Molecular Characterization of Breast Cancer: The Clinical Significance

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The Plan

- To present an overview of molecular characterization of breast cancer and its impact on clinical care and patient outcome
Molecular Characterization of Breast Cancer

The Way We Were

- Pathology diagnosis was based on
  - Gross examination
  - Light microscopy

- The most common diagnosis was:
  - Undifferentiated malignant neoplasm
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Recent Advances

- The emergence of new technologies
- Molecular characterization of tumors
- Stratification of patients for therapy based on tumor characteristics
- A paradigm shift in patient care
Integration of Breast Health Services

Survivorship
Patient and Physician Education
Data Management
Research
Psychosocial Support

Nurse Navigator Breast Care Coordinator

Radiology
Pathology
Surgery
Medical Oncology
Radiation Therapy

Plastic and Reconstructive Surgery
Genetic Counseling
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Changing Role of Pathologists

- Establish a diagnosis
- Classify a neoplasm
- Differentiate between a primary versus a metastatic tumor
- Predict a response to therapy
- Provide a prognosis
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Prognosis: Treatment Planning

- Tumor size/type
- Histologic grading
- Lympho-vascular invasion
- Lymph node status
- Status of surgical margins
- Presence or absence of ductal carcinoma in situ
- Multicentricity/multifocality
- Presence or absence of nipple involvement and ulceration
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Prediction of Response to Therapy

- Assessment of the status of expression of estrogen and progesterone receptors
  - Endocrine therapy
- Assessment of the pattern of expression/gene amplification of Her-2/neu oncogene
  - Herceptin therapy
- Assessment of new genetic molecular pathways
  - Molecular/adjuvant chemotherapy
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The Significance of Accurate Prognostic/Predictive Testing

- Selection of those patients who will most likely benefit from systemic therapy
- Offering personalized medicine with greater safety and effectiveness
- Providing affordable and cost-effective care
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The Issue

- Up to 30% of women with node negative breast cancer die of the disease regardless of adjuvant therapy
- Up to 70% survive without adjuvant therapy
- Heterogeneity in breast cancer can not be captured by traditional prognostic factors
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The Rationale

- Better definition of the biological complexity of breast cancer
- Development of more sophisticated and sensitive testing to better stratify patients for systemic therapy
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The Process

- Gene expression profiling provided an opportunity to classify tumors at a genomic level into subclasses of potential prognostic and predictive significance
Kaplan-Meier analysis of disease outcome in two patient cohorts

(A) Overall survival for 72 patients with locally advanced breast cancer in the Norway cohort. The normal-like tumor subgroups were omitted from both data sets in this analysis.

(B) Time for development of distant metastasis in 97 sporadic cases.
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Breast Cancer is a Family of Diseases

- ER+ (Luminal A) (56%-61%)
- ER+ (Luminal B) (9%-16%)
- Her-2/neu + (8%-16%)
- Basal-like/Triple negative (8%-20%)
- Unclassified/normal breast-like (6%-10%)
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Luminal Subtypes

- Luminal A/B – generally carry a good prognosis and show a favorable response to endocrine therapy
- Luminal A – better prognosis than type B
- Luminal B – show a moderate expression of gene expressed by the breast luminal cells, higher proliferation rate and lower progesterone receptor
Immunohistochemical profile of luminal A subtype. ER positive (A), PR positive (B), HER2 negative ©, CK8/18 positive (D), Bcl-2 positive (E), and EGFR negative (F)
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Her-2/neu + Type

- Presents as two distinct forms
  - ER-
  - ER+

- Frequently associated with DCIS

- Associated with poor prognosis
Immunohistochemical profile of HER2 subtype. ER negative (A), PR negative (B), HER 2 positive +3 ©, EGFR focal positive (D), CK5 negative (E), CK8/18 heterogeneous and moderate positive (F)
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Basal-Like Breast Cancers

- No internationally accepted definition
- Triple negative phenotype (ER-, PR-, and HER2-/neu-)
- Expression of high molecular weight cytokeratin (CK 5/6, CK 14, CK 17), EGFR, CKIT, P63, E-Cadherin, SMA
Immunohistochemical profile of basal-like breast carcinoma. ER negative (A), HER2 negative (B), p53 positive (C), CK5 positive (D), EGFR positive (E), and Bcl-2 negative (F)
Basal-Like Breast Cancers
Morphologic Features

