Obesity, Stem Cells and Cancer

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University of Michigan
Discuss cancer stem cell model of carcinogenesis, metastasis, recurrence

Models for study of stem cell biology; limitations/strengths/needs

Screening system for potential interventions: dietary components eg. curcumin/piperine or drugs eg. Metformin/IL6 receptor Ab/Akt inhibitors

Complexities of human clinical trials assessing stem cell endpoints
Epithelial and mesenchymal stem cells in carcinogenesis, metastasis and recurrence

- Cancers Originate From Tissue Stem Or Progenitor Cells
- Cancers Are “Driven” By Epithelial Cells With Stem Cell Properties supported by mesenchymal stem cells in the niche
- Stem cell properties: self renewal, differentiation, epithelial to mesenchymal transition and mesenchymal to epithelial transition
- Stem Cell Hypothesis:

  Inhibit/kill cancer stem cells  →  Decreased vulnerability to metastasis/recurrence  →  Improved Survival
Development of the Mammary Gland and Mammary Tumors

Stem Cell

Early Progenitors
- Ductal Epithelial
- Alveolar
- Myoepithelial

Late Progenitors

Proliferating Tumor Stem Cell

Dontu, 2004
Example of dysregulated clonal expansion of stem cells

Red: ALDH1; Brown: ER
ALDH1 in breast epithelium from BRCA1-carriers

Ginestier, Wicha 2008
Breast Cancer Development

Normal duct  Hyperplasia  In situ carcinoma  Invasive carcinoma

Polyak, 2008
### Cancer Stem Cell Markers

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>CD44</th>
<th>CD24</th>
<th>CD133</th>
<th>ALDH</th>
<th>ESA</th>
<th>B1</th>
<th>Beta-6</th>
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<tbody>
<tr>
<td>Breast</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Colon</td>
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<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td></td>
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<tr>
<td>Pancreas</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Prostate</td>
<td>+</td>
<td></td>
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<td>+</td>
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<td></td>
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<tr>
<td>Brain</td>
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<td>+</td>
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<tr>
<td>Head/Neck</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
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</tr>
<tr>
<td>Melanoma</td>
<td>+</td>
<td>+</td>
<td></td>
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</tbody>
</table>

Wicha, 2009
Mammosphere Assay and ALDH breast stem cell marker

2D culture
- i) Serum/collagen
  - Mixed
  - Myoepithelial
  - Epithelial
- ii) Matrigel + prolactin
  - Triple lineage
  - Alveolar

3D culture
- iii) Matrigel + prolactin
  - Acinar structure
  - Ductal-acinar structure

Culture in suspension, serum-free, EGF, bFGF,

Mammospheres

Dissociated to single cells

N generation of spheres

Differentiation and self renewal in vivo

Don'tu, Wicha 2003
ALDEFLUOR+ population and tumorigenicity

ALDEFLUOR+ population regenerates heterogeneity of the initial tumor

Ginestier, 2007
ALDEFLUOR+ cells have stem cell properties

Ginestier, 2007
Supportive role of adipocytes and adipose derived mesenchymal cells in the niche in clonal expansion, proliferation, and dissemination of cancer stem cells

Adapted from image by Korkaya 2011
EMT and MET in tumorigenesis, recurrence and metastasis

Thompson, Haviv 2011
IGF1, Leptin, adiponectin and IL-6 are prosurvival cytokines for epithelial and mesenchymal stem cells

Adapted from Korkaya 2011; Pochampally 2009, Hursting 2010
Signaling pathways mediating the effect of the pro-inflammatory state of obesity upon cancer stem cells

Korkaya 2011
Proinflammatory, proangiogenic and hematopoietic cytokines secreted by human adipose derived stem cells

Kilroy, Gimble 2007
The Human Stem Cell Model and Cancer Biology

- A screening system for cancer treatment/risk reductive intervention efficacy
- Assay for mechanism, microRNA, profiling, protein sequencing, toxicant effects, nutrient/toxicant interactions
- In vivo biomarker of efficacy

DMSO control

10 µM curcumin

Kakarala, 2010
Regulation of stem cell self renewal and clonal expansion

**Figure 1.** Proposed pathways involved in breast epithelial stem cell self-renewal
Curcumin’s Anticarcinogenesis Mechanisms

