Targeting mammary stem cells for breast cancer prevention

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Cancer Stem Cell Hypothesis

Cancers Arise from Cells with Dysregulated Self-Renewal

Cancers Are “Driven” By Cells With Stem Cell Properties
Stem cells as targets for cancer prevention

HYPOTHESES

If cancers arise in self-renewing cells then:

• Stem/Progenitor cell number may be a cancer risk factor
• Targeting tissue stem/progenitor cell self-renewal pathways may reduce cancer risk
• Tissue stem cell and self-renewal pathway components may be useful biomarkers for cancer prevention studies
characteristics of stem cells

• self renewal
• differentiation into multiple cell types

• types of stem cells
  embryonic
  adult

• cancer
Stem cells and breast carcinogenesis

- Stem cell
  - Quiescent pool of stem cell
  - Self-renewal
  - Differentiation
  - Early Progenitor
  - Late Progenitor
  - Luminal cells
  - Myoepithelial cells
  - Alveolar cells
  - Cancer stem cell
  - Self-renewal
“Mammosphere” Culture Systems

Reduction mammoplasties

Suspension culture (Serum-free, EGF, ...)

Mammospheres

Dissociated to single cells

N generation of spheres

A. Self-renewal in vitro (Suspension)
   - Mixed
   - Myoepithelial
   - Epithelial

B. Differentiation in vitro in 2-D culture (Collagen)

C. Differentiation in vitro in 3-D culture (Matrigel)

D. Differentiation and self-renewal in vivo (NOD/SCID mice)

Regeneration of the mammary gland in the cleared fat-pad

Dontu & Wicha, 2003
Dontu & Wicha, 2003
Weaver & Bissell, 1999
Dontu & Wicha, 2003
Kuperwasser & Weinberg, 2004
Liu & Wicha, 2006

"Mammosphere" Culture Systems
ALDEFLUOR + cells in Normal breast epithelium

6% of ALDEFLUOR positive cells in Normal breast epithelium

Ginestier et al
Cell Stem Cell 2007
ALDEFLUOR+ cells have stem cell properties

Culture in suspension

With DEAB

Without DEAB

Only ALDEFLUOR+ cells generate mammospheres in suspension
ALDEFLUOR+ cells have stem cell properties

Without DEAB

ALDEFLUOR+

CK18

SMA+

Luminal cells

Myoepithelial cells

CK18+

SMA+

Ginestier, 2007
Models of Tumor Heterogeneity

Cancer cells are heterogeneous, but most cells can proliferate extensively and form new tumors.

Cancer cells are heterogeneous, and only rare cancer stem cells have the ability to proliferate extensively and form new tumors.

Reya et al 2001
Tumor Formation by Human Breast Cancer
“Stem Cells”

20,000 CD44- CD24+ Cells

200 CD44+ CD24- Cells

Al Hajj et al PNAS 2003
Stem Cell Markers

- CD44
- CD24
- Aldehyde Dehydrogenase (Christophe Ginestier)
- Oct-4
- Bmi-1
- Nuclear B-Catenin
ALDEFLUOR+ population and tumorigenicity

5% to 10% of ALDEFLUOR+ population in Breast tumors
ALDEFLUOR+ population and tumorigenicity

ALDEFLUOR+ population regenerates heterogeneity of the initial tumor
Overlap between CD44+CD24- and ALDEFLUOR+ Populations

Ginestier et al
Cell Stem Cell 2007
Breast Cancer Development

Normal duct → Hyperplasia → In situ carcinoma → Invasive carcinoma

Luminal

Myoepithelial
Aldefluor + cells are invasive and metastatic
Cancer Stem Cells: Implications For Metastasis

CSC = Cancer Stem Cell
TDC = Terminally Differentiated Cell

1° Tumor

CSC with FULL malignant potential
Metastases in months to few years
Subsequently to other sites

CSC with PARTIAL malignant potential
Dormancy followed by Metastases after many years:

No Metastases
Secondary Oncogenic “Hits” and/or Changes in Microenvironment
Carcinogenesis

Organogenesis
Stem cell regulation

Inflammation

Dysregulation
Cancer
Stem Cell Generation

TUMOR Microenvironment
Tumor Microenvironment and the “CSC Niche”

- The **Microenvironment** ("Stem cell niche") contains fibroblasts, endothelial cells, inflammatory cells and **mesenchymal stem cells** recruited from bone marrow.
- The Microenvironment plays an important role in normal mammary development as well as tumor growth and metastasis.