- High mitotic rate
- Geographic, central necrosis
- Lymphoplasmacytic infiltrate with the medullary carcinoma - like features
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Biology of Basal-Like Breast Carcinoma

- Account for 10%-20% of all breast cancer
- More frequently affects younger patients
- More prevalent in African American women
- More prevalent in those with germline BRCA-1 mutation carriers
- Are biologically more aggressive
- Have a unique metastatic pattern
- Majority of death occurs in the first 5 years after primary treatment
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Triple Negative Breast Cancer

- Triple-negative tumors represent the majority of cancers within the basal-like subtype

- Not all triple negative breast cancers display the basal-like phenotype and vice versa

- Currently no specific targeted approach is available for triple negative tumors
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Possible Molecular Targeted Therapies for the Treatment of Triple Negative Breast Cancer

<table>
<thead>
<tr>
<th>Molecular Targets</th>
<th>Agents tested in clinical phase trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Anti-EGFR antibody: cetuximab</td>
</tr>
<tr>
<td></td>
<td>EGFR tyrosine kinase inhibitor: erlotinib</td>
</tr>
<tr>
<td>c-kit</td>
<td>Multiple tyrosine kinase inhibitors: imatinib, sunitinib</td>
</tr>
<tr>
<td>Src</td>
<td>Multiple tyrosine kinase inhibitors: dasatinib</td>
</tr>
<tr>
<td>mTOR</td>
<td>mTOR inhibitor: everolimus</td>
</tr>
</tbody>
</table>

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Current Status

- Patients with hormone receptor positive, Her-2/neu oncogene negative tumors benefit from adjuvant hormone therapy
- Patients with Her-2/neu positive tumors, any ER/PR or menopausal status derive major benefits from the administration of Herceptin® therapy in combination with chemotherapy
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Current Status

- There is a need to develop additional forms of systemic therapy for those tumors that fail to express hormone receptors and/or Her-2/neu oncogene.

- It is essential to search for factors that can better stratify patients for systemic therapy.
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Assessment of New Genetic Pathways

- MammaPrint 70-gene assay (Agendia BV, Amsterdam, the Netherlands)
- Oncotype DX 21-gene assay (Genomic Health, Redwood City, California)
- H/I (AvariaDX, Carlsbad, California)
- Others…….
## MammaPrint

<table>
<thead>
<tr>
<th>Biological Function</th>
<th>MammaPrint Gene Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolism</td>
<td>7</td>
</tr>
<tr>
<td>Cell cycle and DNA replication</td>
<td>12</td>
</tr>
<tr>
<td>Extracellular matrix adhesion and remodeling</td>
<td>5</td>
</tr>
<tr>
<td>Growth, proliferation, transformation and cell death</td>
<td>17</td>
</tr>
<tr>
<td>General signal transduction and intracellular transport</td>
<td>3</td>
</tr>
<tr>
<td>Growth factor</td>
<td>7</td>
</tr>
<tr>
<td>Motility or actin filament related</td>
<td>5</td>
</tr>
<tr>
<td>Intracellular hydrolase</td>
<td>1</td>
</tr>
<tr>
<td>Immune response</td>
<td>1</td>
</tr>
<tr>
<td>Neuropeptide</td>
<td>1</td>
</tr>
<tr>
<td>Predicted transmembrane protein with unknown function</td>
<td>2</td>
</tr>
<tr>
<td>Predicted transcriptional control or DNA binding proteins</td>
<td>5</td>
</tr>
<tr>
<td>Unknown function</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total Gene Count</strong></td>
<td><strong>70</strong></td>
</tr>
</tbody>
</table>
Validation of the 70-gene classifier. A and B, Kaplan-Meier analysis of the probability that a patient would remain free of distant metastases and the probability of overall survival among all patients.
OncoType DX® 21-Gene Recurrence Score (RS) Assay

16 Cancer and 5 Reference Genes From 3 Studies

RS =  + 0.47 x Her2 Group Score
- 0.34 x ER Group Score
+ 1.04 x Proliferation Group Score
+ 0.10 x Invasion Group Score
+ 0.05 x CD68
- 0.08 x GSTM1
- 0.07 x BAG1

**Category** | **RS (0 -100)**
--- | ---
Low risk | RS <18
Int risk | RS 18 - 30
High risk | RS ≥ 31

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Recurrence Score as Continuous Predictor