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

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

**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, 2003
Piperine

- Isolate of piper nigrum and piper longum or black pepper/ hot peppers
- Inhibits
  - P-glycoprotein drug efflux
  - First pass enzymes, CYP 3A4
  - Intestinal conjugation
  - NFkB

Shoba 1998
Targeting Breast Stem Cells with the Cancer Preventive Compounds Curcumin and Piperine (1 spheres)

Effect of Curcumin and Piperine on ALDH+ cells (%)
Curcumin/Piperine’s Effect on Wnt Signaling
Piperine in human plasma after 50mg oral dose

Pharmacokinetics of Piperine in Two Healthy Male Volunteers

Kakarala et al. J Ag Food Chem 2010
Is dietary piperine bioavailable?

- 7 day food records, baseline and at 24 wks Mediterranean diet intervention
- Assess sources of piperine, peppers, black pepper, prepared foods, eggs
- Plasma samples to assay for piperine
  - at baseline and
  - at 24 wks

Kakarala, Cheng, Drs Djuric/Brenner
Piperine in human plasma!

- Mediterranean Diet
- 6 mo.s intervention
- 70 subjects, baseline only 9
- Blood drawn after 12 hr. fast
- Piperine detectable in all but 3 blood samples!!
- Range 6-500 ng/mL
- Mean 100.22 ng/mL, baseline 94.04 ng/mL, 6 mo.s
- No intervention effect

Kakarala, Cheng, Dubey, Djuric, Brenner
Metformin, TGF β and EMT
Induction of tumor suppressor Let 7 and suppression of miRNA 181a

Cufi, Menendez 2010, 2011
Assessing stem cell changes as biomarkers in human clinical trials eg. PARP inhibition in women with BRCA1/2 mutations

80 women with a germline BRCA1/2 mutation who are planning prophylactic bilateral mastectomies and are willing to take a PARP inhibitor for 28+4 days just before their surgery.

**SEQUENTIAL dose levels:**

- 20 women at **Level 1**: Veliparib 50 mg po BID d 1-28
- 20 women at **Level 2**: Veliparib 100mg po BID d 1-28
- 20 women at **Level 3**: Veliparib 150mg po qAM, 50mg qPM d 1-28
- 20 women at **Level 4**: Veliparib 200mg po BID d 1-28

Blood sample and Prophylactic bilateral mastectomies preceded in the OR by RPFNA. Tissue collected after clinical processing:

**Dr. Kakarala to assess stem and progenitor cell changes post dosing**

PI: J. Garber, DFCI, Susan G. Komen Foundation
M. Kakarala UM Site PI and Collaborator
Clinical Trial Design to Assess Cancer Stem Cell Endpoints

- Conventional imaging or molecular biomarkers such as Ki67 do not work
- Need large volumes of fresh/frozen tissue for dynamic endpoints
- Profiling potentially powerful tool
- Epigenetics key to assessing environmental/dietary exposure effects
- New tools in development for in situ imaging of individual cells for multiple markers
<table>
<thead>
<tr>
<th>Funding</th>
<th>Past</th>
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<tr>
<td>Innovative</td>
<td>Innovative concepts in stem cell research CC - $50,000</td>
<td>NIH K07 (9/10) 75% salary x 5 yrs. + 30K/yr research funds</td>
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<td>Trehan Foundation - $75,000</td>
<td>Komen Foundation Promise grant 20% salary x 5 yrs. + 75K/yr research</td>
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<td>NIH KL2 – 75% salary 9/07- 1/10</td>
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<td>VA 5/8 appt</td>
<td>VA CDA 100 % salary + 65K/yr research funds 9/10 awarded but declined</td>
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<td>research</td>
<td>Donations from Coady family</td>
<td>Komen Career Catalyst Award</td>
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- Drs. Max Wicha, Hasan Korkaya, and Breast Stem Cell Research Group

- Drs. Kathy Cooney, John Carethers, Eric Fearon, Robb Todd, Scott Gitlin, Lisa Newman, Jennifer Griggs, Daniel Hayes, Celina Kleer

- Drs. Sharon Hoerr, Arshad Majid, Judy Garber, Kornelia Polyak, Jenny Chang, Krishna Misra, Rajendra Mehta, Kishore Chaudhry, Sunita Saxena