Impact of Age on the Biology of Breast Cancer

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Breast Cancer: a model disease of aging

• Aging USA population = more cancers
  \( \geq 65\text{y pop.} = 4\% \text{ in 1990, 12}\% \text{ in 1998, 20}\% \text{ in 2025} \)
  12\% pop. increase in 20y will bring a 60\% cancer increase

• Women are majority of elderly
  55\% of \( \geq 60\text{y} \); 65\% of \( \geq 80\text{y} \)
  Up to 80\% of breast cancers occur after age 50y

• Only \( \sim 6\% \) of breast cancers occur before age 40
  Up to 25\% of these associated with BRCA1/2 mutations

• Poor biological understanding of link with aging

  Altered cancer biology or host defenses?

Better Understanding = Better Treatment
Breast Cancer Epidemiology

❖ Age and geographic variations in incidence?
❖ Age-dependent outcomes and risk factors?
Breast Cancer Incidence Worldwide

- Correlates with development and affluence.
- Adjusted for age, but not ethnicity.
Breast Cancer Incidence Worldwide

- Correlates with development and affluence.
- Adjusted for age, but not ethnicity.
- *Generally increasing over past 30 years.*
Geographic Variations in Breast Cancer Incidence Occur Primarily in Women Over Age 40

Age-dependent Breast Cancer Incidence Rates

Younger Onset Incidence More Geographically Stable Than Older Onset Rates

- SEER database
- USA: 1992-1997

Invasive Breast Cancers 1997-2001

(Benz; Crit Rev Oncol/Hemat, 2008)

(Phipps, Clarke, Ereman; BCR, 2005)
Age-dependent Breast Cancer Incidence Rates

Younger Onset Breast Cancer: Less age-dependent ER/PR variability

"Clemmesen's Hook"

SEER database
USA: 1992-1997

(Benz; Crit Rev Oncol/Hemat, 2008)
Age-dependent Breast Cancer Incidence Rates

Younger Onset Breast Cancer: Less age-dependent histologic & ethnic variability

(Anderson et al.; CEBP, 2006)
Age-dependent Breast Cancer Incidence Rates

“Clemmesen’s Hook” = superimposition of two different incidence rate curves

Bimodal Age-density Distributions

Early onset breast cancer
Inherited or early-life initiating events?

Late onset breast cancer
Later-life promoting events?

(Anderson et al.; CEBP, 2006)
Age-dependent Breast Cancer Incidence Rates

Are There Early vs. Late Onset Differences in Breast Cancer Outcome?

Bimodal Age-density Distributions

Early onset breast cancer
Inherited or early-life initiating events?

Late onset breast cancer
Later-life promoting events?

(Anderson et al.; CEBP, 2006)
Early Onset Breast Cancer = Worse Outcome

Age cohorts selected from four public data sets and 784 clinically annotated breast tumor samples, heterogeneous with regard to stage, grade, ER status, and adjuvant therapy.

N = 211, > 65y

N = 200, < 45y

(HR 1.69; P = .013)

(Anders et al., J Clin Oncol 26: 3324-3330, 2008)
### What Are the Known Risk Factors?

<table>
<thead>
<tr>
<th>&quot;Not modifiable&quot;</th>
<th>&quot;Modifiable&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Parity/Age 1&lt;sup&gt;st&lt;/sup&gt; live birth*</td>
</tr>
<tr>
<td>Age*</td>
<td>Mammographic density</td>
</tr>
<tr>
<td>Family history (1&lt;sup&gt;st&lt;/sup&gt; degree relatives)*</td>
<td>Breastfeeding</td>
</tr>
<tr>
<td>Age at menarche*</td>
<td>Obesity/weight gain</td>
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<tr>
<td>Age at natural menopause</td>
<td>Hormone therapy (E+P)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>Radiation exposure</td>
</tr>
<tr>
<td>Prior benign biopsies*</td>
<td>Alcohol consumption</td>
</tr>
<tr>
<td></td>
<td>Physical activity</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
</tr>
</tbody>
</table>

*Incorporated into Breast Cancer Risk Assessment Tool (BCRAT)/Gail Model*
What Are the Known Risk Factors?