  > Bone marrow derived mesenchymal stem cells are recruited to murine breast carcinomas (*Karnoub 2007*).

- **Cytokines** regulate breast cancer stem cells.
  > IL6 increases breast cancer stem cells (*Sanson 2008*).
  > IL8/CXCR1 axis regulates breast cancer stem cells (*Charafe-Jaffret Cancer Research 2009*)
Effects of Cytokines on breast cancer stem/progenitor cell population
Model of cytokine networks between MSC and breast cancer cells

S. Liu Canc. Res. 2011
Regulation of CSC’s by cytokine loops in the tumor microenvironment

Korkaya & Wicha JCI, 121(10):3804-09, 2011
Diet, exercise, and breast cancer risk

[Diagram showing the relationship between body fat, exercise, and cancer risk through various metabolic factors such as BMI, androgens, estrogens, leptin, adiponectin, TNF-α, IL-6, C-reactive protein, insulin, and inflammation.]
Stem Cell Equilibria

EMT

Quiescent
Vimentin +
Epcam (ESA) -
p53
p21
CD44+/CD24-
E Cadherin -

miR-93
TGF-b
Il6

MET

Cycling
Vimentin -
Epcam (ESA) +
p53
p21
Aldefluor +
E Cadherin +

Differentiation

44+/24-
Aldefluor+

Highly Tumorigenic
Recapitulation of Tumors Via CSC Metastasis

Breast Tumor

Micrometastasis

Macrometastasis

Aldefluor +

CD44+/CD24-

Invasive Edge of Tumor

Vasculature

Distal Healthy Organ

Distal Colonized Organ

Necrotic Zone

Time

EMT

MET
Strategic Approach to Preventive Therapeutics

- Toxicity
  - Genetic Background
    - Sporadic
    - Haplotype-SNP I1307K
    - Familial Syndrome (BRCA)

- Estimated Lifetime Risk
  - NUTRITION BOTANICAL BASED
    - <10%
  - PHARMACEUTICAL BASED
    - 10%-40%
  - Kakalara
    - >40%
Self Renewal and Differentiation Pathways In Breast Stem Cells

A Conceptual Link Between Hereditary & Sporadic Breast Cancers
Breast Tumor

IL-6R

gp130

IL-8

IL-8

IL-8

IL-8

CXCR1

AKT

GSK3

β-catenin

β-catenin

TCF

FAK

PI3-K

PTEN

Cancer stem cell

CXCR2

CXCL7

IL6

Frizzled

Wnt

LRP

DSH

Src

RTKs

HH

HH

HH

HH

HH

HH

GLI

PTCH

SMO

SUFU

γ-secretase

γ-secretase

γ-secretase

GSI

Notch

DSL

DSL

HES

GLI

Bmi-1

TCF

STAT3

STAT3

Stat3

JAK3

gp130

IL-6

IL-6

IL-6

IL-6

IL-6

IL-6

IL-6

Trastuzumab

Repertaxin

Perifosine

Monoclonal Abs

Targeting Self-Renewal Pathways in CSC’S

Mesenchymal Cells
Broccoli could stop breast cancer spreading by targeting stem cells

Broccoli contains sulforaphane, which seemed to kill off cancer stem cells in tests
**Figure 2.**

(A) Primary Mammospheres

B) Mammosphere Size

<table>
<thead>
<tr>
<th>Concentration of SF (µM)</th>
<th>MCF7</th>
<th>SUM159</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µM SF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 µM SF</td>
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<td></td>
</tr>
<tr>
<td>1 µM SF</td>
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<td></td>
</tr>
<tr>
<td>5 µM SF</td>
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Sphere formation normalized to control (%)

C) 2nd Passage

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<th>Concentration of SF (µM)</th>
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</tbody>
</table>

Sphere formation normalized to control (%)

Li CCR 2010
Figure 3.  

