Investigating genetic events in the progression of ductal carcinoma *in situ*

Sanaz (Sunny) Jansen PhD
Mouse Cancer Genetics Program
National Cancer Institute
Outline

• Introduction to ductal carcinoma in situ (DCIS)
• Modeling DCIS progression in mice
• Techniques for noninvasively tracking preinvasive cancer progression in mice
• Summary
Genes and pathways in progression of ductal carcinoma *in situ* (DCIS)

Molecular subtype: luminal A, luminal B, HER2, basal

**Grade:** low, intermediate, high nuclear grade

**Growth pattern:** solid, papillary, micropapillary, cribriform, comedo

**Necrosis:** prominent in comedo, focal in others (if present)

**Differentiation:** well, moderately or poorly differentiated

Jansen SA 2015
Telomere crisis model of DCIS progression

- Implications:
  - DCIS and IDC are genetically similar

Jansen SA 2015
Cancer stem cell model of DCIS progression

Fig. 3 The precancer stem cell model of DCIS progression. DCIS lesions arise as a result of tumorigenic events (e.g., oncogene activation, loss of tumor suppressor) occurring initially in a precancer stem cell. The molecular and biological properties of the ensuing DCIS lesion including its potential for progressing to invasive disease are pre-encoded within the initial target cell. In this way, the bulk of malignant transformation has occurred by the DCIS stage.
Breast cancers evolve along genetic pathways defined at or before DCIS stage
DCIS is a disease revealed by imaging

• Because of early detection, DCIS comprises 25-30% of all newly diagnosed breast cancers
DCIS has *interlesion* heterogeneity

- Subtypes based on growth pattern

[Images of histological sections labeled A to F]
DCIS has \textit{intralesion} heterogeneity

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
& Grade 1 & Grade 2 & Grade 3 \\
\hline
\text{29.2\%} & \text{22.5\%} & \text{2.5\%} \\
\text{30.0\%} & \text{9.2\%} & \text{6.6\%} \\
\end{tabular}
\end{center}

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
\text{\textbf{Case \#012}} & \text{\textbf{Case \#020}} & \\
\hline
\text{\textbf{\approx 30\%}} & \text{\textbf{\approx 10\%}} & \\
\text{\textbf{\approx 60\%}} & \text{\textbf{\approx 85\%}} & \\
\text{\textbf{\approx 10\%}} & \text{\textbf{\approx 5\%}} & \\
\end{tabular}
\end{center}
Finding biomarkers for DCIS progression is critical

NIH State-of-the-Science Conference Statement on Diagnosis and Management of Ductal Carcinoma In Situ (DCIS)

“The primary question for future research must focus on the accurate identification of patient subsets diagnosed with DCIS, including those persons who may be managed with less therapeutic intervention.”

“#14: Are there definable properties of a non-malignant (in situ) lesion that predict the likelihood of progression to invasive or metastatic disease?”
But biomarkers for progression remain elusive

### Biology of DCIS and Progression to Invasive Disease

#### Table 1  Summary of the molecular markers used to characterize DCIS

<table>
<thead>
<tr>
<th>Molecular markers</th>
<th>Functions</th>
<th>Molecular signatures correlating with increased risk of recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, PR</td>
<td>Steroid receptors</td>
<td>ER—</td>
</tr>
<tr>
<td>HER2</td>
<td>Regulates proliferation and apoptosis</td>
<td>HER2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ER—/HER2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ER—/HER2 +/Ki-67+</td>
</tr>
<tr>
<td>p53</td>
<td>Regulates cell-cycle, apoptosis, and genomic stability; p53 is an important tumor suppressor</td>
<td>p53+</td>
</tr>
<tr>
<td>Rb/p16 pathway</td>
<td>Regulates cell-cycle; Rb is an important tumor suppressor</td>
<td>p16+</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Proliferation marker</td>
<td>Ki-67+</td>
</tr>
<tr>
<td>COX-2</td>
<td>Enzyme for prostaglandin synthesis; expressed during inflammatory response</td>
<td>COX-2+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p16+/COX-2-/Ki-67+ (DCIS recurrence)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p16+/COX-2+/Ki-67+ (invasive recurrence)</td>
</tr>
<tr>
<td>Akt/PTEN pathway</td>
<td>Regulates proliferation, survival and motility; PTEN is an important tumor suppressor</td>
<td></td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>DNA damage repair</td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>Transcription factor that can activate proliferation; c-myc is a proto-oncogene</td>
<td></td>
</tr>
<tr>
<td>VEGF, vascular patterns</td>
<td>Angiogenesis and vascular markers</td>
<td></td>
</tr>
<tr>
<td>Cyclin A, cyclin E,</td>
<td>Cell-cycle regulators</td>
<td></td>
</tr>
<tr>
<td>p21, p27</td>
<td></td>
<td>p21+</td>
</tr>
<tr>
<td>Bcl-2, Bax, Survivin</td>
<td>Apoptosis regulators</td>
<td>Bcl-2—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survivin+</td>
</tr>
</tbody>
</table>

