The Breast 24 (2015) S26–S35

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

# Clinical implications of the intrinsic molecular subtypes of breast cancer

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ARTICLE INFO

*Article history:*
Available online 5 August 2015

*Keywords:*
Breast cancer
Subtype
PAM50
Gene expression

ABSTRACT

Gene-expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last 15 years, 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Claudin-low) have been identified and intensively studied. In this review, we will focus on the current and future clinical implications of the intrinsic molecular subtypes beyond the current pathological-based classification endorsed by the 2013 St. Gallen Consensus Recommendations. Within hormone receptor-positive and HER2-negative early breast cancer, the Luminal A and B subtypes predict 10-year outcome regardless of systemic treatment administered as well as residual risk of distant recurrence after 5 years of endocrine therapy. Within clinically HER2-positive disease, the 4 main intrinsic subtypes can be identified and dominate the biological and clinical phenotype. From a clinical perspective, patients with HER2+/HER2-enriched disease seem to benefit the most from neoadjuvant trastuzumab, or dual HER2 blockade with trastuzumab/lapatinib, in combination with chemotherapy, and patients with HER2+/Luminal A disease seem to have a relative better outcome compared to the other subtypes. Finally, within triple-negative breast cancer (TNBC), the Basal-like disease predominates (70–80%) and, from a biological perspective, should be considered a cancer-type by itself. Importantly, the distinction between Basal-like versus non-Basal-like within TNBC might predict survival following (neo)adjvuvant multi-agent chemotherapy, bevacizumab benefit in the neoadjuvant setting (CALGB40603), and docetaxel vs. carboplatin benefit in first-line metastatic disease (TNT study). Overall, this data suggests that intrinsic molecular profiling provides clinically relevant information beyond current pathology-based classifications.



## Introduction

Despite that breast cancer mortality has been moderately reduced by current treatments, more than 450,000 estimated deaths due to breast cancer are expected annually worldwide [1]. The most plausible explanation for this scenario is that we lack a complete picture of the biologic heterogeneity of breast cancers. Importantly, this complexity is not fully reflected by the main clinical parameters and pathological markers (oestrogen receptor [ER], progesterone receptor [PR] and human epidermal growth factor 2 [HER2]), all of which are routinely used in the clinic to stratify patients for prognostic predictions, to select treatments and to include patients in clinical trials.

Gene expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last 15 years, we and others have extensively characterized 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER-2 enriched, Basal-like and Claudin-low) and a normal breast-like group [2–6]. These entities have shown significant differences in terms of their incidence, risk factors, prognosis and treatment sensitivity. Regarding prognosis, the Luminal A subtype has shown repeatedly

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http://dx.doi.org/10.1016/j.breast.2015.07.008

to have a better outcome than the rest of subtypes across many datasets of patients with early breast cancer, including 6 phase III clinical trials (TransATAC, GEICAM9906, CALGB9741, ABCSG08, NCIC-CTG MA.5 and NCIC-CTG MA.12), where patients received various adjuvant systemic treatments.

A particular piece of data that highlights the importance of intrinsic subtyping in breast cancer comes from one of the most complete molecular characterization studies that have ever been performed in breast cancer. In this study, led by The Cancer Genome Atlas Project (TCGA), more than 500 primary breast cancers were extensively profiled at the DNA (i.e. methylation, chromosomal copy-number changes and somatic and germline mutations), RNA (i.e. miRNA and mRNA expression) and protein (i.e. protein and phosphor-protein expression) levels using the most recent technologies [6]. In a particular analysis of over 300 primary tumours (i.e. shown in Figure 2 of that publication [6]), 5 different data-types (i.e. all except DNA mutations) were combined together in a cluster of clusters in order to identify how many biological homogenous groups of tumours one can identify in breast cancer. The consensus clustering results showed the presence of 4 main entities of breast cancer but, more importantly, these 4 entities were found to be very well recapitulated by the 4 main intrinsic subtypes (Luminal A, Luminal B, HER2-enriched and Basal-like) as defined by mRNA expression only [7]. Overall, these results suggest that intrinsic subtyping captures the vast majority of the biological diversity occurring in breast cancer.

Since 2011, the St. Gallen international expert consensus panel adopted an intrinsic subtype-based approach for recommending adjuvant systemic therapies (i.e. endocrine, chemotherapy and anti-HER2 therapy) in early breast cancer [8]. Although the panel acknowledged the superior accuracy and reproducibility of multigene expression molecular assays, these assays are not readily available for all our patients. Thus, over the years, we and others have proposed pathology-based surrogate definitions especially for distinguishing Luminal A from B tumours [9–11]. However, despite important efforts to improve the various pathology-based surrogate definitions of the intrinsic subtypes, these continue to be suboptimal.

