Of Extracellular Matrix, The Genome and the Microenvironment: No Cell is an Island

Mina J. Bissell
Division of Life Sciences
Lawrence Berkeley National Laboratory
University of California, Berkeley, CA
FASEB, Excellence in Science Award, San Diego, April 8, 2008
The Warburg Theory:

Tumor cells are always more glycolytic than Normal cells

But how do we define glycolysis???
The pattern changes regardless of whether the cells are normal or virus-transformed

(Bissell, et al. 1972, JNCI)
Steady-state apparatus for animal cells (SAFAC)
Comparison of metabolite patterns of chick embryo fibroblasts and chick embryo liver

Fibroblasts

Liver cells

5.5 mM U-$^{14}$C glucose, 1 hr
Transformed cells have both the higher rate of glucose intermediates than 'normal' cells and higher steady-state.

![Graph showing comparison between transformed and normal cells over time.](image-url)
Transfer of tritium from the 1-position of GAP to the $\beta$ position of NADH

(Bissell et al. 1976, Science)
Glycerol Phosphate Shuttle

“The glycerol phosphate shuttle is shown not only to be present and functional in virus-transformed cells, but its level is higher than in normal cells in culture. The increased aerobic glycolysis that has been demonstrated for these cells after transformation, therefore, is not due to an impairment of hydrogen transfer pathways.”

(Bissell et al. 1976)

Science
Scanning electron micrograph of normal and transformed chick cells in Meth C suspension

Normal cells

Transformed cells

Meth C suspension, 24 hrs

Bissell, 1976
Cell rounding (shape) reduces the rate of glucose uptake, but the difference between normal and transformed cells remain
$^{14}$C – carbon flow into glucose metabolites is drastically reduced as a function of reduced glucose concentration.

Bissell et al. (1977) Cell Reg. CHX: cycloheximide
Glucose uptake regulates the pattern of glucose metabolism. The data indicated that the level of glucose in the medium could override the metabolic pattern regardless of whether the cells were normal or virus-transformed.
RSV Tumor in Chicks
RSV Expression in the Embryo

Dolberg and Bissell, Nature, 1984
Dolberg et al, Science, 1985
Siewke et al, Science, 1990
Stoker et al, JCB, 1990,
Siewke and Bissell, Critical Reviews in Carcinogenesis. 1994
Thus Context (the microenvironment) Determines What Even a Potent Oncogene Can Do

But how???
Questions:
1-How is tissue specificity maintained?
2-How does one study the problem in mammals?
3-How is the program lost in cancer and aging?
4-How can one use the information for therapy?
The Mammary Gland as an Experimental “Organism”

It develops again and again in adult female mammals just like an organism!
The Pregnancy Cycle in the Mammary Gland

- Virgin branching
- Pregnant growing
- Lactating differentiated
- Involuting apoptosing

Beta. Casein
V  P  L  I
Structure of the mammary gland:
Ducts and acini
Structural Organization of Mammary Epithelial Cells

In vivo

“in vitro”: on tissue culture plastic

(Joanne Emerman)
Formation of acini in a laminin rich extracellular matrix (ECM)

*Matrigel*

*In vivo*

*3D culture*

(Hynda Kleinman)

(Barcellos-Hoff et al.1989)
Milk
got milk?
Model Of Dynamic Reciprocity:
‘The ECM signals to the chromatin via ECM receptors and the cytoskeleton. The chromatin responds by reorganizing, and signals back, and ...continuously. Thus the unit of function in higher organisms is the cell plus its ECM and the microenvironment’.

