Adult and Cancer Stem Cells during Development and Tumor Progression

Zena Werb
Department of Anatomy
University of California, San Francisco

Stem Cell Models for Environmental Health
National Academy of Sciences, Washington DC
6/3/10
Adult tissues that regenerate have both rapidly and slowly proliferating stem cells.
Visvader G&D 2009
The mouse mammary gland is an good model to investigate adult stem cells.
Important advantages for using adult stem cells from mammary development and breast cancer

1. Can regenerate the natural tissue at 1/25-1/50 cells, including structure and function. For mammary gland this is important for functional assays. Do not form teratomas if not differentiated

2. The role of the different cellular subtypes can be assessed, e.g., luminal, myoepithelial, stem cell, alveolar secretory cells.

3. The functions of the microenvironment/stroma/inflammation can be assessed

4. In vitro assays can be linked with in vivo growth

5. Assays be used to involving treatment in vivo or in vitro can be used analyze effects of environmental agents

6. Assays are amenable to both mouse and human cells

7. Can adult stem cells be reprogrammed?
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
Mammary stem cell hierarchy during mammary development
Mammary Epithelial Cell Types

Luminal
*Keratin 18 (Green)*

Myoepithelial
*Keratin 14 (Red)*

Luminal Cell Subtypes

*Keratin 6*  
*Sca-1*

Welm, B., et al., Dev Biol. 245:42-56
Undifferentiated mammary epithelial progenitor cells are GATA-3 negative

Adapted from Nature Cell Biology (2007) 9:201-9

Kouros-Mehr et al., Cell (2006) 127:1041-1055
Primary Mammary Epithelial Cells Contain a CD24/CD49f Cell Population that contains stem cells

Ma-CFC will grow as cysts in culture

MRU will form colonies in culture and grow in vivo into a full ductal tree

Myo are myoepithelial cells

Stingl, J., et. al., Nature 2006
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
Mammary epithelial stem cells can be studied in vivo because even a single stem cell can repopulate the mammary gland.

Transplantation of mouse primary MECs infected with lentiviruses that express red or green fluorescent proteins into the cleared mammary fat pad.

Welm et al., CSC 2008
Quiescent Cell Populations are Maintained in the TEB

Transplantation of mouse primary MECs infected with lentiviruses that express red or green fluorescent proteins into the cleared mammary fat pad.

Welm et al., CSC 2008
Patterning in Lobular-Alveoli During Pregnancy

Welm et al., CSC 2008
Xenografts of human organoids form ductal structures

Human organoids mixed with human fibroblasts in matrigel transplanted into Nod/Scid mice 10 wk

Audrey Brenot
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
Modeling normal and tumor mammary epithelial morphogenesis in vitro: complex niches

In Vitro Analysis

1 Mouse
10 Glands

Dissociate into Cells, Reaggregate and Culture Epithelial Pieces in 3D

Culture 1-5,000 Epithelial Pieces in 3D

Genetic or Pharmacological Interventions

In Vivo Analysis (Histology, Biochemistry, etc)

1. Compare different epithelia in same conditions.
2. Compare same epithelia under different conditions

Welm et al., Cell Stem Cell 2:90-102, 2008

Andy Ewald  Audrey Brenot  Bryan Welm

Long Term Parallel Timelapse Imaging

Long Term Parallel Confocal Imaging

Friday, June 11, 2010
Primary Mammary Epithelial Cells form Cellular Aggregates in Suspension

Single Cell Suspension

Overnight Low Adhesion Plate

Primary Aggregates

Red Keratin 18

Green Keratin 14

Green Keratin 6

Blue Nuclei

Blue Nuclei

Blue Nuclei

FACS Analysis = 80% K18, 15-20% K14

Bryan Welm

Friday, June 11, 2010
Primary Mammary Aggregates Contain the CD24/CD49f Cell Population and Can Branch in Matrigel


Bryan Welm
Why Use 3D Culture?

Primary Mammary Epithelial Cells in 2D or in 3D Matrigel Culture

Mammary Epithelium in Matrigel, 2.5 nM FGF2, Filmed every 15 minutes for 46 hours

Andrew Ewald
Ductal elongation in normal mammary epithelium proceeds through a multilayered elongation front.

Red = All Cells (CellTracker Dye)
Green = Mosaic Cell Label (GFP knockin to Sca-1 locus)

Andrew Ewald et al., Dev. Cell 2008
In Vitro Program of Mammary Branching Morphogenesis
Epithelial Fragments ("Organoids") in 3D Culture

Remodeling and Lumen Formation

New Duct Initiation and Extension

Friday, June 11, 2010
Pattern Results from the Coordinate Motility of 2 Cell Types

3D Reconstruction

Red = All Cells
Green = Myoepithelial Cells

Red = CellTracker Dye, Labels Cytoplasm
Green = Keratin14-GFP-actin (courtesy of E. Fuchs)

Ewald et al. Dev. Cell 14: 570-581
In Vivo, Bilayered Mammary Ducts are Elongated by a Multilayered, Incompletely Polarized Epithelium

Andrew Ewald et al., Dev. Cell 2008
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?