Here, we review the current and the potential future clinical implications of the intrinsic molecular subtypes of breast cancer beyond the pathological-based surrogate classification endorsed by the 2013 St Gallen Consensus Recommendations [8].

## Intrinsic subtyping based on gene expression versus histopathology

To date, numerous studies have evaluated and compared the classification of tumours based on the PAM50 gene expression predictor with the pathology-based surrogate definitions [6,10,12–26]. To better understand the concordance between the 2 classification methods, we have combined the data from all of these studies for a total of 5994 independent samples (Table 1). Of note, the vast majority of these studies performed central determination of pathology-based biomarkers, so this needs to be taken into account since this is not what is currently being done in the clinical setting where each hospital determines these biomarkers. Of note, large discrepancies (~20%) between local and central determination of ER, PR, Ki67 and HER2 are expected [27–31].

In this combined analysis, the discordance rate between both classifications was found to be present in almost 1 out of 3 patients (rate = 30.72% across all patients; kappa statistic = 0.564, "moderate agreement"; rate = 44.0% within non-triple-negative disease; kappa statistic = 0.314, "fair agreement"). Across the IHC-based subtypes, the discordance rate was 37.8%, 48.9%, 53.8%, 33.9% and 13.9% for the IHC-Luminal A, IHC-Luminal B, IHC-Luminal B/HER2+ (to identify PAM50 Luminal B), HR−/HER2+ (to identify PAM50 HER2-enriched) and triple-negative (to identify PAM50 Basal-like) subtypes, respectively. These results clearly suggest that the 2 methods to identify intrinsic biology should not be considered the same. The most likely explanation is that 3 or 4 biomarkers do not fully recapitulate the intrinsic subtypes of breast cancer. For example, we compared the prognostic and predictive ability of a 3-gene subtype classifier based on ESR1, ERBB2 and AURKA compared with the 50-gene PAM50 intrinsic classifier, and the 50-gene assay was significantly better [4]. In fact, during the development of the clinically applicable PAM50 intrinsic subtype predictor, 50 genes was found to be the minim number of genes needed to robustly identify the 4 main intrinsic subtypes without compromising its accuracy [4].

## Main molecular features of the intrinsic subtypes

Four main intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched and Basal-like) have been characterized over the last 15 years. At the RNA and protein level, Luminal A and B subtypes are largely distinguished by the expression of two main biological processes: proliferation/cell cycle-related and luminal/hormone-regulated pathways (Fig. 1). Compared to Luminal A tumours, Luminal B tumours have higher expression of proliferation/cell cycle-related genes or proteins (e.g. MKI67 and AURKA) and lower expression of several luminal-related genes or proteins such as the progesterone receptor (PR) [32] and FOXA1, but not the oestrogen receptor [10], which is found similarly expressed between the two luminal subtypes and can only help distinguish luminal from non-luminal disease. At the DNA level, Luminal A tumours show a lower number of mutations across the genome, lower number of chromosomal copy-number changes (e.g. lower rates of CCND1 amplification), less TP53

**Table 1**
Distribution of the PAM50 intrinsic subtypes within the pathology-based groups.[a]

| IHC-based group | References | N | PAM50 intrinsic subtype distribution | | | |
|---|---|---|---|---|---|---|
| | | | Luminal A | Luminal B | HER2-enriched | Basal-like |
| HR+/HER2− | [10,14,16–22] | 4295 | 60.3% | 31.9% | 6.6% | 1.2% |
| Luminal A | [10,14,17,21] | 637 | 62.2% | 27.0% | 10.2% | 0.6% |
| Luminal B | [10,14,17,21] | 317 | 34.1% | 51.1% | 11.0% | 3.8% |
| HER2+ | [6,23–26] | 831 | 17.6% | 26.8% | 44.6% | 11.0% |
| HER2+/HR+ | [25,26] | 182 | 33.0% | 46.2% | 18.7% | 2.2% |
| HER2+/HR− | [25,26] | 168 | 19.0% | 4.2% | 66.1% | 10.7% |
| TNBC | [12–15] | 868 | 1.6% | 3.2% | 9.1% | 86.1% |

[a] The data has been obtained from the different publications. Several studies have performed a standardized version of the PAM50 assay (RT-qPCR-based or nCounter-based) from formalin-fixed paraffin-embedded tumour tissues [10,14,17,19–22], while others have performed the microarray-based version of the PAM50 assay [6,16,18,23–26].