Rate of Distant Recurrence at 10 Years

Low Risk
Intermediate Risk
High Risk

Recurrence Score

Rate
95% Confidence Interval
Rate of Distant Recurrence at 10 Years by Oncotype DX Risk Group in the Clinical Validation Study

- Low Risk: 6.8%
- Intermediate Risk: 14.3%
- High Risk: 30.5%
Population Distribution by Oncotype DX Risk Group in the Clinical Validation Study

Rate of Distant Recurrence at 10 Years

- 51% Low Risk
- 27% High Risk
- 22% Intermediate Risk
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Current Clinical Trails

- TAILORRx
  - Trail Assigning Individualized Options for Treatment

- MINDACT
  - Microarray In Node-Negative Disease May Avoid Chemotherapy Trial

- Rx PONDER
  - Rx for Positive Node Endocrine Response Breast Cancer
## Comparison of the TAILORx, MINDACT, and RxPONDER trials

<table>
<thead>
<tr>
<th>Trial/Characteristic</th>
<th>TAILORx</th>
<th>MINDACT</th>
<th>RxPONDER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinating group</td>
<td>ECOG</td>
<td>EORTC</td>
<td>SWOG</td>
</tr>
<tr>
<td>Design</td>
<td>Prospective, randomized, open-label trial</td>
<td>Prospective, randomized, open label trial</td>
<td>Prospective, randomized, open-label trial</td>
</tr>
<tr>
<td>No, of patients registered/randomized</td>
<td>10,263/6908</td>
<td>6000/1920</td>
<td>8800/4400</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Oncotype DX</td>
<td>MammaPrint</td>
<td>Oncotype DX</td>
</tr>
<tr>
<td>Key eligibility criteria</td>
<td>ER+, HER2-, LN-</td>
<td>LN- or 1~3 LN+</td>
<td>ER+, HER2-, 1~3 LN+</td>
</tr>
<tr>
<td>Randomize group</td>
<td>RS 11~25</td>
<td>Discrepant risk between MammaPrint and Adjuvant! Online</td>
<td>RS &lt;25</td>
</tr>
<tr>
<td>Randomized treatment</td>
<td>Endocrine vs endocrine + chemotherapy</td>
<td>Treatment by clinical criteria (Adjuvant! Online) or gene expression (MammaPrint)</td>
<td>Endocrine vs endocrine + chemotherapy</td>
</tr>
<tr>
<td>Primary end point</td>
<td>Disease-free survival</td>
<td>Distant metastasis-free survival</td>
<td>Disease-free survival</td>
</tr>
<tr>
<td>Status</td>
<td>Completed accrual</td>
<td>Completed accrual</td>
<td>Accruing</td>
</tr>
</tbody>
</table>

Tissue banking is a common feature for all 3 trials.

ECOG indicates Eastern Cooperative Oncology Group; EORTC, European Organization of Research and Treatment of Cancer; ER, estrogen receptor; LN, lymph node; RS, recurrence score; SWOG, Southwest Oncology Group
A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast

- The Oncotype DX breast cancer assay was performed for 327 patients with DCIS treated without radiation in the (ECOG) E5194 study

- A DCIS score was established for each case

A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast

A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast

- DCIS score predicts the risk of local recurrence and invasive local recurrence and provides information that complements traditional clinical and pathologic factors

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Future Directions

- Emerging classification system with clinical relevance based on
  - Morphology
  - Phenotype
  - Molecular genetics

- Routine provision of prognostic and predictive information

- Identification of key therapeutic targets
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Future Directions

- Translation of research technology into routine clinical practice
- Robust validated and standardized routine methods
- Establishment of quality control measures assuring the accuracy of pathology reporting
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Summary

- Breast cancer includes at least five discrete, molecularly defined subgroups with distinct natural histories, drug sensitivities and specific molecular therapeutic targets.
- Systemic treatments are estimated to reduce annual odds of recurrence by 50% - 60% and annual odds of death by about 40% - 50%.
- Combined modality therapy is the therapeutic approach of choice for management of early breast cancer.
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Summary

- As more advances are made in molecular genetics and more molecular targeted therapies become available, the responsibility of pathologists to find the right answers for the right patients will become greater.

- This approach will form the foundation of the delivery of quality, personalized breast health care.