Stem cells and Cancer

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The Tumor Microenvironment

Littlepage et al Cancer Cell 2005
Cancer Stem Cells

- A small group of cells within the larger tumor bulk is responsible for the maintenance of tumor replication.
- This small percentage of Cells has properties that are different from the bulk of the tumor cells.
- These properties include limitless replication potential and the ability to differentiate into multiple different cell types.
The first Cancer Stem cell
Figure 1. Labeling pattern of leukemic cells in marrow of patient 1. Patient 1, a patient with acute myelomonocytic leukemia, received a continuous 10-day infusion of tritiated thymidine. Leukemic cells were arbitrarily divided into types I, II, and III based on increasing levels of morphologic maturity (type I indicates primitive blast forms; type III, most differentiated cells). At the end of the 10-day infusion, most type II and type III cells were labeled in both marrow (shown here) and blood (not shown), but only 40% of type I cells were labeled, reflecting their slow proliferative rate. Many of the type I cells remained highly labeled for over 3 weeks after infusion.

A cell initiating human acute myeloid leukaemia after transplantation into SCID mice
Tsvee Lapidot Christian Sirard Josef Vormoor Barbara Murdoch Trang Hoang Julio Caceres-Cortes Mark Minden Bruce Paterson Michael A. Caligiuri & John E. Dick

AML initiating cells represented approximately 1 in 10^6 of the total leukemia cells, they could be characterized by cell surface markers.

http://www.stemspec.ca/Project/History/Uhn.html
Example
Glioblastoma Multiforme

• Most common primary brain tumor in adults.
• Poor prognosis.
• Approximately 50% of patients will have some tumor response with radiation therapy and chemotherapy but will have tumor recurrence.
Isolation of cancer stem cells from adult glioblastoma multiforme
Xiangpeng Yuan, James Curtin, Yizhi Xiong, Gentao Liu, Sebastian Waschsmann-Hogiu, Daniel L Farkas, Keith L Black and John S Yu
The cancer stem cell hypothesis: a work in progress

Brenton Thomas Tan, Christopher Yongchul Park, Laurie Elizabeth Ailles and Irving L Weissman
Other Cancer Stem Cells

- Breast Cancer
- Leukemia
- Prostate Cancer
- Lung Cancer
What are the clinical implications?

• Even if a therapy kills most of the cancer cells that are present, they may not be the cells that should be targeted to cure the patient.

• Cancer stem cells have properties that are different than the bulk of cells in a tumor (resistance to chemotherapy, a slower division rate, etc.)
The Lancet.


The Distribution of Secondary Growths in Cancer of the Breast.

By Stephen Paget, F.R.C.S., Assistant Surgeon to the West London Hospital and the Metropolitan Hospital.

An attempt is made in this paper to consider "metastasis" in malignant disease, and to show that the distribution of the secondary growths is not a matter of chance. It is urged both by Langenbeck and by Billroth that the question ought to be asked, and, if possible, answered: "What is it that decides what organs shall suffer in a case of disseminated cancer?" If the remote organs in such a case are all alike passive and, so to speak, helpless—all equally ready to receive and nourish any particle of the primary growth which may "slip through the lungs," and so be brought to them—then the distribution of cancer throughout the body must be a matter of chance. But if we can trace any sort of rule or sequence in the distribution of cancer, any relation between the character of the primary growth and the situation of the secondary growths derived from it, then the remote organs cannot be altogether passive or indifferent as regards embolism.
The Tumor Microenvironment

Littlepage et al Cancer Cell 2005
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Questions about the tumor stroma

• Where does it come from?
• How does it get there?
• What does it do?
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
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.
Migration of MSCs to tumor site in vivo

CFDA-SE labeled MSCs (panel b) were detectable in the TMEN (marked as S in Fig 3). DAPI staining (panel a) revealed that the MSCs surrounded the tumor mass (marked as T in both panels Fig 3).
Human MSCs -- Mesenchymal Differentiation in vitro

Culture expanded human MSCs

osteogenesis  adipogenesis  chondrogenesis

Deans and Mosely Experimental Hematology 2000
The Tumor Microenvironment

Littlepage et al Cancer Cell 2005
Orima et al. Cell 2005
Are MSCs the Source of CAFs?

- CAFs may be derived from the bone marrow
- MSCs look like fibroblasts
- MSCs localize to solid tumors
- Both MSCs and CAFs produce SDF-1
hMSCs express alpha smooth muscle actin following exposure to tumor cell CM

1: NIH 3T3 cells; 2: hMSCs in TCM 6 days; hMSCs plus 5aza; hMSCs neg control
Luciferase Activity (relative light units)

MDA-MB-231 LUC + hMSCs
Preexposed to TCM for 1 to 30 days

Naive hMSCs

MDALUC+MSC (5-Aza)

