The Role of Microenvironment in Susceptibility to Age-related Breast Cancers

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City of Hope

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Disclosures:
I own stocks in Medicustech, and am a consultant for Thrive Biosciences. Issues pertaining to neither entity are being discussed by me.
CD227+
K14+: K19+
1:1

Axl+cKit+
K14+ : K19+

Myoepithelial
CD10+K14+

Luminal
CD227+
K19+ K8+

Stem cell
Progenitor

Villasen et al. JCB 2007
LaBarge and Lorens in review

Contractile &
Tumor suppressive

Secretory

Microenvironment=

Cell-Cell and
Cell-ECM and
Cell-soluble factor
interactions
The role of aging in breast cancer is a huge problem.

~80% diagnosed in women aged >50 years.

New cases per 100,000 women.

Age at diagnosis

The role of aging in breast cancer is a huge problem

CDC predicts a 21% increase in all cancer cases by 2020 due to a larger proportion of aged individuals.

The economic burden of breast cancer treatment to the US healthcare economy may cost as much as $24B by 2020.
Are age-related breast cancers preventable?

Adapted from Matsuno et al., Can Epidem Biomarkers & Prev 2007
Aging impacts transcriptomes but not genomes in hormone-dependent breast cancers, and normal breast.

Breast tumors

Normal mammary epithelia

Changes in breast microenvironment

Fat content increase

Connective tissue decrease

Discontinuities in Extra Cellular Matrix (ECM)

Low mRNA expression

High mRNA expression


How bad can it get when normal architecture is disrupted?

<table>
<thead>
<tr>
<th>Not so bad (normal cells)</th>
<th>Really bad (w/ pre-existing mutations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue polarity</td>
<td>Overt disruptions unleash malignancies</td>
</tr>
<tr>
<td>+Laminin</td>
<td></td>
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<tr>
<td>Laminin Blockade</td>
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</tbody>
</table>

Wound healing

Irradiated stroma

0cGy

40cGy

Dolberg and Bissell, Nature, 1984

LaBarge & Cahjar et al

Gudjonsson JCS 2002

Barcellos-Hoff Can Res 2000

LaBarge & Cahjar et al
Risk of recurrence in age-related breast cancers is protracted.

Represented majority of age-related BC.

Esserman et al and Benz, Br Can Res Trt 2011
Aging raises questions aplenty…

What is the relationship between normal changes that occur with age and increased susceptibility to breast cancers?

Why are there disproportionately more Luminal subtype BCs in older women?

How might age- (and cancer)-related tissue microenvironments impact responses to cancer therapy?
A resource of normal, pre-stasis HMEC for functional interrogation of aging.

Long-term growth of primary cells

Large, standardized batches of normal, pre-stasis HMEC from more than 70 reduction or mastectomy patients.

Multi-lineage maintenance

Self-organizing

Progenitor activity in 3-D culture

Wiring diagrams (transcriptomes) change with age:
What are the functional consequences?
Why are the aging phenotypes so stable?
Mammary stem cells accumulate with age because they lose sensitivity to microenvironment differentiation directives.

Distinction stiffness-dependent differentiation responses

Did not differentiate in response to stiffness changes
Aging alters the trigger points of proteins used by cells to communicate mechanical information into the nucleus, which initiate differentiation programs.

In young progenitor cells...

MST2 levels

200 Pa 2350 Pa

<30y 200Pa 2350Pa >55y

Makes Myoeps

Cell Reports 2014
Aging alters the trigger points of proteins used by cells to communicate mechanical information into the nucleus, which initiate differentiation programs.

But in **older** progenitor cells...

MST2 levels

Makes basal-like Luminals!!
Hypothesis: As tissue ages the new microenvironments establish a continuum of metastable epigenetic states.

Length of time repressed = likelihood of promoter methylation

Oyer... , and Turker PlosONE 2009
Luminal cell DNA genome-wide methylation patterns cluster according to chronological age.

Analysis of primary HMEC with Infinium450K arrays.
Transcriptionally and epigenetically epithelial cells lose tissue specificity with age. Luminal genes and myoepithelial genes are shown in the diagram. The graphs indicate a comparison between <30 years (n=8) and >55 years (n=8). The x-axis represents the percentage of methylation, while the y-axis shows relative expression. Miyano et al., Aging 2017.
Maintenance of luminal (LEP) cells requires the correct microenvironment.

Miyano et al., Aging 2017
Aged microenvironments drive loss of lineage fidelity that are made metastable by epigenetic regulatory states.

Miyano et al., Aging 2017
Promoter DNA methylation changes in key luminal-specific genes driven by age of myoepithelial cells

Primary LEP

Primary LEP in Co-culture with MEP

Miyano et al, Aging 2017
Blockade of cell-cell communication proteins with higher and more variable expression with age prevents transmission of the aged phenotype into luminal cells by older myoepithelial cells.

Miyano, unpublished
Epithelia loses specificity with age due largely to epigenetic changes, what are the consequences for transformation?
Signs of accelerated aging in high-risk breast tissue

Young and middle-aged epithelia that were incorrectly predicted by machine learning to be “old” were from high-risk women.