(Ecm et al, J. Theoretical Biol.1982)
(Discovery of ECM-response element)

(Both biochemical and mechanical signaling are involved)

(Laminin)

(β-Casein)

(Both biochemical and mechanical signaling are involved)

(ECM signaling directs chromatin structure)
The Influence of the ECM on nuclear organization, chromatin structure, and transcription of ECM-responsive genes.
Reactivation of STAT5 correlates with β-casein expression

(Ren Xu, Submitted)
Model Summarizing The Role of ECM on Chromatin organization

- β-casein
- β-globin
- Brg1
- C/EBPb
- STAT5
- RNA Pol II
- AcH4
- GR

Nuclear Matrix/Nucleoid

+ Laminin111

Xu et al, JBC, 2007)
Conclusion

Extracellular Matrix and Chromatin Remodeling Are Essential for Continued Activation of transcription factors (Stat5) and Organ-Specific Gene Expression in the Mammary Gland
Are these concepts applicable also to human breast?
Lone Ronnov-Jessen          Ole Petersen          (Copenhagen)
The 3-Dimensional Basement Membrane Assay

Acinus in Tissue Explant

Cultured Primary hMEC

hMECs in rBM

Petersen/ Bissell, 1992
The mammary acinus *in vivo* is surrounded by myoepithelial cells and not lrBM!
Luminal Epithelial Cells (LEP) in:

lrBM

Collagen I
Laminin-1 (111) is essentially lost in many human breast cancers.
Can these 3-D concepts be used for an assay to distinguish normal and malignant cells from each other?

(Petersen/Bissell, PNAS, 1992)
The HMT-3522 Breast Tumor Progression Series

(Ole Petersen, Per Brian et al.)

Human Breast Cells

“S1”
Non-malignant

Comparison
If tissue structure is the message, we can hypothesize:

1-tumor cells with abnormal genomes should be capable of becoming phenotypically normal if the structure is restored.

2-destruction of tissue structure by itself could be a carcinogenic event
“Normal”  

Malignant
“Normal”  Malignant  “Reverted”

(Weaver et al., JCB, 1997)
T4-2, malignant

T4-2, Reverted, phenotypically normal
Reversion is reversible

Phenotype is dominant over genotype
Growth and malignant behavior are regulated at the level of tissue organization.

Integration of signaling: 3D vs. monolayer

- **β1-integrin**
- **EGFR**
- **EGFR (act.)**
- **Loading control**

Samples: S1, T4, T4 + A1B2, T4 + IgG, T4 2D + A1B2
Expression of receptors involved in adenovirus infection in 2D and 3D

(CAR)

αv-integrin

(S1 T4 T4R)

2D 3D

What then is an oncogene and what does addiction mean?

Can revert metastatic cell lines as well, but need two of the inhibitors.
If so, what about metabolic pathways?
mRNA Expression of glycolytic enzymes

(Yasuhito Onodora)
mRNA Expression of glycolytic enzymes
mRNA Expression of glycolytic enzymes
mRNA Expression of glycolytic enzymes

Arbitrary unit

S1_3D
T4_3D
T4I1B2_3D
T4mAb_3D
T4Tyr_3D
T4PD_3D

HK1, HK2, HK3, HK4, GPI, PFKP, PFKM, PFKL, ALDOA, ALDOB, ALDOC, TPI1, GAPDH, GAPDHs, PGK1, PGK2, PGAM1, PGAM2, ENO1, ENO2, ENO3, ENO1L, PKM2, PKLR, LDHA, LDHB, LDHC, LDHAL6B
mRNA Expression of glycolytic enzymes
mRNA Expression of glycolytic enzymes
mRNA expression of citric acid cycle enzymes (composite)
mRNA expression of citric acid cycle enzymes
mRNA expression of citric acid cycle enzymes
mRNA expression of citric acid cycle enzymes
mRNA expression of citric acid cycle enzymes
mRNA expression of citric acid cycle enzymes
Polarization of acini (\(\alpha 6\)) integrin by 2-DG
Proof of Principle: Do all Pathways lead to Rome?!  

