Molecular Pathology of Breast Cancer

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Biology of breast cancer

• Breast cancer is not a single disease but a collection of diseases with different molecular characteristics and clinical outcomes
Why do we need molecular characterisation of breast cancer

• To identify patients whose prognosis is so good that adjuvant therapy after local surgery would not be beneficial
• To identify patients whose prognosis is so poor that a more aggressive adjuvant approach would be warranted
• To identify patients likely to be responsive or resistant to particular forms of therapy (= predictive factors)
• => Individualised patient management
Traditional classification of breast cancer

- Assessment of the extent to which the appearance of a carcinoma resembles normal breast glandular tissue
- Tumour type
- Histological Grade
- ER and HER2 status
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

• Gene expression patterns of breast carcinomas distinguish tumour subclasses with clinical implications

• ‘Intrinsic subtypes’
  • Sørlie, Perou et al.
  • PNAS, 2001; 98(19):10869–74
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Immunohistochemical correlates
- **ER positive - Luminal breast cancers**
  - type A – PgR +, HER2 -, low proliferation
  - type B – PgR +/-, HER2 +/-, high proliferation
- **ER negative**
- HER2 positive
- Basal breast cancers – ER/PgR/HER2 -, CK5/ CK14 / EGFR +
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

• Intrinsic subtypes are reproducible across platforms, however assignment of individual cancers to a molecular subtype shows only moderate reproducibility

• Dependent upon platform used, expression thresholds, and composition of the population

• Basal-like group most reproducible, luminal B and HER2 enriched least reproducible
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Concordance between gene expression and IHC defined subtypes modest at best
- Luminal A versus Luminal B – Ki67 cut point of 14% [Cheang et al., JNCI 2009]. Sensitivity 72%, specificity 77%
- Follow up study looking at 2 large clinical series [Prat et al., JCO 2013]:
  - 81-85% of luminal A correctly identified
  - 35-52% of luminal B misclassified as Luminal A
  - Improvement if include PR with cut off of 20%
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

Prat A and Perou C, Mol Oncol 2011
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

• Heterogeneity within HER2 positive disease, largely driven by ER status
• Clinically HER2 + and – tumours within each intrinsic subtype differ only in expression of genes in or near the HER2 amplicon on 17q
• Highest levels of HER2 pathway activation in cHER2+ HER2 enriched tumours

Prat et al., JNCI 2014;106(8).
Newer classification systems for invasive breast cancer – Intrinsic Subtypes

- Retrospective analysis of NOAH study looking at PAM50 subtypes
- Only 55% of HER2+ tumours HER2-E subtype; 21% luminal, 7% basal-like, 18% normal-like
- Better pCR rates in HER2-E vs luminal HER2+ tumours (53% v 29%) with larger improvement in EFS with addition of Trastuzumab

Emerging landscape of oncogenic signatures across human cancers

Giovanni Ciriello, Martin I. Miller, Bülent Arman Aksoy, Yasin Senbabaoglu, Nikolaus Schultz & Chris Sander
A new molecular taxonomy of breast cancer

ARTICLE

The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups
## Integrative clusters

<table>
<thead>
<tr>
<th>IntClust</th>
<th>Molecular Features</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amp 17q23, mut GATA3, High instability</td>
<td>Intermed</td>
</tr>
<tr>
<td>2</td>
<td>Amp cyclin D1, High instability</td>
<td>Poor</td>
</tr>
<tr>
<td>3</td>
<td>Mut PIK3CA and CDH1, low instability</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>Low instability, immune upregulation</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>HER2 Amp</td>
<td>Poor</td>
</tr>
<tr>
<td>6</td>
<td>Amp ZNF703, high instability</td>
<td>Intermed</td>
</tr>
<tr>
<td>7</td>
<td>16p gain, 16q loss, amp 8q</td>
<td>Good</td>
</tr>
<tr>
<td>8</td>
<td>1q gain, 16q loss</td>
<td>Good</td>
</tr>
<tr>
<td>9</td>
<td>8q gain, 20q amp, high instability</td>
<td>Intermed</td>
</tr>
<tr>
<td>10</td>
<td>TNBC; high instability, DNA damage repair</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Mukherjee et al., NPJ Breast Ca 2018
Integrative clusters