"Not modifiable"
- Gender
- Age*
- Family history (1st degree relatives)*
- Age at menarche*
- Age at natural menopause
- Race/ethnicity
- Prior benign biopsies*

"Modifiable"
- Parity/Age 1st live birth*
- Mammographic density
- Breastfeeding
- Obesity/weight gain
- Hormone therapy (E+P)
- Radiation exposure
- Alcohol consumption
- Physical activity
- Diet

*Incorporated into Breast Cancer Risk Assessment Tool (BCRAT)/Gail Model

Strong associations with early onset breast cancer
What are the effects of aging on breast cancer biology, assessed by prognostic and predictive biomarkers?
Growth receptors ERBB2/HER2 & ER

Inverse relationships

(Benz; Crit Rev Oncol/Hemat, 2008)
Markers of invasiveness & metastatic potential

angiogenic factors: VEGF, bFGF
proteases: Cath. D, uPA, uPAR, PAI-1

No association with age after 40 y

Markers of proliferation & genetic instability

Ki-67/MIB-1

p53-positvity

(N = 802; r = -0.216, p < 0.0001)

(N = 823; r = -0.111, p = 0.0014)

Decline significantly with age after 40 y
Absent age-expression relationship does not preclude age-dependent prognostic effect

<table>
<thead>
<tr>
<th>N (uPA):</th>
<th>151</th>
<th>541</th>
<th>782</th>
<th>702</th>
<th>813</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (y):</td>
<td>22-39.9</td>
<td>40-49.9</td>
<td>60-69.9</td>
<td>70-69.9</td>
<td>70-85</td>
</tr>
<tr>
<td>N (VEGF):</td>
<td>21</td>
<td>107</td>
<td>178</td>
<td>170</td>
<td>103</td>
</tr>
</tbody>
</table>

uPA & VEGF
prognostic only in early onset breast cancer

(Benz; Crit Rev Oncol/Hemat, 2008)
Biomarker results from retrospective analysis of ~4,000 breast cancer cases...

- Most show no association between age and level
  - PR, pS2, Bcl-2, VEGF, uPA, uPAR, PAI-1, Cath-D
- Some are strongly associated with age
  - Negative: grade, MI/Ki67, AI, p53, ErbB1&2
  - Positive: ER positivity & content

Quong et al., Age-dependent changes in breast cancer hormone receptors and oxidant stress markers. Breast Cancer Res. Treat., 2002

- Demonstrate that aging affects breast cancer biology and its clinical behavior.
- Since ER-positivity correlates inversely with other biomarkers, what is more important… Aging or ER status?
Among the more prevalent forms of ER+ breast cancer, are there age-associated biological differences?
Pilot Retrospective Outcome Analysis: *Impact of Aging*

ER-positive, $T_{1/2} N_0$, ductal BrCa:  $n = 83$; Older ($\geq 70$ y) vs. Younger ($< 45$ y) cases

[A. Thor FFPE archive of 828 breast cancers; >16y follow-up; no adj. tx]
Pilot Retrospective Outcome Analysis: *Impact of Aging*

ER-positive, $T_{1/2} N_0$, ductal BrCa: $n = 83$; Older ($\geq 70$ y) vs. Younger ($< 45$ y) cases

[A. Thor FFPE archive of 828 breast cancers; >16y follow-up; no adj. tx]

Even for ER+ breast cancers, age is a significant breast cancer risk factor

(Benz; Crit Rev Oncol/Hemat, 2008)
Study Design: ER-positive, early-stage (T1/2, N0) ductal breast cancers

• Cohort comparison: YOUNGER (≤ age 45) vs. OLDER (≥ age 70) age-at-diagnosis Cauc. cases
• Cryobanked tumor samples for DNA and RNA (+ protein fractions); sample sources from:
  -- UCSF/BOP; n = 83 (Y = 21, O = 62) for DNA, 68 for RNA; 54 with RFS (Y<<O; p < 0.04)
  -- NCI-Bari, Italy; n = 70 (Y = 27, O = 43) for DNA, 30 for RNA; no RFS data
  [from larger collective of ER+ & ER- cases with matching blood sample]

Specific Aims:

• Identify genomic differences between Older and Younger ER+ cohorts using DNA samples.
  - Genome copy number phenotypes (2.5 K BAC CGH arrays)
  - p53 mutations in DNA core (microsequence exons 5-8)

• Identify gene expression differences between Older and Younger ER+ cohorts using RNA samples.
  - Expression array signatures & phenotypes (Affy arrays)

(Yau and Benz, BCR, 2007)
Array CGH Analysis of Breast Cancers

#19, Y, ER+/PR+

Genome Order

Genomic Locus Gain/Loss Frequency
No Age Associated Differences in Genomic Locus Aberration Frequencies
Unsupervised Hierarchical Clustering of 70 ER+ IDC Shows no Age Association with Subgroups
Copy Number Transitions
Old = Young

p = 0.64
Number of Amplifications: Old ~ Young

(ERBB2, MYC, CCND1, MDM2, EGFR, AIB1, TOPO2, ZNF217, etc.)