1- Response to chemotherapeutic agents: Weaver et al. Cancer Cell, 2002  

2- Utility of inhibitory antibodies to $\beta_1$ integrin Park et al. Cancer Research Feb. 2006  

3- Using the logic of the acini to find new targets for therapy Fournier et al. Cancer Research, Aug. 2006
Pro-apoptotic stimuli

<table>
<thead>
<tr>
<th>Signaling proteins</th>
<th>Chemical signals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trail</td>
<td>Etoposide</td>
</tr>
<tr>
<td>Fas-R</td>
<td>Cytochalasin B</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Paclitaxol</td>
</tr>
</tbody>
</table>

(Weaver et al. Cancer Cell, 2002)
Only non malignant cells within mammary acini are resistant to receptor-linked and chemically-induced apoptosis.

Monolayer
“nonpolarized”

2D-N growing
apolar

3D-N quiescent
(polar)

2D-T growing
(apolar)

3D-T growing
(apolar)

rBM
3D

Immune Chemical

Immune Chemical

3D
Phenotype overrides genotype in response to apoptotic stimuli

- **3D-N polar**
- **3D-N apolar, growing**
- **3D-T apolar**
- **3D-T polar**

**Apoptotic Labeling Index**

- No treatment
- Fas-R
- Etoposide
Luminal Epithelial Cells (LEP) in:

IrBM

Collagen I
Interaction with BM is necessary for apoptotic protection, and growth is not necessary.
Summary
1-Phenotype overrides genotype in response to apoptotic agents
2-Hemidesmosome and α6/β4 integrins, and NFkB are involved in polarity-induced apoptotic resistance in human mammary epithelial tumor cells
3-These findings have important implications for dormancy, drug resistance, and chemotherapy
“All OF A SUDDEN, STUDYING CANCER CELLS IN TWO DIMENSIONS APPEARS TO BE QUAIN'T, IF NOT ARCHAIC”!

Jacks and Weinberg,
Cell,
Mini Review, Dec. 2002
If tissue structure is the message, we can hypothesize:

1-tumor cells with abnormal genomes should be capable of becoming phenotypically normal if the structure is restored.

2-destruction of tissue structure by itself could be a carcinogenic event
Do Basement Membrane and Tissue Architecture Regulate Form and Function in Vivo as well??

*If so, How?*
The Pregnancy Cycle in the Mammary Gland

Virgin branching

Pregnant growing

Lactating differentiated

Involuting apoptosing

B Casein

V P L I
Milk protein promoter (WAP)-Stromelysin1 (MMP-3) construct to make “conditional” transgenic

Collaboration with Zena Werb, UCSF

Carolyn Sympson et al. JCB,1994
Basement membrane is important for tissue – specificity in vivo

Control

Transgenic

Beta casein
MMP-3 mice develop mammary tumors as they age

Does MMP 3 select already mutated cells, or does it by itself cause genomic instability?
<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Line(s)</th>
<th>Chr 4</th>
<th>Chr 7</th>
<th>Chr 6</th>
<th>Chr 15</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5, 20, 21</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Hyperplasias</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1) Moderate</td>
<td>20</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2) Severe</td>
<td>21</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#3) Atypical</td>
<td>5</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#4) Atypical</td>
<td>5</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#5) Atypical</td>
<td>5</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>Carcinomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1) Adenoca.</td>
<td>5</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#2) Adenoca.</td>
<td>21</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#3) Metastatic</td>
<td>20</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#4) Mixed</td>
<td>20</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#5) Mixed (Carc.)</td>
<td>21</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
<tr>
<td>#5) Mixed (Sarc.)</td>
<td>21</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>
MMP-3-induced genomic instability in mouse mammary epithelial cells

**Graph:**
- **Y-axis:** colonies/1E5 cells
- **X-axis:** days of incubation prior to PALA selection

**Lines:**
- SCp2
- SCp2+MMP3

**Images:**
- Untreated
- MMP-3 only
- MMP-3; PALA

**FISH**
Rac1b levels depend on MMP-3 treatment

QPCR primers selective for Rac1b

MMP-3 treatment and wash experiment

Graph showing Rac1b/GAPDH levels over time with MMP-3 treatment and wash.
MMP-3 causes mitochondrial depolarization (JC-1)