Mukherjee et al., NPJ Breast Ca 2018
Integrative clusters

Mukherjee et al., NPJ Breast Ca 2018
Different clinical behaviour

- Basal
- HER2
- Normal
- Luminal B
- Luminal A
- IntClust 1
- IntClust 2
- IntClust 3
- IntClust 4
- IntClust 5
- IntClust 6
- IntClust 7
- IntClust 8
- IntClust 9
- IntClust 10

No pCR vs pCR
ILC - Molecular

- Distinct morphology but genetic heterogeneity
- Gene expression profiling and copy number analysis reveals two subgroups
- Immune related –immune signalling and cytokine pathways, with upregulation of immune response inhibitors
- 78% have moderate to severe lym infiltrate
- Hormone related –ESR1 and PGR, cell cycle and ER target genes

ILC - Molecular

- Mutations:
  - \textit{CDH1} – 43-63%
  - \textit{PIK3CA} – 35-48%
  - \textit{TP53} – 5-27%
  - \textit{PTEN} – 14% (more common than NST – 3%)
  - \textit{GATA 3} – 3-5% (less common than NST – 20%)
  - \textit{ERBB2} – 4-18%
  - Remainder low frequency

Ciriello et al., Cell 2015;163:506-19.
Case 1

- 63 y.o. female
- Presented clinically with a lump in the left UOQ
- Core biopsy – grade 3 invasive cancer, mixed NST and micropapillary; ER/ PR 8, HER2 borderline/ FISH non-amp
Case 1

- Proceeded to WLE and SLNB
- Final histology:
  - 19 mm grade 3 invasive ca, mixed NST and micropapillary
  - Clear of margins
- SLN – 0/2
Case 1

10 year chemotherapy benefit = 4.5% -> discuss
Gene based prognostic tests

- Gene expression profiling
- cDNA array or RT-PCR based
- Several gene signatures have been proposed
  - 21 gene – Oncotype Dx® - TAILORx
  - 70 gene – Mammaprint® - MINDACT
  - PAM50 – uses intrinsic subtypes
  - 12 gene - EndoPredict
- Little overlap in specific genes that make up the signatures, but all include genes involved in proliferation and ER signalling
### Oncotype DX

<table>
<thead>
<tr>
<th>HER2 GROUP</th>
<th>HER2  GRB7</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER GROUP</td>
<td>ER  PgR</td>
</tr>
<tr>
<td></td>
<td>Bcl2  SCUBE2</td>
</tr>
</tbody>
</table>

#### HER2 GROUP
- HER2
- GRB7

<table>
<thead>
<tr>
<th>ER GROUP</th>
<th>Beta-actin</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>RPLPO</td>
<td>GUS</td>
</tr>
<tr>
<td>PgR</td>
<td>TFRC</td>
<td></td>
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</tbody>
</table>

#### REF GROUP
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

#### INVASION GROUP
- Cathepsin L2
- Stromelysin 3

#### PROLIFERATION GROUP
- KI67
- STK-15
- SURVIVIN
- CYCLIN B1
- MYBL2

Combine results in an algorithm to get the recurrence score:

<table>
<thead>
<tr>
<th>Score</th>
<th>Recurrence Rate</th>
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</thead>
<tbody>
<tr>
<td>&lt;18</td>
<td>6.8%</td>
</tr>
<tr>
<td>18-30</td>
<td>14.3%</td>
</tr>
<tr>
<td>&gt;30</td>
<td>30.5%</td>
</tr>
</tbody>
</table>

10yr distant recurrence rate
Case 1

Recurrence score = 12 – low risk
Endocrine therapy and RT only

WGS as part of PBCP – validated PIK3CA mutation
TNBC – Genomic Subtypes

- Gene expression array analysis of TNBC identified 6 subtypes, now refined to 4 (immunomodulatory group reflects TILs and MSL reflects stromal contamination)
- Basal like 1 – elevated cell cycle and damage response genes
- Basal like 2 – growth factor signalling and myoepithelial genes
- Mesenchymal – epithelial-mesenchymal transition and growth factor pathways
- Luminal Androgen Receptor
  - luminal gene expression driven by AR
  - lower grade
  - higher incidence of lymph node and bone metastasis
  - high incidence PIK3CA mutations (40%)

TNBC – response to NACT

- Different rates of pCR between subtypes
- No difference in OS – LAR group had low pCR rate but best survival at 3 years
Basal Breast Cancers

