Molecular Classification of Breast Cancer

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Key Questions

• How many different subtypes of breast cancer are there?
  – Which classification system to use?
• How are the “intrinsic molecular subtypes” defined/tested?
  – How can we translate intrinsic subtypes into routine practice?
• What is the clinical utility of the intrinsic subtypes?
  – What are limitations/unanswered questions?

Clinical subtypes of breast cancer

• Early stage vs locally advanced vs late stage
• Treatment sensitive vs not:
  – Hormone treatments
  – Chemotherapy
  – Radiotherapy
  – Biologics (HER2-targeted)
Histologic Classification

- In situ vs Invasive
- Histologic subtype
  - Mostly Ductal, NOS
  - ILC, special subtypes
- 3 Nottingham Grades
- Size categories
- LN+ vs LN-

Protein expression subtypes

- What proteins does the cancer express in abnormal levels?
- Hormone receptors
- HER2 over-expression
- Proliferation markers

Gene expression based subtypes

- Based on similarity of gene expression profiles
- 4 Distinct Subtypes:
  - Luminal A (ER+)
  - Luminal B (ER+)
  - Her2+
  - Basal-like
Molecular Subtype is associated with a poor relapse-free survival and overall survival


Combining genetic and transcriptional information:

The genomic and transcriptomic architecture of 2,000 breast tumors reveals novel subgroups

- Copy number aberrations and gene expression
- 10 breast cancer subtypes associated with outcome differences

Curtis 2012  doi 10.1038/nature10883

Comprehensive molecular portraits of human breast tumors

- Over 500 breast cancers
- DNA mutations, copy number alterations, methylation, RNA expression, protein
- 50% of driver mutations are present in < 10% of breast cancers (TCGA)
- Many mutations are unique
- If we want to personalize therapy with more targeted drugs --- have to get very specific...
Multiple Molecular Subtypes of Triple Negative Breast Cancer

- Basal-like 1
- Basal-like 2
- Immunomodulatory
- Mesenchymal
- Mesenchymal stem-like
- Luminal androgen receptor
- Unstable

Linked to pCR rates and survival


Why so many subtypes?

- Increasing samples sizes = greater phenotypic heterogeneity and rare types captured
- More diverse –omics data (DNA, gene expression, epigenetics, proteomics, etc)

Changing paradigms?

- Mutations are more common across cancer types
- Blurring lines between current cancer categories?
- Will molecular profiles tell us how to treat (and IF to treat?)

Which classification system to use?

- Reproducible
  - As applied to Populations
  - As applied to Individuals

- Relevant:
  - To Biology
  - To Outcomes:
    - Prognosis
    - Prediction of therapy benefit

Comprehensive molecular portraits of human breast tumours

- Using 5 different data types (protein, RNA, DNA copy number, methylation) on 300 breast cancers
- Cancers clustered into four main "types"
- These 4 types correlated well with 4 intrinsic subtypes as defined by mRNA expression only
  - Luminal A and B, HER2-
  - Enriched, Basal-like
Four “Intrinsic” Molecular Subtypes are a Robust Classification System

- Most reproducible subtypes across platforms
- Fit well with biologic pathways to cancer
- Significant differences in incidence, risk factors, prognosis and treatment sensitivity

St Gallen Guidelines 2013

- Treatment recommendations are based on intrinsic subtypes
What Drives Intrinsic Subtypes?

- Proliferation is a major driver
- Hormone/Luminal Pathways
- HER2-related pathways
- Basal “myoepithelial” pathways

Intrinsic Subtype Characteristics

<table>
<thead>
<tr>
<th>Molecular Subtypes</th>
<th>Basal</th>
<th>HER2-E</th>
<th>Luminal B</th>
<th>Luminal A</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of breast cancers:</td>
<td>15-20%</td>
<td>10-15%</td>
<td>20%</td>
<td>40%</td>
</tr>
<tr>
<td>Receptor expression:</td>
<td>HER2+</td>
<td>ER+</td>
<td></td>
<td></td>
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<tr>
<td>Histologic grade:</td>
<td>High grade</td>
<td>Low grade</td>
<td></td>
<td></td>
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<tr>
<td>Prognosis:</td>
<td>Poor</td>
<td>Good</td>
<td></td>
<td></td>
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<tr>
<td>Response to therapy:</td>
<td>Chemotherapy, HER2 Rx</td>
<td>Hormone Rx</td>
<td></td>
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</tr>
</tbody>
</table>

Basal subtype

- Most unique and robust molecular subtype
- More similar to ovarian serous carcinoma than other breast cancer subtypes
- Most frequent subtype in BRCA1+ patients
- Different ethnic distribution = more common in African Americans
- Different age range = younger
- Risk factors: Increased parity, less time breast feeding

Basal Subtype: Molecular features

- High expression of proliferation-related genes
- High expression of “basal-type” cytokeratins (5, 14, 17)
- Intermediate expression of HER2-related genes
- Very low expression of luminal-related genes
- Second highest number of mutations across genome (HER2-E = #1)
- 80% p53 mutant
- Only 9% PI3KCA mutant

Basal Cancers

- Defect in homologous recombination DNA repair pathway
- Seen in both BRCA-1 mutation carriers and sporadic basal-like breast cancers
- May be a target for specific therapies:
  - PARP-inhibitors/poly(ADP-ribose) polymerase
  - Platinum based chemotherapy = cross-linking agents

Translation into clinical practice

- How do we define these cases clinically?
- Gene expression profiling – not practice
- Basal-markers:
  - CK5/6, EGFR
  - Negative for ER, PR and HER2
  - <1% of cells with weak staining the right threshold?