SCp2

SCp2+MMP3

(green is indicative of mitochondrial depolarization)
Superoxides do it as well
Conclusion

MMP3 causes genomic instability and epithelial to mesenchymal transition de novo through Rac1b, a potent spliced form of Rac1 (cell shape change)

Nature, July 7th issue, 2005
Organ specificity is plastic, dynamic and context-dependent; homeostasis is all about balance in signaling and maintenance of tissue structure and not absolute.
Mais...

Ce qui est vrai pour Escherichia, l’est-ce vraiment pour moi ?

hum....
How about the ductal branching??!

Human

Mouse
The positional control of branching morphogenesis

What prevents the branches from going clear through the fat pad?!

Could we use the normal to understand invasion and metastasis?
Aim: Develop a new branching assay that:

- Specifies \textit{a priori} and uniformly the locations of cells in 3D
- Allows quantification of the positions of cells before and after branching
- \textit{Allows analysis of statistically significant results}
Why mammary gland branches here and not there?!

Celeste Nelson

(Jamie Inman)
Micropatterning

Clusters of mammary epithelial cells patterned in 3D collagen

Method to quantify the position of cells

Phase contrast  Nuclei  Probability map

Binarize  Stack 50 images  Index color

Quantifying branching morphogenesis

in collagen + EGF
Day 0
Phase image

Day 2
Phase image

Probability map (nuclei) in collagen + EGF
Day 0 | Day 2 | Day 4
---|---|---
Phase image
Probability map (nuclei) in collagen + EGF
in collagen + EGF
Branching (locally) depends on shape of structure (globally)

in collagen + EGF
Branching pattern consistent with gradient of inhibitors

in collagen + EGF (24 hrs)

COLLABORATION:
Martijn van Duijn & Dan Fletcher
Martijn van Duijn & Dan Fletcher

Predicting the spatial pattern of branching from analytical results

in collagen + EGF (24 hrs)
Disrupting TGF\(\beta\) signaling yields unpatterned branching and vimentin promoter activation.

Cells or organoids in collagen + EGF
The vimentin promoter is activated specifically in regions that later branch.

Mammary cells/vimGFP in collagen + EGF (8 hrs)
Water penetrating a beach: from NASA satellite

Coral
What an opportunity for the young and the passionate old! A whole new horizon to explore. Go to it!
In memory of Sonia Maria Mueller, one of the most beautiful, courageous and feisty people I have known, 1968-2003
Chloe, When she was five
NEVER, EVER THINK OUTSIDE THE BOX.
Deconstructing microenvironment guided progenitor cell fate decisions in mammary gland

Mark LaBarge

Bissell Laboratory at Lawrence Berkeley National Laboratory
Shifts in keratin phenotype are relative to (p) progenitors on collagen I alone. Microenvironments of overlapping composition generate trends in keratin expression that identify effectors of cell fate.
Lawrence Berkeley National Laboratory
The Advanced Light Source
Genee Lee

Dinah Levy

Keith Vann

Eva Lee

Wei

They get younger every day!
Funding Acknowledgement

Office of the Biological and Environmental Research, (OBER), DOE
(Thank Goodness for multiple sources of funds!)

National Science Foundation

National Cancer Institute

Dept. Defense, Innovator, BCRP

Calif./UC Breast Cancer Program
Conclusion

*In vivo*, it is myoepithelial cells that make the laminin-1 and signal for tissue polarity.

Thus cell-cell and cell-ECM interactions are crucial in establishment of correct tissue structure and function.

As such a number of us have argued that myoepithelial cells are the ultimate tumor suppressors in the breast.
Where to Now?

