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Review

# Genomic profiling of inflammatory breast cancer: A review

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ABSTRACT

Inflammatory breast cancer (IBC) is a rare but aggressive form of breast cancer. Despite efforts in the past decade to delineate the molecular biology of IBC by applying high-throughput molecular profiling technologies to clinical samples, IBC remains insufficiently characterized. The reasons for that include limited sizes of the study population, heterogeneity with respect to the composition of the IBC and non-IBC control groups and technological differences across studies. In 2008, the World IBC Consortium was founded to foster collaboration between research groups focusing on IBC. One of the initial projects was to redefine the molecular profile of IBC using an unprecedented number of samples and search for gene signatures associated with survival and response to neo-adjuvant chemotherapy. Here, we provide an overview of all the molecular profiling studies that have been performed on IBC clinical samples to date.


## Introduction

Inflammatory breast cancer (IBC) is a particularly aggressive form of breast cancer because of its strong metastatic potential. The diagnosis is based on clinical signs of inflammation (redness, edema, "peau d'orange") arising quickly and involving more than one-third of the breast [1]. The presence of tumor emboli in dermal lymphatic vessels, which is the pathological hallmark of the disease, is neither mandatory nor sufficient for diagnosis. The relative subjectivity of the clinical symptoms, and the occasional diagnosis using only pathological criteria (i.e. the presence of tumor emboli) have led to serious heterogeneity with respect to the IBC study population across different clinical and scientific reports, causing a serious challenge for researchers working on IBC. Despite multimodality treatment, the results remain insufficient. The rate of pathological complete response (pCR) after primary anthracycline-taxane-based chemotherapy (CT) ranges from 15 to 30% only (63% for ERBB2+ IBC) when trastuzumab and bevacizumab are added [2], and the 5-year survival remains around 40% despite a multimodality treatment. Prognostic features [3] are contested; the response to CT is an imperfect indicator of survival [4,5]. In this context, a molecular characterization of IBC is crucial.

Because of its scarcity (~5% of breast cancers) and the small size of diagnostic biopsies, in depth molecular studies are difficult to perform, and therefore the biological basis of IBC aggressiveness remained poorly understood. Until 2004, only a limited number of genes or proteins with known or potential prognostic value in other types of breast cancer were investigated. This was done using classical analytic tools (gene-by-gene approach) applied to clinical samples (i.e. tumor biopsies) and preclinical *in vitro* (i.e. SUM149 and SUM190 cell lines) and *in vivo* models (two human IBC xenografts designated MARY-X and WIBC-9). These studies have led to the identification of potentially relevant genes or pathways [6]. Higher incidence of certain molecular alterations have been reported in IBC: negativity of hormone receptors, overexpression of ERBB2 and EGFR, presence of *TP53* mutations, high proliferation and angiogenesis levels, overexpression of E-cadherin and dysfunction of MUC1, overexpression of eIFG4I [7] and overexpression of chemokines and chemokine receptors. Nevertheless, despite these novel insights, the routine clinical applications

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remained limited to the use of trastuzumab in the case of ERBB2-positive tumors. A few other targeted therapies are being assessed in clinical trials such as lapatinib [8] or bevacizumab [2] and have shown promising results.

Ten years ago, high-throughput molecular analyses were applied in IBC research in order to better define the differences between IBC and other types of breast cancer, hereafter collectively referred to as non-IBC. As in non-IBC, the most often applied technology was DNA microarray-based expression profiling, which provides massively parallel quantification of mRNA expression levels for thousands of genes in one sample. Gradually, alternative high-throughput profiling technologies were applied in IBC research. Here, we review the main results described during the last decade and discuss the current perspectives.

## Gene expression profiling of clinical samples: past unicentric studies

Expression profiling studies were reported by 6 different groups worldwide (see Bertucci and colleagues [9] for review). These studies all demonstrated the feasibility of profiling small IBC samples and revealed that IBC is a heterogeneous disease comprising of all classical molecular subtypes previously described in non-IBC. These results suggest that cancer cells from patients with IBC are derived from the same cells-of-origin as cancer cells from patients with non-IBC. More important, the reported data indicate that the molecular subtypes and IBC phenotype are biological independent characteristics.

Overall, most of the reported expression profiling studies demonstrated that a molecular signature of IBC is definable [10–17]. Nevertheless, only a limited number of genes and biological pathways were commonly identified across all studies. Potential reasons for this observation included the small number of tested samples when compared to the large number of tested genes (low statistical power), distinct case definitions of IBC used across different studies, differences in the characteristics of the IBC and non-BC groups between different studies, notably with respect to the hormone receptor status, and technological differences such as the use of different platforms and different input gene lists [9]. Despite these limitations, a few identified candidate genes were further validated at the functional level, including *RHOC* and *WISP3* [18], *NFkB* [19], and *GLI1* [20]. However, due to the unbalance with respect to the molecular subtypes between the IBC and non-IBC patient series in these studies, the possibility remains that the observations are primarily related to the molecular subtypes instead of the IBC phenotype.

