Targeting Prostate Cancer Stem Cells

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Outline

• Historical Research, Identification of Cancer Stem Cells in Leukemia

• Techniques to Identify Cancer Stem Cells

• Prostate (Cancer ) Stem Cells
  – Identification
  – Therapy
Definition of (Cancer) Stem Cells

Benign and Cancer Stem Cells:

Self-renewal - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state

Potency - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type.

Cancer Stem Cells:

Generate all heterogeneous lineages of cells within a tumor

Suggests a hierarchy of tumor initiating capabilities

Modified from Wikipedia.org
Cancer Stem Cell Hypothesis is NOT New

1950-1970’s
G. Barry Pierce: proposed organ cell hierarchy of organogenesis of tumorigenesis

1990’s
John Dick: evidence for the existence of cancer stem cells in Acute Myeloid Leukemia
Cancer Stem Cells in the Headlines

**BBC August 1, 2012**
Cancer stem cell discovery could signal 'paradigm shift'
By Pallab Ghosh

**Fox News September 27, 2012**
Common cancer treatments may create dangerous cancer stem cells
By Charles Q. Choi

**Forbes October 09, 2012**
Cancer Stem Cell Therapy: Real Or Just Hype?
By Nathan Sadeghi-Nejad

**The New Yorker September 7, 2014**
The Transformation
By Jerome Groopman
Cancer Stem Cell Hypothesis

Cancer Stem Cell Identification

Disassociate Tumor → Tumor Generating Assay

Cancer Stem Cell → Tumor

Majority of Cancer Cells → No Tumor
Cell Identification with Antibodies

Antigens

Antigen-binding site

Antibody

[Image of a microscopic view of cells, possibly showing the identification process with antibodies]
Flow Cytometry

1. Dissociate Tumor
2. Label Cells with Fluorescent Antibody
3. Identify Cells
4. Analyze Cells with Flow Cytometry

Tumor Cell Suspension
Green Intensity
Red Intensity
Flow Activated Cell Sorting

[Diagram of Flow Activated Cell Sorting process]

KEY
- Fluorescent cells
- Nonfluorescent cell
- Fluorescent cell droplets
- Nonfluorescent cell droplet

Lodish et. al., NCBI.
Bone Marrow Transplants in Mice

[Diagram showing bone marrow transplant process, including bone (B6), stromal cells (HSC-Chemotactic Factor), HSCs, Pseudo-emperipolesis, 8.5 Gy radiation, MRL/lpr, HSCs (B6), subcutis, and injection (i.v.).]
First Demonstration of Cancer Stem Cells

*In vivo* assay for human leukemia – Demonstrated different stages of AML could engraft in irradiated mice

had colony formation

CD34+CD38-, but not CD34+CD38+ cells could recapitulate human AML in mice

Stem Cell Protection Mechanisms

Common protective mechanisms between benign and cancer stem cells assays for discrimination and isolation

- **Multidrug resistance pumps**
  - Hoechst Efflux Side Population
  - Vybrant® DyeCycle™ Violet Side Population

- **High Aldehyde Dehydrogenase Activity**
  - ALDEFLOUR ®

- **Radiation Protection**: ↑Chk1 & Chk2 mediated DNA repair capacity

- **Telomerase Activity**
Hematopoietic Side Population Phenotype

+ verapamil

ABC Transporters

- ATP binding cassette transporters - the largest family of drug transporters
- Evolutionarily conserved
- Present in plasma membrane and membranes of intracellular compartments
- Use of cellular ATP to drive transport
- 7 subfamilies and 50 ABC transporters