Cancer is an organ specific, polymorphism-dependent disease. We do not have enough patients (luckily) and resources (unluckily!) to do the required testing of combination drugs for different classes of patients, and 2D models do not reflect the realities in vivo. Thus need alternative models of human (in addition to mouse) disease.
Proof of Principle:

1- Response to chemotherapeutic agents: Weaver et al. Cancer Cell, 2002

2- Utility of inhibitory antibodies to $\beta_1$ integrin Park et al. Cancer Research Feb. 2006

3- Using the logic of the acini to find new targets for therapy Fournier et al. Cancer Research, Aug. 2006
Pro-apoptotic stimuli

**Signaling proteins**
- Trail
- Fas-R
- TNF-\(\alpha\)

**Chemical signals**
- Etoposide
- Cytochalasin B
- Paclitaxol

(Weaver et al. Cancer Cell, 2002)
Only non malignant cells within mammary acini are resistant to receptor-linked and chemically-induced apoptosis.

Monolayer
“nonpolarized”

2D-N growing
apolar

2D-T growing
(apolar)

3D-N quiescent
(polar)

3D-T growing
(apolar)

rBM
3D

Apoptotic Labeling Index

Immune Chemical

Immune Chemical

Apoptotic Labeling Index

3D

* * *
Phenotype overrides genotype in response to apoptotic stimuli

- **3D-N polar**
- **3D-N apolar, growing**
- **3D-T apolar**
- **3D-T polar**

Apoptotic Labeling Index

- **No treatment**
- **Fas-R**
- **Etoposide**

* indicates statistical significance.
Luminal Epithelial Cells (LEP) in:

IrBM

Collagen I
Interaction with BM is necessary for apoptotic protection, and growth is not necessary
Summary

1- Phenotype overrides genotype in response to apoptotic agents
2- Hemidesmosome and α6/β4 integrins, and NFκB are involved in polarity-induced apoptotic resistance in human mammary epithelial tumor cells
3- These findings have important implications for dormancy, drug resistance, and chemotherapy
“All OF A SUDDEN, STUDYING CANCER CELLS IN TWO DIMENSIONS APPEARS TO BE QUAIN'T, IF NOT ARCHAIC”!

Jacks and Weinberg, Cell, Mini Review, Dec. 2002
Proof of Principle:

1- Response to chemotherapeutic agents: Weaver et al. Cancer Cell, 2002

2- Utility of inhibitory antibodies to \( \beta_1 \) integrin Park et al. Cancer Research Feb. 2006

3- Using the logic of the acini to find new targets for therapy Fournier et al. Cancer Research, Aug. 2006
The Influence of the ECM on nuclear organization, chromatin structure, and transcription of ECM-responsive genes.

Ren Xu

Virginia Spencer

(Bissell Lab)
Differentiated function depends on both prolactin and lRECm

Quantitative RT-PCR for the endogenous milk protein

Promoter analysis of beta casein
Polarity in 2D and 3D

(a) 

(b) Apical membrane
Tight junction
Basolateral membrane

(c)
PrlR localizes at basolateral surface

PrlR (Green); ZO-1 (Red); Nuclei (Blue)

Apical
Basolateral
z-section
Apical addition of ligand fails to activate PrlR

Transwell experiment

Polarized cells in 2D
EGTA treatment disrupts tight junction

Control     EGTA
Apical addition of prolactin activates STAT5 after EGTA treatment
Prolactin binds to the receptor on polyHEMA and in 3D IrECM cultures
Only laminin-111 induces STAT5 reactivation
Mammary cells on polyHEMA and in 3D lrECM differ in morphology and polarity distinct

Poly-HEMA

3D lrECM

PrlR (Green); ZO-1 (Red); Nuclei (Blue)
Laminin111 can polarize polyHEMA and induce milk protein gene expression

PrlR (Green); ZO-1 (Red); Nuclei (Blue)
Reactivation of STAT5 correlates with β-casein expression
STAT5 activation *in vivo*

Mol Endo, 1996; Vol 10 (12)

Xiuwen Liu, Lothar Hennighausen *et al*
PrlR-Stat5 pathway
Sustained-activation of STAT5 is required for β-casein expression.

