Breast Cancer Prognostic and Predictive Genomics: Histology/Markers/Grade/Stage/Molecular profiling: The case for integrated representation

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The challenge

- Breast cancer is a highly heterogeneous and complex set of diseases.
- Every tumor is driven by numerous and varied genomic (DNA mutations/alterations) and epigenetic changes (methylation, histone acetylation, phosphorylation...) and is virtually unique.
- The above changes manifest themselves in various ways and every analysis gives us a particular snapshot of the cancer.
The challenge...

- The challenge is to create an integrated representation of the snap shots that will enhance our understanding and impact the disease course.
- As such, classification of breast cancer attempts to define the prognosis of the cancer and who benefits from what type of therapy by defining commonly behaving and responsive tumors.
- Providing the right therapy to the right person!
The Solution...comprehensive analysis

- Characterization of breast cancer traditionally relied on histologic diagnosis and classification, grading, immunohistochemical and limited copy number profile (ER, PR, Her-2, Ki-67), staging (TNM), and in the last two decades increasingly molecular characterization (multi-omic approach). Some of these characteristics are related to: diagnostic, prognostic, predictive features of cancer ....
Breast cancer by Histology
WHO defined 21 subtypes

D. Vuong ... Virchows Arch (2014) 465
Markers, Grade and Stage

- ER, PR, Her-2 and Ki67
- Grade: The degree to which the histologic pattern resembles normal breast ducts and lobules
- Nottingham (Elston and Ellis modified Scarff-Bloom Richardson) grade: Based on a tubular score (1-3), nuclear score (1-3) and mitotic rate (1-3).
  - Well differentiated has a cumulative score of 3-5, Moderate diff 6-7, and poorly diff 8-9.
- Stage: AJCC 7th Edition: T=tumor size, N= nodal status, M=metastatic status
Adjuvant! Online/data integrator

- Online tool predicting 10 year survival and degree of benefit from hormonal and/or chemotherapy based on age, grade, tumor size and lymph node status, ER status, comorbidities...
- Survival estimates based on SEER and response to therapy based on Early Breast Cancer Trialists’ Collaborative Group
- Criticized for being based on insufficient data and overestimating risk of recurrence in some patients. Her-2 status not considered....
Enter Molecular classification of breast cancer

- By interrogating cancer on the basis of gene expression, mutation, copy number changes, methylation, proteomic expression, miRNA...
- A molecular based classification emerged
- Various signatures, expression of cohort of genes, have been identified and validated... that are of diagnostic, prognostic and predictive importance in breast cancer
Lessons learned from the intrinsic subtypes of breast cancer in the quest for precision therapy
Intrinsic subtypes and correlation
J H Norum ... T. Sorlie BJS 2014; 101

Table 1 Characteristics, surrogate immunohistochemical definition and treatment recommendations for the intrinsic molecular subtypes of breast cancer

<table>
<thead>
<tr>
<th>Intrinsic subtype</th>
<th>Characteristics</th>
<th>Immunohistochemical definition</th>
<th>Recommended treatment</th>
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</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>(ER^+), highly express luminal epithelial genes, (PIK3CA) mutations, diploid, low grade, cyclin D1 overexpression, whole chromosome arm aberrations</td>
<td>(ER^+) and/or (PR^+) (HER2^-) (Ki-67) low</td>
<td>Hormone therapy</td>
</tr>
<tr>
<td>Luminal B</td>
<td>(ER^+) (low), proliferative, high grade, whole chromosome arm aberrations and complex rearrangements, (TP53) and (PIK3CA) mutations, alterations in retinoblastoma and MAPK pathways, some are (HER2^+)</td>
<td>(ER^+) and/or (PR^+) (HER2^-) (or (HER2^+)) (Ki-67) high</td>
<td>Hormone therapy, chemotherapy, anti-(HER2) if (HER2^+)</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>(ER^-), most tumours show (HER2) amplification, overexpression of genes on 17q22, highly proliferative, (TP53) mutations, focal high-level amplifications</td>
<td>(HER2^+) (amplified or overexpressed)  (ER^-)</td>
<td>Anti-(HER2), chemotherapy</td>
</tr>
<tr>
<td>Basal-like</td>
<td>(ER^-), (HER2^-), highly express basal keratins, express EGFR, highly proliferative, aneuploid, high grade, (TP53) mutations, complex genomic rearrangements, WNT pathway activation increased</td>
<td>(ER^-), (PR^-), (HER2^-) (triple negative)</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Normal-like</td>
<td>Express basal and myoepithelial genes, adipose tissue-specific genes</td>
<td>Not relevant</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>

ER, oestrogen receptor; \(PIK3CA\), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit \(\alpha\); \(PR\), progesterone receptor; \(HER\), human epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor.
Intrinsic subtypes survival by stage

D. Pracella et al Disease Markers Vol 35 (2013), 6
Prognostic and Predictive Multigene signatures

- Developed to improve prognostication beyond conventional clinical pathological parameters like grade and stage.
- Collection of genes correlated with outcome and or response to therapy, frequently associated in part to proliferation
- Review four examples: OncotypeDx, MammaPrint, PAM50 ROR and IHC4+C
**OncotypeDx (21 gene Recurrence Score) (Genomic Health)**

Performed on ER+, mostly LN- cancers, <5cm. Prognostic and predictive assay.
FFPE, qRT-PCR based, 21 genes. Assesses, proliferation, ER, HER-2...
10 year risk for distant disease recurrence - assigns recurrence score (RS) which is translated to risk of distant recurrence.