The issues of IBC prognosis and prediction of response to primary CT have been addressed in only two studies encompassing small patient series and without independent validation series. We have identified an 85-gene set that divided the IBC patients in two groups with different pCR rate [10]. Bieche and colleagues reported a 3-gene expression profile, which discriminated subgroups of patients with good, intermediate and poor outcome [14].

## Gene expression profiling of clinical samples: multicentric studies within the World IBC Consortium

In 2008, the World IBC Consortium was founded with the goal of fostering collaborations between international research groups with focus on IBC. The first project gathered gene expression profiles of clinical tumor samples generated on Affymetrix (HGU133-series) platforms from 3 different sites in Europe (Institut Paoli-Calmettes, Marseille, France; General Hospital Sint-Augustinus, Antwerp, Belgium) and in the USA (MD Anderson Cancer Center, Houston, Texas). With 137 IBC samples (and 252 non-IBC samples), this series is by far the largest series of IBC samples ever analyzed and allowed resolving the issues identified with respect to previous studies: international consensual definition for IBC [1], same technological platform, and a large number of patient samples. The latter point allowed us for the first time to take into account the differential distribution pattern of the molecular subtypes between IBC and non-IBC (first study), to address prognostic and predictive issues (second study), and to investigate heterogeneity in triple-negative IBC (third study).

In the first study [21], expression profiles derived from all 389 samples were investigated in order to redefine the molecular profile of IBC by taking into account the points of criticism raised in the previous studies. First, we showed that IBC is indeed transcriptionally heterogeneous, but to a lesser extent as compared to samples from patients with non-IBC. All molecular subtypes were confirmed, but with a distribution pattern different between the two tumor groups. Overall, 75% of IBCs belonged to aggressive subtypes (basal-like, ErbB2+, claudin-low and luminal B), whereas these subtypes account for 53% of the non-IBCs. The luminal A subtype represented 19% of IBCs, but 42% of non-IBCs (Fig. 1A). Whole-genome clustering showed that molecular subtype classification, and not the tumor phenotype, is a predictor of the cluster outcome (Fig. 1B), suggesting that differences in gene expression between IBC and non-IBC are dominated by the molecular subtype-related differences.

Supervised analysis between IBC and non-IBC identified significant differences in gene expression, which are similar regardless of the type of stage-matching performed on the non-IBC group (early stages only, locally advanced stages only, or all stages pooled). Thus, the influence of the composition of the control group according to tumor stage appeared to be limited, allowing us to retain all non-IBC control samples for the analysis. We also compared the IBC and non-IBC groups for pathway and transcription factor activation signatures. Out of 19 tested pathways (Fig. 2A), 12 (63%) were differentially activated: 8 were more activated in IBC (CTNB, ERBB2, MYC, RAS, INFα, INFγ, TNFα, and VEGF), whereas 4 were attenuated (ER, PR, P53, and TGFβ). We identified 78 out of 234 (33%) differentially activated transcription factors, from which 38 (49%) were more activated in IBC and 40 (51%) were more activated in non-IBC. Among the transcription factors hyperactivated in IBC, we identified RELA, corroborating our previous results [19,22]. However, because of the correlation between IBC/non-IBC phenotype and molecular subtypes, we suspected that these differences could be related to the molecular subtypes, and hence were not actually IBC-specific. Thus, we reiterated these three IBC/non-IBC comparative analyses using linear regression models to identify molecular subtype-independent differences. In the training set, we identified 491 IBC-specific probe sets (443 unique genes). For comparison, 2743 genes (6.2-fold more) were differentially expressed when molecular subtype-dependent gene expression differences were not considered, clearly demonstrating that the differential distribution of the molecular subtypes between IBC and non-IBC needs to be considered. Within the list of 491 probe sets, 79 probe sets (79 genes) were uniquely IBC-specific, whereas the remaining probe sets ($N = 412$) showed additional molecular subtype-specific gene expression variation. Thus, the number of genes with a uniquely IBC-specific gene expression profile represented only 3% of the global expression differences. The robustness of both models (491 and 79 probe sets) was confirmed by cross-validation in the training set and by external validation in an independent validation set of 139 samples including 53 IBCs set apart at the beginning of the analyses. Further indirect validation of the biological relevance of our 79-gene model came from its independent prognostic value in a public series of 871 chemotherapy- and hormone therapy-naive patients with node-negative non-IBC, With a median