# ABC Transporter Super Family

<table>
<thead>
<tr>
<th>Subfamilies</th>
<th>Most studied member</th>
<th>Role of the most studied member</th>
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</thead>
<tbody>
<tr>
<td>ABCA1-12</td>
<td>ABCA1 and 2</td>
<td>Cholesterol efflux (A1), Drug resistance (A2)</td>
</tr>
<tr>
<td>ABCB1-11</td>
<td>ABCB1,6,11</td>
<td>Multi drug resistance, <em>Hoechst</em> (B1) Iron transport (B6) Bile salt transport (B11)</td>
</tr>
<tr>
<td>ABCC1-13</td>
<td>ABCC1-5</td>
<td>Drug resistance (C1,3) Nucleotide transport (C4,5) Chloride channels (C7)</td>
</tr>
<tr>
<td>ABCD1-4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ABCF1-3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ABCE1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ABCG1-8</td>
<td>ABCG1, 2, 5, 8</td>
<td>Cholesterol efflux (G1) Toxins, drugs, dyes e.g. <em>Hoechst</em>, rhodamine, DCV, <em>steroids</em> (G2)</td>
</tr>
</tbody>
</table>
Add Antibody to Recognize Cancer Stem Cells (red) and Cancer Cells (blue)

Optimize dissociation to preserve cell viability and marker integrity

Exclude debris and dead cells
Exclude non-cancer cells

Shackleton M et al., Cell 2009;138(5):822-829
Identification of Cancer Stem Cells in Solid Tumors

Optimize dissociation to preserve cell viability and marker integrity

Exclude debris and dead cells
Exclude non-cancer cells

CANCER CELLS
Prostate Cancer is Second Cause of Cancer Related Death in American Men

Estimated Cancer Deaths in the US in 2013

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>306,920</td>
<td>273,430</td>
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<tr>
<td>Prostate</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>All other sites</td>
<td>24%</td>
<td>25%</td>
</tr>
</tbody>
</table>

26% Lung & bronchus
14% Breast
9% Colon & rectum
7% Pancreas
5% Ovary
4% Leukemia
3% Non-Hodgkin lymphoma
3% Uterine corpus
2% Liver & intrahepatic bile duct
2% Brain/other nervous system
25% All other sites
Clinical Significance: Castration Resistant Prostate Cancer
Organ Stem Cells

Bladder

Urethra

Normal Prostate

Normal adult stem cell

Cancer Stem Cells

Bladder

Prostate

Cancer

Cancer stem cell
Schematic Depiction of the Prostatic Duct

Luminal cell markers:
- AR,
- CK8,
- CK18

Basal cell markers:
- p63,
- CK5,
- CK14

Non-epithelial cells
(Lineage markers):
- Endothelial cells: CD31
- Fibroblasts: CD45 and CD34
- Erythroid cells: Ter119

Stem Cell?

Abcg2+ Cells are Located in Stem Cell Compartment

ABCG2 Inhibition Increases Intracellular Androgen and Androgen Receptor

Androgen Receptor (AR) Regulation by Androgens

Cell with a functional ABCG2 transporter

Cell with a non-functional ABCG2 transporter

Differentiated cell

Nov FTC KO143
Prostate Benign and Cancer Stem Cells

Secretory
Basal
NE
Adenocarcinoma
Transit-Amplifying Cell
ABC transporter
Prostate Stem Cell Niche
Tumor Stem Cell
Stroma

Differentiation
Stroma Signaling
Androgen Deprivation Therapy
Recurrent Prostate Cancer

Prostate Stem Cell Niche

Tumor Stem Cell

Recurrent Prostate Cancer

Basal

NE

Differentiation

Stroma
Secretory Adenocarcinoma

Prostate Stem Cell Niche

Basal NE

Transit-Amplifying Cell

Stroma

Inhibition of ABC Transporters Before Androgen Deprivation to Target Cancer Stem Cells

Differentiation

ABC transporter

Prostate Stem Cell Niche

Tumor Stem Cell

Stroma

Adenocarcinoma
Inhibition of ABC Transporters Before Androgen Deprivation to Target Cancer Stem Cells
Hypothesis: ABCG2 efflux of androgen inhibits prostate stem cell differentiation to maintain stem cell properties

Specific aims:

1. Determine the mechanism of androgen efflux to maintain stem cell properties. ABCG2 mediated androgen efflux inhibits AR induced prostate stem cell differentiation.

2. Identify regulators of the side population phenotype that contribute to maintaining prostate stem cell properties. Prostate stem cells within the side population require ABCG2 expression.

