Molecular Classification of Triple-Negative Breast Cancer (TNBC)

Emerging genomic and clinical data

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Lineberger Comprehensive Cancer Center
University of North Carolina, USA
Disclosures

• I have no relevant relationships to disclose.
Deconstructing the molecular portraits of breast cancer

- Luminal A and B
- Normal-like
- HER2-enriched
- Basal-like
- Claudin-low
Distribution of the intrinsic molecular subtypes (+/- Claudin-low) within TNBCs

- **Triple Negatives with Claudin-low**
  - Basal-like: 49%
  - Claudin-low: 30%
  - 9%
  - 5%
  - 6%
  - 1%

- **Triple Negatives without Claudin-low**
  - Basal-like: 72%
  - Claudin-low: 8%
  - HER2-enriched: 6%
  - Luminal A: 5%
  - Luminal B: 9%

N=87

Prat & Perou Mol Oncol 2011
Phase III TNBCs comprised of diverse molecular subtypes (CT +/- Iniparib, NCT00938652)

Affymetrix gene expression profiling of FFPE samples
Intrinsic subtypes assigned using Sorlie et al, PNAS, 2003 data set and claudin-low classifier (Prat et al., BCR, 2010) [courtesy of J. Theilhaber and D. Bergstrom, Sanofi]

Modified from O'Shaughnessy et al. ASCO 2011
Basal-like subtype

Data Highlights
1. 10-25% of all tumors.
2. Highly proliferative (RB-loss).
3. TP53 mutations.
4. BRCA1-associated.
5. Highly aneuploid.
6. Distinct cell type of origin or developmental stage of arrest.
Claudin-low subtype

Data Highlights

1. 5-10% of tumors.
2. Typically TNBCs.
3. Low expression of cell-cell junction proteins
4. Stem cell + EMT (mesenchymal) features
5. Lymphocyte infiltrates
6. Distinct cell type of origin or developmental stage of arrest.

Immune infiltrate
Gene Expression Characteristics of Claudin-low Tumors

Differentiation/Proliferation Markers

Mesenchymal Markers

Stem Cell Markers

Prat et al., Breast Cancer Res 2010
Claudin-low tumors show low DNA copy number changes

- Basal-like
- Claudin-low
- HER2-enriched
- Luminal A
- Luminal B

aCGH landscape
Weigman et al. submitted
Identification of the Claudin-low subtype in a panel of breast cancer cell lines

52 cell lines from Neve et al. Cancer Cell 2006

Prat et al., Breast Cancer Res 2010
Claudin-low/Basal B cell lines show low DNA copy number changes (CNA)

Kao et al., Plos One 2009
Association between metaplastics and medullary-like features and Claudin-low tumors


<table>
<thead>
<tr>
<th></th>
<th>Claudin-low</th>
<th>Basal-like</th>
<th>HER2-enriched</th>
<th>Luminal B</th>
<th>Luminal A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaplastic</td>
<td>8 (40%)</td>
<td>11 (55%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary</td>
<td>2 (20%)</td>
<td>8 (80%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ILC</td>
<td>2 (9%)</td>
<td>1 (5%)</td>
<td>5 (23%)</td>
<td>3 (14%)</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>Tubular</td>
<td>1 (11%)</td>
<td>-</td>
<td></td>
<td>-</td>
<td>7 (78%)</td>
</tr>
<tr>
<td>Adenocystic</td>
<td>-</td>
<td>4 (100%)</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Apocrine</td>
<td>-</td>
<td>-</td>
<td>3 (50%)</td>
<td>1 (17%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>-</td>
<td>-</td>
<td></td>
<td>2 (20%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>IDC with OGC*</td>
<td>-</td>
<td>-</td>
<td></td>
<td>4 (80%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Micropapillary</td>
<td>-</td>
<td>-</td>
<td>2 (25%)</td>
<td>3 (38%)</td>
<td>3 (38%)</td>
</tr>
<tr>
<td>Mucinous A</td>
<td>1 (10%)</td>
<td>-</td>
<td></td>
<td>3 (30%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Mucinous B</td>
<td>-</td>
<td>-</td>
<td></td>
<td>2 (22%)</td>
<td>7 (78%)</td>
</tr>
</tbody>
</table>

*ILC, invasive lobular carcinoma; IDC with OGC, invasive ductal carcinoma with osteoclastic giant cells.

