Cyclophilin B

Source of CAFs

SDF-1

\[ \text{cxcr} \, 4 \quad \text{cxcr} \, 7 \]

SDF-1 induced signal transduction via Jak2

MSC

Source of CAFs?
Acknowledgements

NJCCR

NJCST

The Childrens Brain Tumor Foundation