ERBB2 amplifications:

Young = 11%
Old = 9%

p = 0.28
Age, ER status & p53 Mutations?

~ 20% p53mut frequency reported among all breast cancers

~ 90% missense mutations, >90% in DNA-binding core (exons 5-8, aa 126-306)

<table>
<thead>
<tr>
<th></th>
<th>ER-/p53wt</th>
<th>ER+/p53wt</th>
<th>ER-/p53mut</th>
<th>ER+/p53mut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset (&lt; 45 y) n=135</td>
<td>49 (36.3%)</td>
<td>64 (47.4%)</td>
<td>14 (10.4%)</td>
<td>8 (5.9%)</td>
</tr>
<tr>
<td>Late onset (≥ 70 y) n=154</td>
<td>25 (16.2%)</td>
<td>107 (69.5%)</td>
<td>12 (7.8%)</td>
<td>10 (6.5%)</td>
</tr>
</tbody>
</table>

No age link with p53mut when ER status considered

P = 0.004, Fisher Exact
Microarrays Identify Multiple Breast Cancer Subsets

(Sørlie et al., PNAS 98: 10869-10874, 2001)

78 breast cancers (+7 benign breast samples)
clustered by 456 genes from ~8K array

<table>
<thead>
<tr>
<th>ER+, type A</th>
<th>ER+, type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>82%</td>
<td>71%</td>
</tr>
<tr>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>13%</td>
<td>67%</td>
</tr>
</tbody>
</table>

%mutated p53

Overall Survival (Mo.)
Relapse-free Survival (Mo.)

N = 49 breast cancer patients (Stage II/III, uniform adj. treatment)
Microarrays Identify Several ER+ Br Ca Subsets

(Sørlie et al., PNAS 98: 10869-10874, 2001)

78 breast cancers (+7 benign breast samples)
clustered by 456 genes from ~8K array

82%                    71%                33%                       67%                                         13%
%mutated p53

ER+, type A
ER+, type B
ER+, type A
ER+, type B

N = 49 breast cancer patients (Stage II/III, uniform adj. treatment)
Microarray Unsupervised Clustering of ER+ BrCa

N = 102 RNA samples from O + Y age cohorts of node-neg ER-pos ductal BrCa
Unsupervised Analysis of ER+ Ductal BrCa

Group 1A: older patients (68%)
- 89% PR+

Group 1B: older patients (55%)
- 74% PR+

Group 2: younger patients (77%)
- 56% PR+

Group 3: younger patients (65%)
- 83% PR+

p<0.05 for age cohort difference between Group 1A/B & 2
Unsupervised ER+ Clusters: *Not as prognostic as PR status*

Adjuvant tamoxifen use (>60%) balanced in all comparison groups

$p = 0.09$

$p = 0.02$
Supervised Analysis: **Differentially expressed genes**

59 unique genes, including ER, are significantly up-regulated in the Older Age cohort (FDR, p<0.05)

