CCR Breast Cancer Stamp Fund Program

Barbara K. Vonderhaar, PhD
Co-Chair Breast & Gynecologic Malignancies Faculty
Program Coordinating PI
Drugs that kill tumour stem cells

CSC

Drugs that kill tumour cells but not cancer stem cells

Tumour shrinks but grows back

CSC

Tumour loses its ability to generate new cells

Tumour degenerates
• Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer

• Project 2: Development and Characterization of Affibody®-Based Bioconjugates for Molecular Imaging and Targeted Therapy of HER2-Positive Breast Cancers
• **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

  – **Hypothesis:** Breast cancer stem cells can be characterized by unique cell surface markers that can be used for targeting molecular imaging probes and directing molecular therapy.
Project 2: Development and Characterization of Affibody®-Based Bioconjugates for Molecular Imaging and Targeted Therapy of HER2-Positive Breast Cancers

- **Hypothesis:** Delivery of therapeutic substances to HER2-positive breast cancers can be optimized using conjugates of HER2-specific Affibody® molecules with multifunctional thermosensitive liposomes.

Jacek Capala, PhD  
Robert Blumenthal, PhD  
Peter Choyke, MD
• **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

• **Specific Aims**
  
  • Identify, localize and characterize stem/progenitor cells in human breasts from normal and high-risk women, as well as those from malignant neoplasms,
  
  • Define a rigorous **functional assay** for the normal stem cell and its niche in humanized mouse mammary fat pads,
  
  • Develop **targeted imaging** methods that will allow the detection of breast cancer stem cells within tumors at high resolution, as a prelude to developing targeted treatments, and
  
  • Develop **improved chemotherapy** of breast cancer by targeting the breast cancer stem cell niche.
Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer

- Year 1: Expand clinical ductal lavage, duct endoscopy and normal and cancer breast tissue collections
- Year 2: Identify, localize and propagate mammary stem cells
  - Stem cell characterization in nipple fluids/tissues
  - Establish functional assays for normal stem cells in vivo
  - Gene expression profiling, proteomics & DNA methylation studies on stem cells
  - Validation of markers/tissue arrays
  - Targeted therapy studies in vitro and in vivo
    - First targeted stem cell imaging in vitro
    - First targeted stem cell imaging in vivo with radioisotopes and optical probes
    - First targeted stem cell imaging in vivo with iron based nanoparticles
- Year 3: Continuing development of imaging probes based on new stem cell targets
- Year 4: Targeted therapy studies in vitro and in vivo

Additional activities:
- Gene expression profiling, proteomics & DNA methylation studies on stem cells
- Validation of markers/tissue arrays
- Targeted therapy studies in vitro and in vivo
- First targeted stem cell imaging in vitro
- First targeted stem cell imaging in vivo with radioisotopes and optical probes
- First targeted stem cell imaging in vivo with iron based nanoparticles
- Continuing development of imaging probes based on new stem cell targets
• **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

• **PIs**
  - **Breast Stem Cells:** Barbara K. Vonderhaar Ph.D., NCI (Co-Chair BGMF), Gilbert Smith, Ph.D., NCI, John Ortaldo, Ph.D., consultant, NCI retired, David Salomon, Ph.D., NCI, Robert Callahan, Ph.D., NCI, Ron McKay, Ph.D., NINDS; Michael Dean, Ph.D., NCI, Joshua Zimmerberg, M.D., Ph.D., NICHD, Leonid Margolis, Ph.D., NICHD, Michael Gottesman, M.D., NCI, John Niederhuber, M.D., NCI;
  - **Imaging:** Peter L. Choyke, M.D., NCI, Martin Brechbiel, Ph.D., NCI, Hisataka Kobayashi, M.D., Ph.D., NCI, Catherine Chow, M.D., CC, David Thomasson, Ph.D., CC, Brad Wood, M.D., CC, Eva Baker, M.D. Ph.D., CC.
• **Project 1: Isolation, Propagation, Characterization, and Imaging of Breast Cancer Stem Cells to Improve Early Diagnosis and Therapy of Breast Cancer**

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• Our immediate goals for the first year are to:

  – Catalog **existing samples** from various protocols, amend existing protocols and **write new protocols** to expand the tissue collection to include core biopsies, ductal lavage and duct endoscopy samples from normal and high risk patients. This includes establishing a database to collect clinical and biological data from the patient samples;

  – **Standardize processing** of samples and preparation of dispersed cells from tissues, tumors and pleural effusions;
• Our immediate goals for the first year are to:

  – **Optimize growth conditions in vitro** for human breast stem cell (all sources) as mammospheres, as monolayers at clonal density and in the rotating bioreactor;