2. What assays are available to analyze adult stem cell function in vivo?

3. What assays are available to analyze adult stem cell function in culture?

4. Can these assays be used to analyze effects of environmental agents?

5. How do insights about normal mammary stem cells in development help us think about breast cancer?

6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
Formation of the branched organoid structures is sensitive to perturbation of several critical pathways: A potential assay for environmental agents

Cultures in Matrigel. Drugs were added at 24 h and organoids were stained at 7 d.

Andy Ewals
Mammary organoids undergo improper morphogenesis when treated with some environmental agents.

Cultured in Matrigel for 5d with 2.5 nM FGF2.

Control

butylbenzylphthalate 10μM

bisphenol A 10μM

hydrocortisone 100nM

George Lemieux

Friday, June 11, 2010
Putting reporters (e.g., vimentin-GFP) into MMEC allows high throughput analysis if responses, in this case epithelial to mesenchymal transition (EMT)

Radisky et al., Nature 2005
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
There are common themes for development and tumor progression

Normal Mammary Duct

Adipocytes

Collagenous ECM

Zona Occludens 1, Beta-catenin, Nuclei

MMTV-PymT Mammary Hyperplasia

Adipocytes

Collagenous ECM

Zona Occludens 1, Beta-catenin, Nuclei

MMTV-PymT is a mouse model of human luminal type breast cancer.

Adult tissues have myeloid cells (monocytes, macrophages, dendritic cells, PMN), blood vessels and epithelium in the normal mammary gland

c-fms-EGFP; β-actin-ECFP

900x real time,

Mikala Egeblad
Tumors have myeloid cells migrating in extracellular matrix rich tumor areas at the stomal border

Velocity:
4.9 $\mu$m/min $\pm$ 0.08 N=544
(moving cells only)
Compare and contrast normal and neoplastic epithelia

Normal Duct

MMTV-PyMT Carcinoma

Different:
- Mutational State
- Tissue Architecture
- Extracellular Matrix
- Stromal Cells

MMTV-PymT is a mouse model of human luminal type breast cancer.

Compare and contrast normal and neoplastic epithelia, in the same microenvironment: a basement membrane

Normal Duct

MMTV-PyMT Carcinoma

Different epithelia into same microenvironment

Matrigel + FGF2

Andy Ewald
Audrey Brenot
in preparation

Friday, June 11, 2010
Tumors evolve to have a tumor initiating subpopulation. How are these related to stem cells?
Normal mammary stem cells and cancer stem cells
Are “tumor initiating cells” stem cells?

- They self renew
- They are clonigenic
- They have lost many features of differentiation
- In some cases they may give rise to multiple cell types
- They have migration potential
- They are not necessarily rare
- They may not arise from stem cells

BUT

- Are they resistant to chemotherapy?
- Is there a tumor stem cell niche?
Mammary epithelial cells transduced with a Wnt1 lentivirus proliferate but do not form lumens, branch poorly and have few myoepithelial cells.
Wnt1 Transduction in MMEC Leads to Hyperplasia and Carcinoma in vivo

Welm et al., Cell Stem Cell 2008

Friday, June 11, 2010
Loss of GATA-3 marks the loss of differentiation in breast cancer.
Important questions regarding stem cells in mammary development and breast cancer

1. What is currently known about adult stem cells during mammary development?
2. What assays are available to analyze adult stem cell function in vivo?
3. What assays are available to analyze adult stem cell function in culture?
4. Can these assays be used to analyze effects of environmental agents?
5. How do insights about normal mammary stem cells in development help us think about breast cancer?
6. Does thinking about cancer as a stem cell disease help us make progress in breast cancer?
If GATA-3 negative tumor cells are stem cell-like, they should:

1. Have stem cell-like markers

2. Self renew-tumor initiating cells

3. Give rise to multiple cell types

4. Show other properties of stem like cells, like increased motility

Tumor progression involves the expansion of GATA-3 negative progenitor-like cells like those described by Kouros-Mehr, *Cancer Cell* 13:141-51 (2008).
Progenitor-like cells have enhanced migration ability (transwell migration)

Both in normal mammary glands and early tumors

Progenitor-like cells are able to give rise to both luminal and myoepithelial cells when GATA3 is restored.

Future Directions

Are these cancer stem cells?
What signals cause these clones to expand?
Can we target these cells with cytotoxic agents?
Can we induce differentiation and then target
• If CSC are the only cells that can expand then they should be the target of therapies

• Most cells in tumors are quiescent. Quiescent cells are resistant to anti-proliferative drugs. Do CSC cycle slowly like stem cells?

• The proportion of cells with CSC markers increase after irradiation of glioma

• Stem cells and CSC have increased ABC transporters and may efflux drugs (Hoechst 33342)

Seth Bechis
Audrey Brenot
Peter Dijkgraaf
Mikala Egeblad
Andrew Ewald
Hosein Kouros-Mehr
George Lemieux
Pengfei Lu
Vicki Plaks
Mark Sternlicht
Bryan Welm

Funding:
National Cancer Institute
Stewart Trust