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Average Fold Change</th>
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<tr>
<td>NPM1</td>
<td>nuclear matrix protein</td>
<td>2.16771230</td>
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<tr>
<td>CD45RB1</td>
<td>homeobox B6</td>
<td>2.42707016</td>
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<tr>
<td>TMEM53</td>
<td>transmembrane channel-like 6</td>
<td>2.34985049</td>
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<tr>
<td>GUS</td>
<td>mucin 1, transmembrane</td>
<td>2.15628763</td>
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<tr>
<td>STS</td>
<td>alpha-1-antitrypsin-related protein</td>
<td>2.10171060</td>
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<tr>
<td>A2M</td>
<td>alpha-2-macroglobulin</td>
<td>1.04257056</td>
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<td>PYG</td>
<td>phosphoglycoprotein, krukenkrooss</td>
<td>1.03662056</td>
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<td>KAGAA1022</td>
<td>KAGAA1022 protein</td>
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<td>TNFRSF10</td>
<td>tumor necrosis factor (ligand)</td>
<td>1.076381061</td>
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<td>GSTM</td>
<td>glycine amidinotransferase (GAMA)</td>
<td>1.07761938</td>
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<td>RNU54</td>
<td>ribonuclease, Rnase A family, 4</td>
<td>1.043199366</td>
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<td>GLC9</td>
<td>glutathione (thioredoxin)</td>
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<td>FLN2152</td>
<td>hypothetical protein FLN2152</td>
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<td>ERB</td>
<td>estrogen receptor</td>
<td>1.07030226</td>
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<td>ENTPD6</td>
<td>endothelial protein disulfide</td>
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<td>DOSG6</td>
<td>dermatitis sulfate proteoglycan 3</td>
<td>1.069834665</td>
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<td>CDS4</td>
<td>CDS4, interacting transcript</td>
<td>1.034421394</td>
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<td>SH3D1</td>
<td>SH3 domain binding glutamine acid</td>
<td>1.081139451</td>
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<td>TRR11</td>
<td>retinoic acid receptor, type 1</td>
<td>1.080906910</td>
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<td>SSX1</td>
<td>SSX1 and SSX domain containing 1</td>
<td>1.08193964</td>
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<td>ANG</td>
<td>angiotensin, riboside, ribase A family, 6</td>
<td>1.05147141</td>
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<td>H3F3D5</td>
<td>homeobox D5</td>
<td>1.059547306</td>
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<td>MARK1</td>
<td>MARK1 domain containing 1</td>
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<td>IGFAP2</td>
<td>IGF-A protein activating protein</td>
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<td>ARHGEF20</td>
<td>Rho GTPase activating protein (GAP)</td>
<td>1.074930184</td>
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<tr>
<td>FAN</td>
<td>farnesyltransferase</td>
<td>1.053779373</td>
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<tr>
<td>WWX4</td>
<td>WW domain containing oxidoreductase</td>
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<td>COBL1</td>
<td>COBL like 1</td>
<td>1.073895022</td>
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<td>C20orf58</td>
<td>chromosome 20 open reading frame 35</td>
<td>1.05011981</td>
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<td>EMA21</td>
<td>enolase-A1</td>
<td>1.056894611</td>
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<td>C24265A10</td>
<td>catenin (cadherin-like, transmembrane)</td>
<td>1.054815024</td>
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<td>GL2EC3A</td>
<td>type-3 lectin domain family, member A</td>
<td>1.02835474</td>
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<td>P8</td>
<td>P8 protein (candidate of motility 1)</td>
<td>1.02491979</td>
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<td>D0S2141</td>
<td>D0S2141, 21qter</td>
<td>1.052202335</td>
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<td>CSF2R1</td>
<td>chromosome 21 open reading frame 25</td>
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<td>SEF6</td>
<td>sef protein</td>
<td>1.051858507</td>
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<td>RHOB</td>
<td>rac homolog gene family, member B</td>
<td>1.05172247</td>
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<tr>
<td>SODD</td>
<td>store-25-deiodinase (ERG), delta-5-deiodinase, fungal-like</td>
<td>1.051134372</td>
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<td>F11A1</td>
<td>F11A1, plasminogen activator</td>
<td>1.043971074</td>
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<td>TAPBP</td>
<td>TAP binding protein-like</td>
<td>1.049399935</td>
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<tr>
<td>PPP1R2C2</td>
<td>PPP1R interacting protein, binding 2 (protein beta 2)</td>
<td>1.051793099</td>
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<td>CCDC28A</td>
<td>coiled-coil domain containing 28A</td>
<td>1.04926235</td>
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<td>CRM</td>
<td>carboxy-steroid M</td>
<td>1.046262865</td>
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<tr>
<td>CALM3</td>
<td>calcineurin 3 (phosphatase kinase, delta)</td>
<td>1.045830549</td>
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<tr>
<td>SLC25A12</td>
<td>solute carrier family 25 (mitochondrial carrier, Arai, member 12)</td>
<td>1.04571349</td>
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<td>CHL1</td>
<td>chlomerin</td>
<td>1.043699772</td>
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<tr>
<td>MARCO</td>
<td>membrane-associated ring finger (C9H4C4)</td>
<td>1.0434302973</td>
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<td>HOXB8</td>
<td>homeobox B8</td>
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<td>FLJ09266</td>
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<td>PEO2</td>
<td>peroxisomal biogenesis factor 3</td>
<td>1.04788318</td>
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<td>SLC2A8</td>
<td>solute carrier family 12 (lysosomal chloride transporter), member B</td>
<td>1.04515292</td>
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<tr>
<td>SLC34A9</td>
<td>solute carrier family 7 (amino acid transporter, y+ system), member 8</td>
<td>1.03803564</td>
</tr>
<tr>
<td>DBI</td>
<td>diacylglycerol binding inhibitor</td>
<td>1.033872935</td>
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<tr>
<td>PRBPL</td>
<td>prolyl endopeptidase-like 4</td>
<td>1.037664793</td>
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<td>P4AT</td>
<td>phosphatidylinositol glycerol kinase</td>
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<td>LOC337145</td>
<td>promethin</td>
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<td>RANBP2</td>
<td>RAN binding protein 2</td>
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<td>TGOCD2</td>
<td>tango-2 homolog protein 2</td>
<td>1.031864338</td>
</tr>
</tbody>
</table>