  – **Optimize the in vivo growth conditions** for normal breast epithelial cells in humanized NOD/SCID mouse mammary fat pads and for cells from pleural effusions and primary breast tumors in NOD/SCID mice; and

  – **Validate** the practicality of amplification of cDNA from 100, 1000 and 10,000 cells for **expression array** analysis and establish a plan to analyze **protein expression analysis** of total and cell surface proteins.
Sources of Tissue

- Suburban Hospital (SH)
- NIH Clinical Center (CC)
- Bethesda Naval Hospital
- (Walter Reed Hospital)

NOD/SCID mice

- NIH colony
### Suburban Samples- Updated 5/22/06   ** from biopsy**

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STEM CELLS

• Currently defined by function

• Plan to define at molecular level
  • Cancer
  • Normal
Big Challenge

How to maintain and/or expand the stem cell population
STEM CELL

Self-Renewal

Hedgehog
TGFβ Family
EGF Family

Notch
Wnt
LIF

Minimize differentiation

Estrogens & Progesterone
Prolactin
Others TBD
Normal Tissue

Functional assay
Humanize the mouse mammary fat pad

Fibroblasts
(into cleared fat pad)
Immortalized-GFP

4 weeks

Epithelial cells
& Fibroblasts

Outgrowth/isolation
molecular analysis

8-12 weeks
Humanize the mouse mammary fat pad

4 weeks

Fibroblasts (into cleared fat pad)
Immortalized-GFP
Normal
  Intralobular
  Interlobular
Tumor
High risk tissue?

Epithelial cells & Fibroblasts
8-12 weeks
Outgrowth/outgrowth
molecular analysis
Normal Human Breast

- Intralobular stroma
- Interlobular stroma
LCM

Microarrays

Surface markers to distinguish fibroblast types
Fibroblasts

Normal Fibroblasts
Growing from chunk on plastic

Passage 2 on plastic

Tumor Fibroblasts
Growing from chunk on collagen
Big Question

• Will fibroblasts growing on plastic express the surface markers that distinguish the two normal populations?
a. Mammosphere

b. Differentiation along the three adult mammary cell lineages

c. Differentiation in 3D culture

d. Differentiation into functional structures

e. Self-renewal
**Experimental System:**
Staged series of human breast-derived cell lines representing different steps in cancer progression

*Dr. Fred Miller, Barbara Ann Karmanos Cancer Institute, Detroit*

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<td>“MCF10AT1k.cl2” Ha-ras transfected MCF10A</td>
<td>“MCF10Ca1h” Line derived from xenografts of MCF10AT</td>
<td>“MCF10Ca1a.cl1” Line derived from xenografts of MCF10AT</td>
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<td>Well-differentiated tumor</td>
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Increasing malignancy
Mammospheres from MCF10A M-I cells

7-10 days in culture
Tumors & Pleural Effusions

Functional assay
-5 days: etoposide & E2 pellet

3-5 x 10^6 cancer cells or PE

up to 8 months

Tumors/isolation molecular analysis

Tumors

SH-1  →  3/5
CC-1 →  0/5
T47D →  5/5
Cells isolated from SH1 tumors recapitulate the full spectrum of cell types
No tumors after 8 months
Cancer cells or PE

Presort: CD44^+/CD24^+ or CD44^+/CD24^-/low

Cells:
- 200
- 500
- 1K
- 10K

up to 6 months

Tumors/isolation molecular analysis
In vitro gives good tumorspheres
SH 12 after 5 months

up to 6 months

Tumors/isolation molecular analysis

Cancer cells or PE

Presort CD44+/CD24+ or CD44+/CD24−/low

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In vitro gives good tumorspheres

SH8
SH 8 after 5 months

up to 6 months

Tumors/isolation
molecular analysis

Cancer cells or PE

Presort

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No tumors from CD44^-/low/CD24^+
MCF10A M-IV cells

• Parental cells are a mix
  • CD44+/CD24+ and CD44+/CD24low/-

• In vitro gives good tumorspheres at 7-10 days

• In tumorsphere selection media at 24 hr cells are
  • CD44+/CD24+

• Into NOD/SCID mice
  • 1K cells give 100% tumors in 3 weeks

• Grow out on plastic as colonies
  • CD44+/CD24+
  • CD44+/CD24low/-
Conclusion

• CD44\(^+\) appears to be a marker for tumor formation in vivo

• CD24\(^{\text{low/-}}\) does not appear to correlate with tumor formation in vivo

• New/additional markers are needed to better define the tumor stem cell on the molecular level
  – Tumorspheres from PE
  – Microarrays
  – MCF10A IV cells and known markers