Included are the molecular signatures that have been shown to correlate with an increased risk of subsequent recurrence in some reports.
But biomarkers for progression remain elusive

<table>
<thead>
<tr>
<th>Molecular markers</th>
<th>Functions</th>
<th>Molecular signatures correlating with increased risk of recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER, PR</td>
<td>Steroid receptors</td>
<td>ER−, HER2+&lt;br&gt;ER−/HER2+&lt;br&gt;ER−/HER2+ +/Ki-67+</td>
</tr>
<tr>
<td>HER2</td>
<td>Regulates proliferation and apoptosis</td>
<td>p53+</td>
</tr>
<tr>
<td>KI-67</td>
<td>Proliferation marker</td>
<td>p16+&lt;br&gt;Ki-67+&lt;br&gt;COX-2+&lt;br&gt;p16+/COX-2-/Ki-67+ (DCIS recurrence)&lt;br&gt;p16+/COX-2+/Ki-67+ (invasive recurrence)</td>
</tr>
<tr>
<td>COX-2</td>
<td>Enzyme for prostaglandin synthesis; expressed during inflammatory response</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>Regulates proliferation, survival and motility; PTEN is an important tumor suppressor</td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td>DNA damage repair</td>
<td></td>
</tr>
<tr>
<td>VEGF, vascular patterns</td>
<td>Angiogenesis and vascular markers</td>
<td></td>
</tr>
<tr>
<td>Cyclin A, cyclin E, p21, p27</td>
<td>Cell-cycle regulators</td>
<td>p21+</td>
</tr>
<tr>
<td>Bcl-2, Bax, Survivin</td>
<td>Apoptosis regulators</td>
<td>Bcl-2−&lt;br&gt;Survivin+</td>
</tr>
</tbody>
</table>

Included are the molecular signatures that have been shown to correlate with an increased risk of subsequent recurrence in some reports.
These tumor suppressor pathways are key in invasive breast cancer.
These tumor suppressor pathways are key in invasive breast cancer.
Accumulation of p53 correlates with increased heterogeneity

Role of p53 in DCIS heterogeneity and progression?

Increased recurrences in Rb and PTEN deficient DCIS

Knudsen et al. JNCI 2012

Invasive breast cancer recurrences

Role of PTEN and Rb in DCIS progression?
### Decreased incidence of DCIS in BRCA1 mutation carriers

<table>
<thead>
<tr>
<th>Screening trial, Mammo+MRI</th>
<th>No. of tumors in BRCA MC</th>
<th>No. tumors that are DCIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warner et al, 2011</td>
<td>9</td>
<td>0/9</td>
</tr>
<tr>
<td>Sardanelli et al, 2010</td>
<td>21</td>
<td>2/10</td>
</tr>
<tr>
<td>Rjinsburger et al, 2010</td>
<td>21</td>
<td>2/21</td>
</tr>
<tr>
<td>Gilbert et al, 2009</td>
<td>15</td>
<td>0/15</td>
</tr>
<tr>
<td>Shah et al, 2009</td>
<td>11</td>
<td>2/11</td>
</tr>
<tr>
<td>Kaas et al, 2008</td>
<td>39</td>
<td>3/39</td>
</tr>
<tr>
<td>Schrading et al, 2008</td>
<td>23</td>
<td>0/14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>139</strong></td>
<td><strong>9/139 (6%)</strong></td>
</tr>
</tbody>
</table>

**Role of BRCA1 on DCIS imaging properties and progression?**
Outline

• Introduction
• Modeling DCIS progression in mice
• Techniques for noninvasively tracking preinvasive cancer progression in mice
• Summary
Modeling DCIS progression in mice
Xenograft vs. GEM models of DCIS

- Intraductal xenograft models of DCIS

Behbod et al 2009, Valdez et al 2011
Modeling DCIS progression in mice
Genetic transformation may not be linear

- Bulk of genetic transformation has already occurred by DCIS stage (Ma et al PNAS 2003, Chin et al Nature Genetics 2004)
Modeling DCIS progression in mice

Keratin promoters

$Rb_f$

$OR$

$PTEN$

$p53$

$BRCA1$
Modeling DCIS progression in mice
Keratin promoters

K18 \(\text{eGFP} \rightarrow \text{STOP} \rightarrow \text{T121}\)
K19 \(\text{eGFP} \rightarrow \text{STOP} \rightarrow \text{T121}\)

\(\text{Pb} \rightarrow \text{Cre}\)
\(\text{WAP} \rightarrow \text{Cre}\)

K18, K19

Normal

PTEN
p53
PRCA1
Modeling DCIS progression in mice
Keratin promoters: Rationale

- Evidence suggesting that these models can initiate mammary carcinomas

\[ \text{K19-T121tg/+; Pb Cre tg/+} \]

\[ \text{K18-T121tg/+; B-actin Cre tg/+} \]
But these models have weak penetrance and long latency.