Prat et al, Breast Cancer Res 2010
Association between Metaplastics and Claudin-low tumors

Hennessy B. et al., Cancer Res, 2009
Clinical-pathological characteristics of the Claudin-low Intrinsic Subtype

<table>
<thead>
<tr>
<th></th>
<th>Claudin-low</th>
<th>Basal-like</th>
<th>HER2-enriched</th>
<th>Luminal B</th>
<th>Luminal A</th>
<th>Normal-like</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UNC NKI MDACC</td>
<td>UNC NKI MDACC</td>
<td>UNC NKI MDACC</td>
<td>UNC NKI MDACC</td>
<td>UNC NKI MDACC</td>
<td>UNC NKI MDACC</td>
</tr>
<tr>
<td>Num. Patients</td>
<td>37 21 18</td>
<td>73 42 15</td>
<td>39 49 28</td>
<td>62 69 27</td>
<td>99 84 37</td>
<td>10 30 8</td>
</tr>
<tr>
<td>Prevalence</td>
<td>12% 7% 14%</td>
<td>23% 14% 11%</td>
<td>12% 17% 21%</td>
<td>19% 23% 20%</td>
<td>31% 28% 28%</td>
<td>3% 10% 6%</td>
</tr>
<tr>
<td>ER+</td>
<td>12% 33% 22%</td>
<td>11% 19% 0%</td>
<td>36% 59% 29%</td>
<td>91% 100% 96%</td>
<td>91% 100% 97%</td>
<td>44% 93% 100%</td>
</tr>
<tr>
<td>HER2+</td>
<td>23% - 22%</td>
<td>6% - 13%</td>
<td>30% - 25%</td>
<td>53% - 41%</td>
<td>74% - 70%</td>
<td>22% - 63%</td>
</tr>
<tr>
<td>HER2-/ER-</td>
<td>70% - 72%</td>
<td>82% - 87%</td>
<td>25% - 18%</td>
<td>8% - 4%</td>
<td>6% - 3%</td>
<td>13% - 0%</td>
</tr>
<tr>
<td>HER2-/ER-/PR-</td>
<td>71% - 61%</td>
<td>80% - 73%</td>
<td>22% - 14%</td>
<td>9% - 4%</td>
<td>4% - 3%</td>
<td>0% - 0%</td>
</tr>
<tr>
<td>Node-</td>
<td>58% 48% 28%</td>
<td>63% 60% 20%</td>
<td>26% 47% 21%</td>
<td>44% 42% 33%</td>
<td>51% 58% 41%</td>
<td>33% 50% 25%</td>
</tr>
<tr>
<td>Grade 3</td>
<td>77% 38% 61%</td>
<td>88% 86% 93%</td>
<td>55% 61% 89%</td>
<td>62% 41% 46%</td>
<td>30% 13% 27%</td>
<td>63% 20% 50%</td>
</tr>
<tr>
<td>Tumor size &gt; 2 cm</td>
<td>74% 38% 78%</td>
<td>77% 62% 80%</td>
<td>93% 57% 79%</td>
<td>85% 52% 96%</td>
<td>66% 36% 91%</td>
<td>89% 40% 88%</td>
</tr>
<tr>
<td>pCR</td>
<td>- - 39%</td>
<td>- - 73%</td>
<td>- - 39%</td>
<td>- - 19%</td>
<td>- - 0%</td>
<td>- - 0%</td>
</tr>
</tbody>
</table>

**Graphs:**

- **NKI295**
  - Basal-like
  - Claudin-low
  - HER2-enriched
  - Luminal A
  - Luminal B
  - p=1.9e-07

- **UNC337**
  - Basal-like
  - Claudin-low
  - HER2-enriched
  - Luminal A
  - Luminal B
  - p=7.67e-06

Prat et al. Breast Cancer Res 2010
Pathological complete response (pCR) data after neoadjuvant anthracycline/taxane chemotherapy within TNBCs (n=188)

<table>
<thead>
<tr>
<th></th>
<th>RCB0/1</th>
<th>%</th>
<th>RCB2/3</th>
<th>%</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like</td>
<td>51</td>
<td>49%</td>
<td>53</td>
<td>51%</td>
<td>104</td>
</tr>
<tr>
<td>Claudin-low</td>
<td>14</td>
<td>31%</td>
<td>31</td>
<td>69%</td>
<td>45</td>
</tr>
<tr>
<td>HER2-E</td>
<td>4</td>
<td>31%</td>
<td>9</td>
<td>69%</td>
<td>13</td>
</tr>
<tr>
<td>LumA</td>
<td>0</td>
<td>0%</td>
<td>3</td>
<td>100%</td>
<td>3</td>
</tr>
<tr>
<td>LumB</td>
<td>0</td>
<td>0%</td>
<td>6</td>
<td>100%</td>
<td>6</td>
</tr>
</tbody>
</table>

**Basal-like vs. others, p<0.005**
**Basal-like vs. Claudin-low, p<0.05**

Array data obtained from: Hatzis et al. JAMA 2011
Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features

Chad J. Creighton, Xiaoxian Li, Melissa Landis, J. Michael Dixon, Helen Wong, Anna Tsimelzon, Jason I. Herschkowitz, Cheng Fan, Xiaomei Zhang, Xiaping He, Anne Pavlick, Jian Huang, M Carolina Gutierrez, William R Miller, Alexey A Larionov, Susan G. Hilsenbeck, Charles M. Perou, Michael T. Lewis, Jeffrey M. Rosen, and Jenny C. Chang.