Histology of Basal-like subtype

- Nottingham grade 3
- Sheet-like growth pattern with pushing borders
- Associated geographic necrosis
- Frequent lymphocytic response


“Triple Negative Immunophenotype”:
- ER –
- PR –
- Her2 –

Ki67 index HIGH

Basal-Like Immunophenotype:
Expression of basal cytokeratins (CK5,6,14,15,17)
In contrast to simple/luminal cytokeratins (CK7,8,18,19)

Basal-like Subtype is Heterogeneous

- Histologic subtypes: Ductal (NOS), "Medullary", Adenoid cystic, Metaplastic

Some good prognosis subtypes - don't want to base treatment ONLY on subtype!

Triple Negative Breast Cancer: Multiple Molecular Subtypes

- Basal-like 1
- Basal-like 2
- Immunomodulatory
- Mesenchymal
- Mesenchymal stem-like
- Luminal androgen receptor
- Unstable

Some studies linked to pCR rates and survival


Basal-like Subtype

- 23% of basal-like by GEP are non-triple negative
- 29% of triple negative cancers are non-basal by GEP
- Variable degrees of expression of basal cytokeratins
  - What threshold should define the entity?
  - Has not become standard practice

Low ER+ Breast Cancer

Is This a Distinct Group?

Nina C. Glikoza, MD; David J. Dabbs, MD; and Rohit Bhargava, MD

- Features of 49 cases with low ER (1-10%)
- Histologic Features more similar to "Triple Negative" cancers:
  - High grade (92%)
  - Ki67 > 50% (80%)
  - Sheet-like growth (71%)
  - Necrosis (45%)
- CPR to neoadjuvant chemotherapy: 33%

An J Clin Pathol 2014;141:607-611
DOI: 10.1208/s12252-014-0537-2

Effect of ASCO/CAP Guidelines for Determining ER Status on Molecular Subtype

Brenda Boyarsky, RH/ASCP; Jennifer L. Kane, RH/ASCP; Alyson L. Yalunsky, BS; Ryan van Laar, PhD; Christopher Gallagher, MD; Craig D. Shriver, MD; and Rachel E. Ellsworth, PhD

- 88% of low ER staining cases (Luminal by IHC) were classified as "Basal-like" by molecular subtype

DOI 10.1245/s10434-012-2558-8
Explaining Discordance when Borderline ER results

- Grade 3 invasive ductal carcinoma, LN neg
- Core Biopsy outside read by image analysis : ER 2%
- Core biopsy by our review: ER 20%, 1+
- Excision at Stanford: ER 30%, 1-2+
- Sent for Oncotype DX:
  - High RS (54; 34% recur )

Surrogate Definitions of Intrinsic Subtypes: St Gallen

- Use ER-/PR-/HER2- for “Basal-like”
- Acknowledge that only 80% overlap
- Low-ER positive cancers may be included
- Special histologic subtypes

HER2 Enriched Subtype

- High expression of HER2-related and proliferation related genes
- Intermediate expression of luminal-related genes
- Low expression of basal-related genes
- Highest mutation numbers across the genome
- 72% p53 mutant
- 39% PIK3CA mutant
- High frequency of APOBEC3B-associated mutations (source of mutations)
HER2 Enriched Subtype

- Only 60-70% are HER2 positive clinically!
- HER2 Enriched subtype may have higher pCR rates than other subtype that are HER2+ clinically
- Immune signature response also important

![Figure 1. pCR rates of HER2 clinically positive cancers after neoadjuvant trastuzumab + chemotherapy](source)

Original article
Clinical implications of the intrinsic molecular subtypes of breast cancer

**Table 1.** Distribution of the HER2+ breast cancers within the pathology-based groups.

<table>
<thead>
<tr>
<th>-</th>
<th>ER status</th>
<th>HER2 status</th>
<th>pCR rates</th>
<th>BMI</th>
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</thead>
<tbody>
<tr>
<td>HER2+</td>
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Clinically HER2 Positive Cancers have highly variable intrinsic subtypes – Which classification is most predictive of HER2-targeted therapy?


