Cancer Stem Cells in Human Breast Cancer

A new direction in breast cancer therapy

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Embryonic Stem Cells vs. Adult Stem Cell vs. Cancer Stem Cells

• What is the difference?
• Why does it matter?
Embryonic Stem Cell

• Multipotent
  – Can make all the cells in the organism

• Self-renewal
  – Including itself (asymmetric division)

• Long-lasting, quiescent
Adult Stem Cell

- Same, but can only make the organ it came from
• It is possible to select cells that can grow the entire mammary gland based on markers on the cell surface
Sca-1\textsuperscript{pos} Cells Retain Increased Outgrowth Capacity

<table>
<thead>
<tr>
<th></th>
<th>Sca-1 \textsuperscript{pos}</th>
<th>Sca-1 \textsuperscript{neg}</th>
</tr>
</thead>
<tbody>
<tr>
<td>no outgrowth</td>
<td>0/6</td>
<td>6/6</td>
</tr>
<tr>
<td>outgrowth</td>
<td>6/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>
Single Cell Phenotype of Normal Mammary Gland Stem Cell Identified

Generation of a functional mammary gland from a single stem cell

Mark Shackleton¹², François Vaillant¹², Kaylene J. Simpson³†, John Stingl⁴⁵, Gordon K. Smyth¹, Marie-Liesse Asselin-Labat¹², Li Wu¹, Geoffrey J. Lindeman¹² & Jane E. Visvader¹²
Cancer Stem Cell

- Multipotent
  - Can make all the cells in the tumor
- Self-renewal
  - Including itself (asymmetric division)
- Long-lasting, quiescent
Some cancers are sustained by a small minority of cells; their resemblance to normal stem cells might explain why many cancers are so hard to eradicate, and it has researchers rethinking cancer treatments

Mutant Stem Cells May Seed Cancer
Tumor Stem Cells Provide a Novel Therapeutic Target

Weissman I, AACR 2004
Parallels Between Normal and Cancer Stem Cells

Applying cancer stem cell hypothesis to breast cancer

• A new way of thinking about it?
Breast Cancer Subtypes: Gene Expression Profiling and Overall Survival

Sorlie et al, PNAS 100:8418, 2003
Figure 2

- **LT** (left) to **ST** (right)
  - Self-renewal → **ER-, Basal Like**
  - Differentiation → **ER-, ERBB+**
- **Luminal Progenitor**
  - Self-renewal → **ER-, ERBB+**
  - Differentiation → **ER-, Luminal Type C or B**
- **Luminal Progenitor**
  - Self-renewal → **ER+, Luminal Type A**
  - Differentiation → **ER+, Luminal Type C or B**
- **Myoepithelial Progenitor**
  - Differentiation → **Myoepithelial cells**
- **Ductal / Alveolar ER-**
  - Differentiation → **Ductal / Alveolar ER-**
  - Differentiation → **Ductal / Alveolar ER+**
The task at hand…

• Identification of mammary stem cells
• Characterize molecular pathways involved in regulation of stem-like behavior
• Examine the role of these genes in tumorigenesis
• Evaluate and test anti-stem cell therapy in cancer models
Hypothesis

• Breast cancers arise from cancer stem cells, which may be resistant to conventional therapy, and are therefore a critical determinant of recurrence
Radiation Enriches for Progenitor Phenotypes

A

Radiation dose (Gy)

Fold Increase (%SP)

0 2 4 6

MEC

Hoechst Blue

0 Gy 4 Gy

0.18% 0.94%

B

Radiation dose (Gy)

%SP

0 2 4 6

MCF-7

Hoechst Blue

0 Gy 4 Gy

0.08% 0.21%
β-Catenin Mediates Radiation Resistance of Progenitors

**A**

<table>
<thead>
<tr>
<th>Radiation dose (Gy)</th>
<th>MMTV-Wnt-1</th>
<th>Stabilized β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>Wnt</td>
<td>Control</td>
</tr>
</tbody>
</table>

%SP

- **p < 0.05**

**B**

<table>
<thead>
<tr>
<th>Radiation dose (Gy)</th>
<th>Survivin Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Arbitary units</td>
</tr>
<tr>
<td>2</td>
<td>SCA+ and SCA-</td>
</tr>
<tr>
<td>4</td>
<td>SCA+ and SCA-</td>
</tr>
</tbody>
</table>

**p < 0.05**

# Active β-catenin+ cells

- **SCA+ and SCA-**

Survivin Expression
Fewer DNA Damage Foci form in Progenitors
B-catenin regulation is upregulated by radiation in selectively in progenitors – Survivin
Murine Data

• Normal and weakly tumorigenic murine mammary gland progenitors are resistant to radiation
  – Normal stem cell likely are sensitive
• β-catenin and survivin are selectively upregulated in radioresistant progenitors
Impact?