Comparative oncogenomics identifies breast tumors enriched in functional tumor-initiating cells

Jason I. Herschkowitz\textsuperscript{a}, Wei Zhao\textsuperscript{b,c}, Mei Zhang\textsuperscript{a}, Jerry Usary\textsuperscript{c,d}, George Murrow\textsuperscript{e}, David Edwards\textsuperscript{a}, Jana Knezevic\textsuperscript{a}, Stephanie B. Greene\textsuperscript{a}, David Darr\textsuperscript{c,d}, Melissa A. Troester\textsuperscript{c}, Susan G. Hilsenbeck\textsuperscript{e}, Daniel Medina\textsuperscript{a}, Charles M. Perou\textsuperscript{c,d,f}, and Jeffrey M. Rosen\textsuperscript{a,1}

\textsuperscript{a}Department of Molecular and Cellular Biology and \textsuperscript{b}Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, TX 77030; and \textsuperscript{c}Curriculum in Bioinformatics and Computational Biology, Lineberger Comprehensive Cancer Center, \textsuperscript{d}Department of Genetics, and \textsuperscript{f}Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC 27599

P53-null mouse model

Herschkowitz et al. PNAS 2011
Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers

Elgene Lim\textsuperscript{1,2,9}, François Vaillant\textsuperscript{1,9}, Di Wu\textsuperscript{1,2}, Natasha C Forrest\textsuperscript{1}, Bhupinder Pal\textsuperscript{1}, Adam H Hart\textsuperscript{3}, Marie-Liesse Asselin-Labat\textsuperscript{1}, David E Gyorki\textsuperscript{1,2}, Teresa Ward\textsuperscript{1}, Audrey Partanen\textsuperscript{4}, Frank Feleppa\textsuperscript{4}, Lily I Huschtscha\textsuperscript{5}, Heather J Thorne\textsuperscript{6}, kConFab\textsuperscript{7}, Stephen B Fox\textsuperscript{6}, Max Yan\textsuperscript{6}, Juliet D French\textsuperscript{8}, Melissa A Brown\textsuperscript{8}, Gordon K Smyth\textsuperscript{1}, Jane E Visvader\textsuperscript{1,9} & Geoffrey J Lindeman\textsuperscript{1,2,4,9}
232 Human Breast Tumors analyzed for FAC sorted epithelial cell signatures

Mammary Stem Cell (MaSC)  
Luminal Progenitor  
Mature Luminal

Prat & Perou, personal communication
Mammary development meets cancer genomics
Basal-like and Claudin-low tumors have epithelial tumor cells with mesenchymal characteristics (Intra-tumor heterogeneity)

![Images of VIMENTIN and KRT5/19 staining in breast cancer tissues]

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Samples with Dual Negativity</th>
<th>%</th>
<th>Samples with Dual Positivity</th>
<th>%</th>
<th>Total Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin-low</td>
<td>9</td>
<td>45%</td>
<td>11</td>
<td>55%</td>
<td>20</td>
</tr>
<tr>
<td>Basal-like</td>
<td>4</td>
<td>22%</td>
<td>14</td>
<td>78%</td>
<td>18</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>6</td>
<td>86%</td>
<td>1</td>
<td>14%</td>
<td>7</td>
</tr>
<tr>
<td>Luminal B</td>
<td>20</td>
<td>91%</td>
<td>2</td>
<td>9%</td>
<td>22</td>
</tr>
<tr>
<td>Luminal A</td>
<td>19</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>67%</td>
<td>28</td>
<td>33%</td>
<td>86</td>
</tr>
</tbody>
</table>
Selected TNBC/Basal-like cell lines have cells with Claudin-low characteristics

Claudin-low more migratory

Claudin-low less proliferative

Prat et al. AACR 2011
Conclusions

1. TNBCs are a heterogeneous group primarily composed of Basal-like breast tumors.

2. Claudin-low tumors also constitute a significant proportion of TNBCs.

3. The molecular subtypes as of today have no influence over treatment decisions for TNBC patients.

4. Chemotherapy benefit is typically high in Basal-like and Claudin-low tumors, although those with residual disease after treatment (claudin-low features??) have a high risk of relapse.

5. Basal-like and Claudin-low tumors are enriched with tumor cells with mesenchymal/stem cell features (intra-tumor heterogeneity) that may need specific targeted treatments.
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Steve Marron and Andrew Nobel (Statistics)
Lisa Carey, Carey Anders, Neil Hayes, and Ned Sharpless (Oncology)
Bob Millikan (Epidemiology) and Chad Livasy (Pathology)

Washington University
Matthew Ellis Lab
Elaine Mardis, Rick Wilson
and The Genome Center

University of British Columbia
Torsten Nielsen Lab

University of Utah
Phil Bernard Lab