Surrogate Definitions of Intrinsic Subtypes: St Gallen

- Use clinical HER2+/ER- for HER2-E subtype
  - Despite poor overlap
- HER2 clinical status is most well-validated and recommended predictor of response to HER2 targeted therapies currently

![Table](source)
Luminal Subtypes

- High expression of luminal cytokeratins
- Hormone-regulated pathways upregulated
- Most common subtype: 60+% of breast cancers are luminal

Luminal Subtypes Prognosis Differences

- Prognosis different
  - Critical to distinguish
  - OncotypeDX and other assays attempt to segregate Lum A from Lum B
Luminal Subtypes Therapy Response Differences

- Neoadjuvant anastrozole x 2 weeks
  - 97% of Luminal A → Lum A or "normal"
  - 71% of Luminal B → Lum A or "normal"
- Luminal B Subtype can change with treatment

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Chemotherapy Response

- Low pathCR rates in Luminal A cancers (3%)
  - pathCR does not predict outcome in Luminal A
- Luminal B pathCR rates slightly higher (16%)
  - Path CR rates are predictive of outcome in Luminal B

Original article
Clinical implications of the intrinsic molecular subtypes of breast cancer
Aleix Prat a,b,c,d, Estela Pineda e,f, Barbara Adamo a,b, Patricia Galván h.i, Avanzazu Fernández a,b, Lidia Caba a,b, Marc Díez a,b, Margarita Vladut e,f, Ana Arance a,b, Montserrat Muñoz a,b

Survival curves of luminal and basal cross at 10 years

High discordance between Clinically Defined “Luminal A and B” and PAM50 categorization

Surrogate Definitions of Intrinsic Subtypes: St. Gallen

1. Annual A: "St. Gallen A+B" positive
   - High and high-risk
   - HER2 negative
   - Risk low
   - Recurrence risk based on multi-gene expression assay (FISH/Chips)

2. Annual B: "St. Gallen B-like (HER2 negative)"
   - ER positive
   - PR positive and/or low or intermediate risk
   - HER2 negative or low
   - Recurrence risk high based on multi-gene expression assay (FISH/Chips)

3. Annual C: "St. Gallen C-like (HER2 positive)"
   - ER positive
   - PR positive
   - HER2 negative or equal to wild type
   - Low risk

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What is the clinical relevance of the intrinsic subtypes?

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Why do we care?
Predicting therapy benefit

Multiple Tests to Determine Intrinsic Subtype

- PAM-50 (subtype information only reportable outside of US) – ROR score reportable in US

- MammaPrint BluePrint
  - Luminal vs HER2 vs Basal
  - “low risk” vs “high risk”

Multiple testing platforms for prognostic signatures

<table>
<thead>
<tr>
<th>Signature</th>
<th>Description</th>
<th>Validation</th>
<th>Training platform</th>
<th>Coverage (%)</th>
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<tbody>
<tr>
<td>PAM-50</td>
<td>HER2 subtypes</td>
<td>Ref. 2,3</td>
<td>Stanford DNA array</td>
<td>41-52/56</td>
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<td>Ref. 10</td>
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<td>40/70/90</td>
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<td>30-50/60/90</td>
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<td>qRT-PCR</td>
<td>70/100/50</td>
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<tr>
<td>ALDH1</td>
<td>HER2 subtypes</td>
<td>Ref. 24</td>
<td>qRT-PCR</td>
<td>70/100/50</td>
</tr>
</tbody>
</table>

*The original data (US) reported 108 validation sites annotated from 256 clones in the gene expression, of which 177 clones mapped to the Affy probes (46.2%).

(2009) The reported data for the platform is higher than the percentage reported here.
Breast cancer molecular profiling with single sample predictors: a retrospective analysis

• Compared 3 micro-array based predictors
• Only Basal-like showed consistent agreement (K>0.812)
• Assignment to Luminal A, Luminal B, HER2 and normal-like depended on predictors used
• Each test was equally predictive of overall survival

Variability when used for a single patient may be too high to be useful clinically

Clinically relevant breast cancer subtypes

• Identification of treatment targets is most clinically relevant

Prognostic Pathology-Based Factors:

• Histologic Subtype
• Molecular Subtype/ER+HER2+proliferation
• Grade (Nottingham)
• Mets to LN or beyond
• Size/extent
• Margin status
• Associated DCIS
• AVI extensive

Biology-Dependent Factors: How aggressive is it?

Time-Dependent Factors: How long it has been there?

Local-Recurrence Factors: How hard to remove locally?

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Clinical Utility of Intrinsic Molecular Subtypes

- Useful framework for looking at breast cancer populations
- Questions remain about utility as an individual patient predictor
- Has not replaced clinical testing that determines treatment options

1. How many cancer subtypes are there?
2. How to evaluate robustness of classification?
3. How affected by intratumoral heterogeneity and tumor evolution?
4. Can multiple classification systems co-exist?

Biology is a spectrum!
Pathologists as “Diagnostic Oncologists”

Translation and integration of biologic information

Treatment Team

Patient Factors

Individualized Treatment Decisions