- Should we be examining response of stem/progenitors cells to new therapies that are being tested?
- Are they different?
Enhancement of *in vitro* and *in vivo* tumor cell radiosensitivity by valproic acid

Kevin Camphausen¹*, David Cerna², Tamalee Scott¹, Mary Sproull¹, William E. Burgan², Michael A. Cerra², Howard Fine³ and Philip J. Tofilon²

¹Radiation Oncology Branch, National Cancer Institute, Bethesda, MD, USA
²Molecular Radiation Therapeutics Branch, National Cancer Institute, Bethesda, MD, USA
³Neuro-Oncology Branch, National Cancer Institute, Bethesda, MD, USA
• Valproic Acid promotes differentiation and apoptosis in leukemic blasts

• Self-renewal of normal HSCs through inhibition of GSK-3b (Upregulation of Wnt signaling)
2D Clonogenic Assays

![Graph showing the surviving fraction vs dose for Control and Valproic Acid treatments. The graph includes points for different dose regimens:
- Control: 2Gy x 5, 5Gy x 2, 10Gy x 1
- Valproic Acid: 2Gy x 5, 5Gy x 2, 10Gy x 1

The surviving fraction is plotted on the y-axis, and the dose in Gy is plotted on the x-axis. The graph demonstrates a decrease in surviving fraction with increasing dose for both treatments.}
3D Mammosphere Culture

Dose (Gy)

Surviving Fraction

Control

Valproic Acid

Surviving Fraction

Dose (Gy)

Control

Valproic Acid

Surviving Fraction

2 5 10
Relevant in Clinical Material?

• Clinical Protocols
  – Paired normal and tumor mastectomy specimens
  – Pleural effusion cells
  – Bone Marrow, disseminated tumor cells
Pulmonary nurse calls – fresh fluid
Transferred to pathology

Material examined by pathology – blocks stored

1. Grow and passage MS
2. Radiate MS
3. Re-analyse Freeze MS
4. Re-analyze cytospin/block
5. B-catenin (western/IF)

Analysis for circulating tumor cells and stem cell markers; Freeze RNA; Grow remaining cells in mammosphere culture

Active Protocol (LAB 06-0776):
3D Mammosphere Culture from Patient Fluid
Cytospin – pathology review:
Cultured cells are malignant and similar to uncultured, baseline cells
Some cancers are sustained by a small minority of cells; their resemblance to normal stem cells might explain why many cancers are so hard to eradicate, and it has researchers rethinking cancer treatments.

**Mutant Stem Cells May Seed Cancer**

Prospective ID of Breast tumor stem cells: “Al-Hajj Phenotype”
Cancer stem cells are Resistant to Radiation

-Mammmosphere culture enriched for circulating tumor cells (326+)
-Initial (P0) mammmosphere cancer stem cells were resistant to 4Gy
-First Passage (P1) higher percentage cancer stem cells, also resistant
• 3D culture potential system to test targeted stem cell therapies with and without radiation in cell lines and clinical samples

• Clinical material allows correlation to known clinical markers and selection for appropriate patients for future trials
Baseline CTC Counts Predicts Overall Survival

Experimental Design

2 x 3 ml Bilateral Bone Marrows
PBC (n = 23)

Magnetic Bead Enrichment for CD326/EpCAM

FACS

Pathology Morphology (Pap) Cytokeratin

RT-PCR Select Genes (Mammospheres Cell Lines)

CD326/EpCAM Stem Cell Phenotype

FACS Stem Cell Phenotype

ARC-MD
CD326+ Cells Isolated from Bone Marrow Have Tumor Cell Morphology and Express Cytokeratin

PAP Stain

Cytokeratin
Some cancers are sustained by a small minority of cells; their resemblance to normal stem cells might explain why many cancers are so hard to eradicate, and it has researchers rethinking cancer treatments.

**Mutant Stem Cells May Seed Cancer**

Prospective ID of Breast tumor stem cells: “Al-Hajj Phenotype”
Majority of CD326+ Bone Marrow Cells of PBC Patients Are Breast Cancer-Initiating Stem Cells

Stem Cell Phenotypes in CD326+ BoneMarrow Cells

<table>
<thead>
<tr>
<th></th>
<th>% CD326</th>
<th>Al-Hajj (CD44+CD24lo) % Br Cancer-Initiating Cells</th>
<th>Shackleton (CD24+CD29+) % Mammary Br Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td><strong>0.81 ± 0.07</strong></td>
<td><strong>76.3 ± 3.8</strong></td>
<td><strong>13.3 ± 2.4</strong></td>
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Generation of a functional mammary gland from a single stem cell

Mark Shackleton^1,2, François Vaillant^1,2, Kaylene J. Simpson^3†, John Stingl^4,5, Gordon K. Smyth^1, Marie-Liesse Asselin-Labat^1,2, Li Wu^1, Geoffrey J. Lindeman^1,2 & Jane E. Visvader^1,2

“Shackleton Phenotype”
Identical profile to normal mouse mammary gland
In 326+ bone marrow of some pts
CTCs

• Possible a measure of cancer stem cells
• Present even when there is no known metastatic disease
• Predict therapy response?
• Prognostic?
CTC Incorporation Into Trials

• Recurrent PBI
  – Can circulating tumor cells predict for patients with residual disease in the breast and therefore local recurrence?
  – Can patients who will not benefit from aggressive local therapy based on likelihood of DM be identified?

• SBRT for Olgiomets
  – Weichselbaum, Minn, Chmura
Summary

• Cancer may start as a mutation in an adult stem cell, or an adult differentiated cell that allows it to act like a stem cell

• Lots of attention is being direct to evaluating the efficacy of current therapies on cancer stem cells and develop new therapies specifically for cancer stem cells
Summary

• Normal and weakly tumorigenic murine mammary gland progenitors can be resistant to radiation

• β-catenin and survivin are selectively upregulated in radioresistant progenitors
  – Studying whether these will make good targets of new drugs
Summary

• Clinical human breast cancer progenitors can also demonstrate radiation resistance

• CTCs may be a meaningful clinical biomarker of tumor stem/progenitor cells
  – Can we use them to determine who would benefit from radiosensitizer?
  – Chemotherapy?
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