Methodology:
- Plate cells on polyHEMA
- Add PRL and IrECM
- Add AG490
- Collect samples

Time points:
- 24 hours
- 24 hours
- 24 hours

Western blot analysis:
- AG490:
  - -
  - +
- P-Stat5:
  - -
  - +
- Stat5:
  - -
  - +

Graph:
- β-casein/GAPDH
- AG490
- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 0
- 20
- 40
- 60
- 80
- 100
- 120
- -
- +
Introducing a constitutively-activated Stat5

MCB 1998, Vol 18 (7);
Mayumi Onishi, Toshio Kitamura et al

<table>
<thead>
<tr>
<th>PRL (h)</th>
<th>0.25</th>
<th>2</th>
<th>24</th>
<th>0.25</th>
<th>2</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stat5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-Stat5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vec control | Stat5A 1*6
Sustained Stat5 activation is sufficient to induce β-casein expression
Previous model

Akhtar N, Streuli CH, JBC 2006 Jun 5;173
Our model

Laminin-111

Integrin

DG

PI3K

Rac1

Sustain STAT5 activation

Chromatin remodeling

Mammary-specific function

JAK2

Prolactin

PrIR

PrIR

Transient STAT5 activation

No chromatin remodeling
Model Summarizing The Role of ECM on Chromatin organization
(Ren XU and Virginia Spencer)

β-casein

β-globin

Nuclear Matrix/Nucleoid

+ Laminin1

Nuclear Matrix/Nucleoid

β-globin

C/EBPb

STAT5

AcH4

Brg1

RNA Pol II

GR

Ren XU and Virginia Spencer

Model Summarizing The Role of ECM on Chromatin organization
(Ren XU and Virginia Spencer)
Conclusion

Extracellular Matrix and Chromatin Remodeling are Necessary for Continued Activation of Stat5 which in Turn is Necessary for Tissue-Specific Gene Expression in the Mammary Gland
No milk proteins

\(\text{Lactoferrin} \quad \text{Whey acidic protein}\)

- Level 1 (shape/cytoskeleton)
  - (Dystroglycan)
- Level 2, (integrins)
- Level 3 (Morphogenesis) (TGFalpha)
- BM Destruction (MMPs)

\(\beta\) casein (ECM-response element)

No laminin

- Laminin-rich gel
- Single round cell

Tissue-specific protein expression is hierarchical and depends on cell shape and tissue architecture.
Can these concepts be applied to human cells and breast cancer???
Developmental Biology: The other side of the cancer coin
MCF7 
(makes differentiated tumors)

MDA231 
(Is metastatic)
Examples of the 4 classes of morphologies unraveled on 3-D gels

- **Round**
  - Organized nuclei
  - Robust cell-cell adhesion

- **Mass**
  - Disorganized nuclei
  - Robust cell-cell adhesion

- **Grape-like**
  - Disorganized nuclei
  - Poor cell-cell adhesion

- **Stellate**
  - Disorganized nuclei
  - Elongated cell body with invasive processes

[ Kenny, Lee et al. Molecular Oncology, April 2007]
8/9 of these cell lines were isolated from metastases
List of genes that distinguish cells grown in 2D and 3D in cancer cell lines

Tested all 22215 probes for association with culture substratum (cutoff, P=0.00025)

Identified 96 genes which are differentially expressed at this level of significance
Proof of Principle:

1- Response to chemotherapeutic agents: Weaver et al. Cancer Cell, 2002

2- Utility of inhibitory antibodies to $\beta_1$ integrin Park et al. Cancer Research Feb. 2006

3- Using the logic of the acini to find new targets for therapy Fournier et al. Cancer Research, Aug. 2006
Thus Proof of Principle:

3D models of cancer cells are robust surrogates for breast cancer