- ‘Triple negative’ – ER, PgR and HER2 negative
- Express basal cytokeratins – CK 5/6, CK14
- Express EGFR
- Distinct morphology – high grade, central acellular zones, necrosis, high mitotic count
- Heterogeneous group – medullary-like, metaplastic, adenoid cystic carcinoma
- Associated with BRCA1 mutations in young women
- Associated with worse prognosis and distant metastasis, particularly visceral metastases
Basal Breast Cancers
Basal Breast Cancers
Carcinoma with medullary features

- Circumscribed tumour with pushing rather than infiltrating margin
- Interconnecting sheets of large, bizarre and pleomorphic carcinoma cells forming a syncytial network
- Prominent lymphocytic inflammatory cell infiltrate
- Usually ER/ HER2 negative
- Association with BRCA1 mutations
TNBC – LAR Subtype

• Luminal Androgen Receptor
  • luminal gene expression driven by AR
  • lower grade, higher incidence of lymph node and bone metastasis
  • high incidence PIK3CA mutations (40%)

• Molecular Apocrine Subtype
  • Farmer et al., Oncogene2005
  • 3 groups; luminal (ER+), basal and an ‘intermediate’ group -> ER- but with a luminal keratin expression pattern
  • 50% HER2 positive
  • Androgen receptor positive with expression of metabolism related signatures and increased androgen signalling
  • Review of histology – apocrine features but not classical apocrine carcinomas
Carcinoma with Apocrine Features

- Large cells with abundant granular eosinophilic cytoplasm
- Round nuclei with prominent nucleoli
- Pure apocrine carcinoma incidence 1-5%
- Older women
- AR and GCDFP15 positive
- ER/ PR negative
- 10-60% HER2 positive
Androgen Receptor

- AR is the most commonly expressed hormone receptor in breast cancer
- Up to 90% of breast cancers are positive depending upon methods and cut offs used (literature 60-90%)
- ER + - 85-95% AR+ (LumA – 91%, LumB – 68%)
- ER - - 15-70% AR+ depending on series
- ER-/ HER2+ - 50-66% AR+
- TNBC – 32% AR+ (20-55%)
Apocrine Carcinoma and AR

- Apocrine Ca
- AR+ BC
- TNBC
Future Classification

- Hypermutated tumour
- Associated with increased expression of neoantigens -> host immune response with TILS
- BASKET OF BASKETS trial – eligible for immune therapy

Figure 2: IMAGES OF HYPERMUTATED BREAST CANCER – Somatic Rain Plot;

Mutation frequency - Number of mutations per megabase: 16.94. This sample is considered hypermutated. The threshold for hypermutated profile is 12 mutations/Mb
Case

- 49 year old female presented with a painful right breast lump
- Core biopsy – grade 3 invasive carcinoma NST, ER/ PR/ HER2 negative
Case

- Enrolled into PARTNER trial using platinum based chemotherapy with a novel targeted agent, olaparib.
- The patient also consented to WGS in the Personalised Breast Cancer Program
- No germline mutations including BRCA1/2
- Cosmic signature 3 – defective double stranded DNA break repair by homologous recombination
- Likely to respond well to trial therapy with platinum and targeted DNA damage repair agent
Case

- Complete radiological response on midtreatment MRI
- Final histology: pCR following neoadjuvant chemotherapy
Personalised Breast Cancer Programme – whole genome sequencing looking for mutations and copy number alterations

Actionable mutations:
Highly Actionable (Tier 1) - Robust evidence
- Genomic alteration validated in clinical trials
– Clinical evidence of association with response to therapy

Potentially Actionable (Tier 2)
- Evidence mutation is activating (oncogene) or non-activating (tumour suppressor gene) in models
- Pre clinical evidence of association with response to treatment but clinical evidence lacking/ insufficient

Dr Jean Abraham and Prof Carlos Caldas
Future Classification

• Whole genome sequencing -> mutational signatures
• Cosmic signatures – distinct patterns of mutations are associated with different mechanisms of DNA damage
  • Signature 1 = age related
  • Signature 2 = APOBEC family of cytosine deaminases; C>T
  • Signature 3 = double stranded DNA damage repair; BRCA1 and 2 mutation carriers

• Concept of Basket studies – eligibility for trial dependent upon mutational profile or presence of specific mutations rather than tissue type