MDALUC+hMSCs (DMEM 30 days)
Fibroblast Surface Protein and H&E

Naïve hMSCs  Matrigel

5-aza  TCM
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MSC
MSCs migrate towards C85 colon tumor cells as well as to conditioned medium from these cells.
Can we identify patterns of gene expression that are specific for MSC chemotaxis to the tumor microenvironment?
cDNA microarray as a tool to investigate molecular basis of migration of MSCs

MSCs are exposed to CM from tumor cells or control media, RNA isolated and processed for cDNA microarray
cDNA micro array analysis of rMSCs exposed to CM from rat BM (left panel) as compared to CM from tumor cells (right panel)

The two migration conditions are shown side by side, genes upregulated for BM migration appear to be distinct from genes upregulated for tumor migration

Note similarity of gene quadrants up and downregulated in migrating versus non migrating cells
### Table Ib

Expression of mRNA levels of following genes are increased in MSCs exposed to Tumor CM but decreased in MSCs exposed to Bone marrow CM

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene name</th>
<th>Fold Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1387648_at</td>
<td>chemokine (C-X-C motif) ligand IX</td>
<td>184.93</td>
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<tr>
<td>1370634_at</td>
<td>cytokine-induced neutrophil chemoattractant-2</td>
<td>59.17</td>
</tr>
<tr>
<td>1368760_at</td>
<td>chemokine (C-X-C motif) ligand 2</td>
<td>20.03</td>
</tr>
<tr>
<td>1387655_at</td>
<td>chemokine (C-X-C motif) ligand 12, SDF-1</td>
<td>10.52</td>
</tr>
<tr>
<td>1368078_at</td>
<td>endothelial cell-specific molecule 1</td>
<td>9.68</td>
</tr>
<tr>
<td>1375951_at</td>
<td>thrombomodulin</td>
<td>3.33</td>
</tr>
<tr>
<td>1369884_at</td>
<td>fibroblast growth factor 7</td>
<td>3.03</td>
</tr>
<tr>
<td>1370968_at</td>
<td>nuclear factor kappa B p105 subunit</td>
<td>2.27</td>
</tr>
</tbody>
</table>

The table contains only functionally identified genes
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).
Knockdown of SDF-1 inhibits migration of rMSCs to CM from tumor cells

SDF-1 knockdown using 50nM siRNA inhibits migration of MSCs (bars 1 and 3) to CM from tumor cells (bar 2) but not to CM from bone marrow cells (bar 4).
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MSC

- MIP-1α
- IL-8
- Cyclophilin B

SDF-1
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MIP-1a, IL-8, Cyclophilin B

Source of CAFs?

MSC

SDF-1

cxcr 4

cxcr 7

SDF-1 induced signal transduction via Jak2

?
Treatment Targets involved in the Interaction between MSCs and other components of the Tumor microenvironment

Tumor Microenvironment

Source of CAFs?

MSC

SDF-1 induced signal transduction via Jak2
Summary

• The cellular components of tumors are heterogeneous.
• A very small population of cancer stem cells are probably responsible for the propagation of the neoplastic cell.
• Other progenitor cell populations are important in tumor growth and formation.
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Recombinant MIP-1α and IL-8 promote migration of hMSCs.

Migration to MIP-1α

Migration to IL-8
Roles of Heparin Sulfate Proteoglycans

Bishop et al. Nature vol 446 April 2007
Conditioned medium from breast cancer cells

- Heparin column
  - Fractionation by step gradient of NaCl and determining active fractions by MSC migration assay
  - Green dye column
    - Fractionation by step gradient of NaCl to obtain the enriched active fraction based on migration assay
Identification of Candidate Chemotactic Factors from Tumor CM

A

kDa

Green Heparin CM

47
35
25
18
13

B

DFMIQGGDFTRGDGTG
DKPLKDVIIDACGK
DTNGSQFFITTVK
DVIIADCGK
HYGPWGVMANAGK
IEVEKPFIAAKE
IGDEDVGR
FPDENFKLK
LKHYPWGVMANAGK
FPDENFK
SIYGERFPDENFK
TVDNFVALATGEGK
TAWLDGKHVFVFGK
VIKDFMIQGDFTRDFTR
VLEGMEVVRK
VIFGLFGK
TVPKTVDNFKVALATGEGK

C

Green Heparin CM
Anti-Cyclophilin B Antibody Blocks hMSC Migration

% migrating cells of CM

α cyclophilin Ab (μg/ml)

1 2 5 10 boiled Ab CM Control Eot-2 mAB
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MIP-1a, IL-8, Cyclophilin B

MSC
Recombinant MIP-1α and IL-8 promote migration of hMSCs.

The effect of IL-8 on SDF-1 expression in MSCs

The effect of MIP-1 alpha on SDF-1 expression in MSCs

IL-8 and MIP-1α induce expression of SDF-1 in hMSCs
SDF-1 signaling is mediated by Jak2

Effect of SDF-1 on hMSC migration

Effect of Jak2 and MAPK inhibitors on hMSC toward CM
Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

MIP-1α, IL-8, Cyclophilin B

MSC

SDF-1

? cxcr 4 cxcr 7

SDF-1 induced signal transduction via Jak2