A

![Bar graph showing the percentage of ALDH-positive cells at different concentrations of SF. The x-axis represents the concentration of SF (µM), and the y-axis represents the percentage of ALDH-positive cells. The concentrations tested are 0, 1, and 5. The bars indicate a significant decrease in ALDH-positive cells with increasing SF concentration, with p-values of 0.003 and 0.008 for the comparisons of 1 vs. 0 and 5 vs. 1, respectively.]

B

![Flow cytometry plots comparing ALDH-positive cells in control and SF-treated samples. The control shows 3.01% ALDH-positive cells. With 1 µM SF, the percentage drops to 1.47%. With 5 µM SF, the percentage further decreases to 0.49%.]

Concentration of SF (µM)
A 1st Generation

- Control
- 50 mg/kg SF

Tumor volume (mm³)

Days after inoculation

B 1st Generation

- Control
- 50 mg/kg SF

Body weight (g)

Days after inoculation

C

% ALDH-positive cells

P = 0.003

Control

Treatment

D

Control

Treatment

Site Scatter

R1

R2

2.39%

1.08%
A 2nd Generation

Days after inoculation

Tumor volume (mm³)

Control group 1

Control group 2

SF group 1

SF group 2

P = 0.007

P = 0.0006

B

2nd Generation

Days after inoculation

% Tumor-free mice

Control

C

Control SF group
### A

<table>
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<tr>
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<th>SF (µM) 4 d</th>
<th>SF (5 µm)</th>
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<tr>
<td>β-catenin</td>
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<tr>
<td>cyclin D1</td>
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<tr>
<td>β-actin</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
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### B

<table>
<thead>
<tr>
<th></th>
<th>% dGFP-positive cells</th>
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<tbody>
<tr>
<td>TOP-dGFP</td>
<td>18.00 (P&lt;0.0001)</td>
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<tr>
<td>TOP-dGFP+SF</td>
<td>16.56 (P&lt;0.0001)</td>
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<tr>
<td>TOP-dGFP+BIO</td>
<td>2.13 (P=0.002)</td>
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<tr>
<td>TOP-dGFP+BIO+SF</td>
<td>3.04 (P=0.002)</td>
</tr>
</tbody>
</table>

### C

- **TOP-dGFP**
  - R1: 3.04%
- **TOP-dGFP+SF**
  - R1: 2.13%
- **TOP-dGFP+BIO**
  - R1: 16.56%
- **TOP-dGFP+BIO+SF**
  - R1: 6.92%
Stages of Cancer Progression and Its Suppression by Curcumin

**Constitutive activation of transcription factors**
- AP-1, NFκB
- TumorSuppressor Genes

**Modulation of Signaling**
- Wnt/β catenin
- Notch

**Overexpression of**
- Oncogenes
- Her 2
- Growth factors eg. EGF, PDGF
- Survival factors eg. Survivin, bcl 2, bcl-xl
- Cyclin D1

**Overexpression of**
- MMPs
- Cox 2
- Adhesion molecules
- Chemokines
- TNF

**Normal Cells** → **Tumor cells** → **Tumor growth** → **Tumor metastasis**

**Curcumin**

Aggarwal et al Anticancer Research 23, 2003:363-398
Using human stem cells as a screening system for chemopreventive efficacy, in vivo biomarker of efficacy and assay for mechanism


DMSO control

10 µM curcumin
Effect of Curcumin and Piperine on ALDH+ cells (%)
Breast Cancer Prevention Strategy
Curcumin/Piperine Targeting Stem Cells

Preclinical Research:
Stem cell self renewal, differentiation, signaling (ongoing)

→

Phase I Clinical Trial
Bioavailability/Toxicity (Spring 2010)

→

Phase II Clinical Trial
BRCA1 Patients
Curcumin/Piperine (2011)
Stem Cells in Breast Cancer
Risk reduction, Early detection, Prevention and Therapy

Risk reduction
- Polyphenols
  - Example: curcumin, genistein

Early detection
- Detection of expanded stem cells or secreted markers
  - Example: EZH2

Primary prevention
- Apoptosis or differentiation of initiated stem cells
  - Example: Vitamin D

Treatment
- Conventional therapy
  - Residual metastases → Recurrence

Targeting cancer stem cells
- Inhibitors of Hedgehog, Notch, Wnt signaling
  - Example: Gamma secretase inhibitor
  - Residual non-tumorigenic cells → Cure
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