Note: Highlighted in red are genes with implied or established roles in cancer

(From Yau and Benz, BCR, 2007)
Predictive Analysis: *Is there an ER+ age signature?*

**A. ER+ test set:**

<table>
<thead>
<tr>
<th></th>
<th>Test Set (68 tumors)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>old</td>
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</table>

**B. ER+ validation sets:**

<table>
<thead>
<tr>
<th>Validation Set</th>
<th>accuracy</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(35 cases)</td>
<td>80%</td>
<td>0.0003</td>
</tr>
<tr>
<td>(30 cases)</td>
<td>83%</td>
<td>0.0017</td>
</tr>
<tr>
<td>(64 cases)</td>
<td>84%</td>
<td>1.67E-7</td>
</tr>
</tbody>
</table>

**C. Two genes correlating (-,+) most strongly with age:**

![Gene expression graphs](Yau and Benz, BCR, 2007)
Age & ER+ Gene Expression Profiles

Unsupervised Analysis

- ER+ breast cancers are heterogeneous (4 subtypes)
- PR status not reflected in ER+ transcriptional subtypes
- Subset of early onset cases have worse prognosis (RFS).

Supervised and Predictive Analyses

- Early onset ER+ breast cancer associated with:
  - reduced expression of ER and some tumor suppressors (ARHGDIB, SASHI), development regulators (HOXB6/B7), & apoptosis inducer (TNFSF10)
  - increased expression of growth factor (AREG) & receptor (FGFR1), ER-inducible growth regulator (GREB1), mitotic factors (CDC14A, STK6), & serine proteases (PRSS1/2)

- Early onset ER+ cases enriched in poor prognostic signatures:
  - proliferation
  - oxidative stress
Oxidative Stress & Early Onset ER+ Breast Cancer

- Oxidative stress signature (Ox-E/ER) linked to poor-outcome ER+ breast cancers (Yau et al., BCR 2008)
- Early onset ER+ breast cancers enriched with both proliferation and Ox-E/ER gene signatures
- Gene pathways shared by early onset and Ox-E/ER enriched tumors share upstream TNF & TGFβ nodes
- At least 75% of signature genes regulated by TNF & TGFβ contain NFκB and/or AP-1 promoter elements

Pathway Comparisons Between ER+ Age Signature and Ox-E/ER Signature

From ER+ age signature (Yau et al., BCR 9:R59, 2007):

From Ox-E/ER signature (Yau & Benz, BCR 10:R61, 2008):
Signaling Pathways Shared by Oxidatively Stressed and Early Onset ER+ Breast Cancers

Opportunities for Therapeutic Intervention?

Breast Cancer & Aging: Questions

Do ER-breast cancers show age-associated outcome and biology differences?
Maybe not...

Metastasis-free Survival

Pooled outcome analyses comparing ER+ vs. ER- untreated N₀ cases from age-annotated data sets (Y ≤ 39 years; O ≥ 40 years)

Conclusions

- Breast cancer is a heterogeneous disease with early and late onset forms, even within known clinical subtypes (e.g. ER+ vs. ER-).
- Inverse age relationship between ER and biomarkers of breast cancer growth (e.g. Ki-67, ERBB2/HER2) and genomic stability (nuclear grade, p53).
- Among sporadic ER+ breast cancers, age has little effect on cancer genome but predictably alters breast cancer gene expression (epigenome).
- Sporadic, early onset ER+ breast cancer is clinically and biologically more aggressive, with features indicating enhanced NFκB and AP-1 activated gene programs that correlate with endocrine resistance.
• Gary Scott, PhD
• David Britton PhD
• Christina Yau, BS
• Corina Marx, PhD
• Christian Atsiriku, PhD
• Yamei Zhou, PhD
• Crystal Berger, BS
• Chris Benz, MD

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• STB, Basel, Switz.