Low risk of distant recurrence 6.8% (RS <18), intermediate ~14% (RS 18-30), high risk 30% (RS >30) in 10 years (all tamoxifen treated). Local regional recurrence also predicted.
Oncotype DX (Genomic Health)

- RS more accurately predicts recurrence than Adjuvant!Online, overall survival.
- It predicts benefit from tamoxifen in low and intermediate risk groups but not high, and benefit from chemotherapy in high RS group.
- Accepted by insurance, ASCO and National Comprehensive Cancer Network tumor marker guidelines.
- Prospective clinical trial utilizing Oncotype DX: TAILORx underway to determine if a modified intermediate group (11-25) of RS benefit from chemo.
- SWOG S1007 ER+, Her-2-, nodes 1-3, RS<25, tamox +/- chemo
Oncotype Dx Recurrence score
MammaPrint(Agendia): Developed by NKI

- Prognostic and predictive test, ER+/-, node negative, <5cm, FDA cleared
- FFPE/previously fresh only, 70 genes (proliferation, invasion, metastasis, stromal integrity and angiogenesis), array based.
- Dichotomous results: good and poor prognosis group 90% versus 70% overall survival in 10 years.
  Improvement over St. Galen and Adjuvant!Online in predicting high and low risk patients.
  - Evidence of a predictive marker for the addition of chemo only in the high risk group
MammaPrint

- MINDACT (microarray in node negative disease may avoid chemotherapy) prospective, phase III trial, early stage breast cancer examining effect of chemo+/- in patients that are categorized as divergently as high and low risk based on Adjuvant! Online and MammaPrint. Findings reported in AACR recently suggest that low risk MammaPrint and high risk Adjuvant w/o chemo were 94.7% metastasis free in 5 years. Suggesting these patient can avoid chemo
- Also offers Blue print (breast subtypes) and Target print (ER, PR, Her-2 status)
PAM50(Predictor analysis of microarray), PAM 50 ROR(risk of recurrence) score

- Using NanoString technology/Ncounter machine 50 genes and 5 controls used for intrinsic classification of cancer (Luminal A/B, Her-2 enriched and Basal) and risk of Recurrence score in 10 years by taking into consideration tumor size, ER, PR, Her-2 and proliferation. It has been shown to be an independent predictor of survival in multivariate analysis in BC.
- PAM50 ROR has prognostic ability in node neg and pos.
PAM50 ROR

- Has good agreement with OncotypeDx, but fewer patient were scored as intermediate by ROR relative to Oncotype
- Prognostic ability in late distant recurrence in ER+ cancer, between 5-15 years risk of DR was 2.4% in low ROR group and 17.5% in high ROR
- PAM50 ability to predict intrinsic subtype contributes to its ability to predict response to endocrine therapy in luminal subtype and was superior to IHC determination.
IHC4 and IHC4+C

- Utilized IHC results for ER, PR, Her-2 and Ki-67, a “poor man’s” Oncotype or MammoPrint
- In ATAC trial was a predictor of early (0-5 years) and overall (0-10 year) DR but not late distal recurrence.
- Similar prognostic capability to OncotypeDx and separated luminal A and B tumors.
- Considered inferior to PAM50 ROR
- IHC reproducibility and accuracy an issue
- IHC score modified by nuclear grade and mitotic count improves concordance of risk with Oncotype
- Available online in developing countries.
Molecular characterization

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- **Massively Parallel Sequencing**
  - TCGA (>500 tumours)
  - ICGC (>100 tumours)
  - Data analysis across multiple platforms (DNA copy number arrays, DNA methylation, exome sequencing, RNA arrays and sequencing, protein arrays)

- **Integrated gene expression and DNA copy number analysis**
  - METABRIC
  - (2,000 breast cancers)
  - → 10 integrative clusters

- **ER**
  - Dextran coated charcoal beads to quantify ER

- **Immunohistochemistry for ER on tissue sections**

- **New molecular subtypes**
  - Claudin-low
  - Molecular apocrine
  - Interferon-related

- **Loss of Heterozygosity (LOH) and Comparative Genomic Hybridization (CGH)**
  - (losses, gains and amplifications of genomic DNA sequences)

- **cDNA microarray based gene expression profiling**
  - Intrinsic molecular subtypes associated with distinct clinical outcomes:
    - Luminal A & B
    - Basal-like
    - HER2-enriched
    - Normal breast-like

- **Gene expression based prognostic signatures**
  - OncotypeDX®
  - MammaPrint®
  - PAM50

- **Targeted gene sequencing**
  - Cancer gene panels
Which is true?

2. Stage, grade and ER, PR, Her-2 and ki67 IHC studies remain an important means of assessing breast cancer.
3. Molecular characterization of breast cancer has produced at least 4 intrinsic subtypes of breast cancer that are of prognostic and predictive importance.
4. Several commercially available signatures are currently available that enhance our prognostic and predictive capacity beyond traditional clinical pathological parameters.
5. This lecture wasn’t horrible. (choose all or none of the above)
References