Machine learning used to sort CyTOF analyzed HMEC into “young” or “old” classifications.
Two-hit immortalization does not introduce gross genomic errors in HMEC
Immortalization re-sensitizes aged progenitors to a physiological range of stiffness

Primary normal

<30y

>55y

Immortal non-malignant

66 years
122LmY

91 years
805Pp16s

Cell Reports 2014
Immortalization re-sensitized YAP (and TAZ) to the physiological elastic range

66 years
122LMY

91 years
805Pp16S

Differentiation

Log2 fold change From 200Pa

200 Pa 1500 Pa 2400 Pa

LEP MEP

Cell Reports 2014
Immortal post-menopausal HMEC tend to maintain phenotypes of Luminal BC

Keratin 14
Keratin 19
DAPI

β-catenin
Estrogen receptor alpha

DAPI

Front in Cell Dev Bio, 2015
Epigenetic states of chronological age and the route of stasis bypass determines cancer subtype.
The roles of tissue microenvironments in...

- Aging & breast cancer
- Epithelial plasticity
- Drug response
Molecular compositions
ECM
Growth factor

Mechanical properties
Substrata stiffness
Shear force

Cellular

Architecture
Polarity
Dimension
Microenvironment microarray platforms for functional dissection of the microenvironment

**MicroEnvironment MicroArray (MEMA)**
A highly parallel cell-based functional screening platform

Thousands of spots per MEArray
Uses standard microarray robot technology
Functionally assess lineage commitment, cell proliferation, and cell death.
Analyzed with fluorescent mAb

Progenitors on an MEArray
Combinatorial Microenvironments Impose a Continuum of Cellular Responses to a Single Pathway-Targeted Anti-cancer Compound

<table>
<thead>
<tr>
<th>Input</th>
<th>Molecular compositions</th>
<th>Matrix rigidity</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECM</td>
<td>Type I collagen (C1)</td>
<td>X</td>
<td>DMSO</td>
</tr>
<tr>
<td></td>
<td>Fibronectin (FN)</td>
<td></td>
<td>Lapatinib (Lap)</td>
</tr>
<tr>
<td></td>
<td>Type IV collagen (C4)</td>
<td></td>
<td>Lap + Verteporfin (VP)</td>
</tr>
<tr>
<td></td>
<td>Laminin I (L1)</td>
<td></td>
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<tr>
<td></td>
<td>Mix of L1 and C1 (L1C1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteins</td>
<td>AREG IL6 EGF IL8 Gas6 TGFb HGF</td>
<td>2500 Pa 40 kPa</td>
<td></td>
</tr>
<tr>
<td>Output</td>
<td>Morphological feature</td>
<td></td>
<td>EdU incorporation (Proliferation)</td>
</tr>
<tr>
<td></td>
<td>Cellular morphology</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>HER2 pHER2</td>
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</table>

5 x 7 x 2 x 3 = 210 combinations

Visualization of cell phenotypes as functional of ME

Cell Reports 2017
Generalized linear models (GLM) identified individual ME components that had strong effects on Lapatinib responses.

Cell lines that represent:

- **Breast Cancer**
  - HCC1569
  - w/HER2 amp

- **Lung Cancer**
  - A549
  - w/HER2 amp

- **Prostate Cancer**
  - PC3
  - w/HER2 amp

Cell Reports 2017
GLM can identify potentially interesting synergies between ME properties
Microenvironment-driven morphology co-organizes with drug-response phenotypes, providing clues to therapeutic combinations.

HCC1569  A549  PC3
Microenvironments modulate ratios of pHER2 to total HER2 in HER2-amplified BC cells, which correlates with Lapatinib resistance.

Relevant summary from previous figures:
The rigidity and ECM properties independently drove the most variation in HCC1569 responses to Lapatinib, and EGF showed potential synergy with these properties. FN was specifically implicated.

Reports on predictability of anti-HER2 therapy efficacy based on pHER2/HER2 ratios is variable. Context matters....
Adhesion to FN confers resistance to Lapatinib via a Src-YAP related pathway:
Drug-tolerance in defined contexts enables identification of work-arounds.

Adhesion to FN activates a FAK-Src-YAP pathway in mammary epithelial cell lines (Kim and Gumbiner, 2015). AZ = AZ0530, a Src inhibitor
Do the functional results that identified FN as a modulator of Lapatinib stand up in patients?

YAP/FN protein level associations

FN protein level survival associations

SUBTYPE

Basal

HER2-enriched

Luminal A

Luminal B

Tumor GRADE

T1

T2

T3 & T4

Cell Reports 2017
There are many neighborhoods in a tumor that may have the capacity to increase tolerance to a drug by a non-genetic mechanism...

e.g. This is FN staining in 4 different ductal carcinomas.
1) Cellular phenotypes of aging and high-risk, differentiation, and drug responses are significantly determined by the ME in normal and malignant mammary epithelial cells.

2) Failure to achieve durable drug responses in cancers may have as much to do with heterogeneous tumor MEs, as it does with genetic heterogeneity.

3) Cellular phenotypes of aging in breast, which are related to susceptibility, are transmissible and malleable... is aging in breast preventable?
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