**Pb-Cre induction**

- 111 study mice
- 22 tumors
- ~20% penetrance
- 472 days median age at tumor onset

**WAP-Cre induction**

- 84 study mice
- 10 tumors
- ~12% penetrance
- 459 days median post lactation at tumor onset
Tumors have luminal characteristics

ERα  K18  Rb f  p53  K19  Rb f  PTEN  K19  Rb f  PTEN  K19  Rb f  p53

K5, K18
Modeling DCIS progression in mice

MMTV promoter
Modeling DCIS progression in mice

MMTV promoter

\[ \text{Normal} \]

\[ \text{WAP-Cre} \]

\[ \text{MMTV} \rightarrow \text{eGFP} \rightarrow \text{STOP} \rightarrow \text{T121} \]

\[ \text{p53} \]

\[ \text{BRCA1} \]
Modeling DCIS progression in mice

MMTV Promoter

Predominantly basal gene expression profile

Predominantly luminal B gene expression profile

Kumar et al PLOS Genetics 2012
Modeling DCIS progression in mice
Focal induction with lenti-Cre

- MMTV
- eGFP
- STOP
- T121

\[ \text{Normal} + \text{Lenti-Cre} \]
Modeling DCIS progression in mice
Focal induction with lenti-Cre

- Rosa-YFP MEFs treated with lenti-Cre at 200 MOI
Outline

• Introduction
• Modeling DCIS progression in mice
• Techniques for noninvasively tracking preinvasive cancer progression in mice
• Summary
C3(1) Tag mice
C3(1) Tag mice
C3(1) Tag mice
C3(1) Tag mice
Accurate imaging methods to follow preinvasive cancer progression

<table>
<thead>
<tr>
<th></th>
<th>Number of DCIS</th>
<th>Sensitivity of MRI</th>
<th>Sensitivity of MRI-directed ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>All inguinal glands</td>
<td>60</td>
<td>88% (53/60)</td>
<td>72% (38/53)</td>
</tr>
<tr>
<td>Posterior inguinal glands</td>
<td>33</td>
<td>94% (31/33)</td>
<td>97% (30/31)</td>
</tr>
<tr>
<td>Anterior inguinal glands</td>
<td>27</td>
<td>82% (22/27)</td>
<td>36% (8/22)</td>
</tr>
</tbody>
</table>
Rapid whole body MR screening for preinvasive cancer

- **Mouse Mammary** collection on The Cancer Imaging Archive
  https://wiki.cancerimagingarchive.net/display/Public/Mouse-Mammary

10-15 minutes per mouse to screen all mammary glands
MRI can be used to follow progression of DCIS in mice

- Classify as progressing, regressing, indolent

Jansen et al. 2009
Other imaging techniques for DCIS

- Contrast enhanced US
- Quantitative MRI morphology
- Fluorescence Molecular Tomography
- Contrast enhanced MRI
Outline

• Introduction
• Modeling DCIS progression in mice
• Techniques for noninvasively tracking preinvasive cancer progression in mice

• Summary
Summary

• Genetic events in progression of DCIS are not well understood
• We have characterized mouse models to study genetics of DCIS progression and developed noninvasive imaging techniques for interrogating these models
Acknowledgements

**TVD Lab**
Terry Van Dyke
Yurong Song
Amit Adhikari
Zhenye Yang
Nailing Zhang
Yaroslava Ruzankina

**Lucy Lu**
Norene O’Sullivan
Terry Sullivan
Debbie Gilbert
Linda Cleveland
Sophie Wang

**LCDS**
Esta Sterneck
Glenn Summers

**UMass**
Karl Simin

**SAIP/SAIC**
Joe Kalen
Lily Ileva
Lisa Riffle
Yuxi Pang
Marcelino Bernardo

**PHL**
Jenn Matta
Tammy Beachley
Miriam Anver

**CAPR**
Philip Martin

**OMAL**
Stephen Lockett
Alla Brafman

**CBIIT/NCIP**
Brian Hughes
Ed Helton
Eliot Siegel
Paul Mulhern

**LASP**
Stephanie Henderson
Dan Logsdon

**Mentor Committee**
Peter Choyke
Shyam Sharan
Jeff Green
Paul Dayton
Glenn Merlino

**MCGP**
CGS Fellows Program
DOD Postdoc Fellowship