3. Determine the effect of abrogated ABCG2 function on the prostate stem cell niche. ABCG2 inhibition depletes stem cell compartment and the prostate is unable to serially regenerate.
Aim 1: Inhibiting ABCG2 Mediated Androgen Efflux Increases AR Nuclear Translocation

ABCG2 expressing HPr-1-AR cells (Similar Results in CWR-R1)
Aim 1: Inhibiting ABCG2 Mediated Androgen Efflux Increases Expression of Luminal Differentiation Markers

ABC+G2-expressing HPr-1-AR cells
(Similar Results in CWR-R1)

<table>
<thead>
<tr>
<th></th>
<th>CK8</th>
<th>PSA</th>
<th>CK18</th>
<th>SOX 2</th>
<th>Actin</th>
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</thead>
<tbody>
<tr>
<td>DHT</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ko143</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Enzalutamide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Neha Sabnis
Summary Aim 1 and Future Directions

ABCG2 inhibition increases nuclear AR & expression of AR regulated differentiation markers

Determine if differentiation is regulated by AR activation
inhibit AR and determine differentiation capabilities
Hypothesis-
Inhibition of ABCG2-Mediated Androgen Efflux Eliminates the Prostate Cancer Stem Cell Compartment

Aims
• Determine AR Function with ABCG2 Inhibition
• Determine Stem Cell Properties of ABCG2 Expressing Cells
• Determine the Role of ABCG2 in Stem Cell Maintenance
ABCG2 Expression in Side Population from Human Prostate Specimens

Mathew et al., 2009 Cell Cycle 8:1053-61
Testing Prostate Stem Cell Properties with Tissue Recombination

Embryonic Rodent Urogenital Tract

Graft *in vivo* under renal capsule
Tissue Recombination of rUGM and Putative Stem Cells

Side population assay

1.2% F

Trypsinize for 60 minutes at 37°C

Urogenital Epithelium

FACS isolated Epithelial cells

FACS isolated Epithelial cells attached to Rat Urogenital Mesenchyme

Graft under kidney capsule of SCID mouse

Harvest grafts after 8 weeks and analyze histologically

Rat Urogenital Sinus

Rat Urogenital Mesenchyme

Side population cells isolated from radical prostatectomy specimens

Cells + collagen = no growth

Cells + UGM = growth

H&E analysis of side population cells + UGM

FISH analysis for epithelial species identification, IHC analysis for differentiation markers
Serial Tissue Recombinations

Foster et al., 2013 PLoS ONE 8(1): e55062
Summary Aim 2 and Future Directions

Side Population Assay enriches for Prostate Stem Cells

Next-Determine if Side Population Assay enriches for Prostate Cancer Stem Cells
Hypothesis-
Inhibition of ABCG2-Mediated Androgen Efflux Eliminates the Prostate Cancer Stem Cell Compartment

Aims

• Determine AR Function with ABCG2 Inhibition
• Determine Stem Cell Properties of ABCG2 Expressing Cells
• Determine the Role of ABCG2 in Stem Cell Maintenance
ABCG2 Inhibition and Androgen Retention Leads to Delayed Growth Response

Felix unpublished data
Sphere Formation Assay

Sabnis unpublished data
Inhibiting ABCG2 Function Reduces Sphere Forming Capabilities

Primary mouse ventral prostate cells

CWR-R1 cells

Ko143 inhibits ABCG2>ABCB1>ABCC1


The Role of Androgens in Prostate


Apoptosis in the luminal cell compartment
ABCG2 null mouse model

- Abcg2 is deleted embryonically.
- Systemic deletion.
- By replacing exons 3 and 4 with a neomycin cassette via homologous recombination.

Aim 3: Abcg2 null prostate cells were more sensitized to reversan treatment than WT controls

Reversan inhibits ABCC1>ABCB1>ABCG2
Summary

ABCG2 is a marker of prostate (cancer) stem cells

Inhibition of ABCG2 forces prostate (cancer) stem cell differentiation

AR nuclear translocation
Elevated differentiation markers
Decreased cell growth
Decreased sphere formation
Future Directions

Determine if decreased cancer stem cells reduces tumor recurrence
Cancer stem cell treatment leads to no tumor relapse.
Clinical significance: Differentiation therapy for prostate cancer
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