Gene signatures of breast cancer development and the potential for novel targeted treatments

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Breast cancer (BC) is a heterogeneous disease; a single tumor can be classified differently according to its histological grade, stage, appearance and expression of receptors (estrogen, progesterone and HER2). The clinicopathological assessment of the disease is not sufficient to determine the efficacy of a particular treatment to a given patient, which often results in irrelevant and excessive treatments in many patients, without an effective clinical outcome. The advent of -omics technologies has led to the identification of genes distinguishing different classes of BC into molecular subtypes, genes frequently mutated in BC or associated with hereditary BC, leading to an enormous amount of data exploring resistance, metastasis, BC development and progression, and opening many boundaries for personalized patient care.

There are several approaches to come up with a gene signature for different subtypes of BC to provide early detection or therapeutic options. Adjuvant therapy is currently used in clinical practice, but all patients do not display similar results [1]. Keeping this in mind, efforts have been undertaken to understand the molecular signatures that could predict the efficacy of these treatments. There are several genomic tests like Oncotype DX, Mammaprint, EndoPredict, BC Index and Prosigna that screen a set of selected genes [2]. Based on their expression index, a score is attributed to a patient profile, helping clinicians in making decisions regarding chemotherapy. In 2019, the American Society of Clinical Oncology (ASCO) updated their guidelines for the use of adjuvant therapy for early stage invasive BC [3]. These guidelines, based on specific subtypes, have proven beneficial to many patients, but they do not yet consider gene mutations and epigenomic alterations.

There are various alternative approaches that can be used to examine the disease, some of which will be discussed in this editorial (Figure 1).

BC development & progression

There are several biomarkers that have been studied in order to understand the onset of BC: miRNAs, epigenetics and deregulation of tumor suppressor genes. The list is endless, and it would be impossible to analyze these genes one by one in each individual. Another approach is to understand the changes happening at each histopathological stage of BC progression, namely atypical ductal hyperplasia (ADH), ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (IDC). In regard to BC progression, there are different theories. One is that DCIS does not usually transform to IDC; the development of IDC is rather due to the presence of other adjacent lesions, also called independent evolution [4]. Many studies are also evaluating this aspect of BC [4]. A study by Kristensen *et al.* showed

Figure 1. Gene signatures in breast cancer. Each section consists of an approach to study breast cancer. The inner panel shows the available strategy, outer panel shows the possibility to improve each approach using gene analysis.
ADH: Atypical ductal hyperplasia; BC: Breast cancer; DCIS: Ductal carcinoma in situ; IDC: Invasive ductal carcinoma.

that by using pathway-based modeling methodology (PARADIGM), it is possible to differentiate each BC subtype according to its immune signature and thus predict patient survival [5]. Other studies have also evaluated these subgroups separately to understand how their morphological and molecular characteristics differentiate them [6].

However, Burstein *et al.* discuss the possibility that IDC develops from DCIS [7]. This gives rise to two different evolution patterns, evolutionary bottleneck or multiclonal invasion [4]. During the evolutionary phase a single clone arising from primary tumor (DCIS) escapes and becomes invasive while in the latter, each clone arising from primary DCIS has the capacity to become invasive. These evolutionary patterns suggest a linear process involving constant transformation of normal epithelial cells starting with ADH, then to DCIS, and finally resulting in increased risk of IDC [8]. Kothari *et al.* have identified biomarkers to identify these histopathological stages of BC progression and propose gene signatures that could predict whether an ADH or DCIS breast lesion has the potential or not to progress to IDC, to aid clinicians in their decision regarding the requirement for surgery [9].

Hereditary BC

Approximately 10–15% of BC is caused by hereditary factors. The risk increases with the number of events in first-degree relatives; one affected first-degree relative = twofold increase, while two affected first-degree relatives = threefold increase [10]. A family at high risk of BC is composed of multiple cases of ovarian or BC. In general, a family is considered to be at high risk if they have a minimum of three cases of breast or ovarian cancer in their first-degree relatives, or four cases in second-degree relatives [11].

An important aspect to keep in mind when studying inherited breast cancer is founder effect. Founder effect and mutations in *BRCA1/2* genes have been found in many populations, such as the French–Canadians, the Jewish Ashkenazis and Iceland [12]. Vézina *et al.* have characterized the R1443X *BRCA1* founder mutation in the French–

Canadian population [13]. According to BC information core database, mutation 5382insC in *BRCA1* has been found in Jewish, Dutch, Lithuanian, Russian, Hungarian, Germanic, French, Italian, British and French–Canadian ancestry population [12].

Around 25% of hereditary BC is accounted for by deleterious mutations in the *BRCA1* and *BRCA2* genes, and approximately 5% is attributed to other genes such as high-penetrance genes (*TP53*, *CDH1*, *PTEN*, *STK11*, *RAD51C* and *RAD51D*) and low/moderate-penetrance genes (*ATM*, *CHEK2*, *BRIP1* and *PALB2*). However, there are still a large number of families in which the causal gene is not known [11]. Research has also focused on *BRCA1/2* interactors, with the hypothesis that changes in these genes might also affect the proper functioning of *BRCA1/2* genes. An interesting study by Lo *et al.* demonstrated that, in many cases, it is not the effect of mutations in one single gene, but rather a cumulative effect of SNPs in 16 *BRCA1/2* interactors, namely, *CASP8*, *BARD1*, *PIK3CA*, *ESR1*, *RB1CC1*, *TCG101*, *SLC22A18*, *ATM*, *PALB2*, *KRAS2*, *RAD51*, *TP53*, *PHB*, *BRIP1*, *PPM1D* and *CHEK2* [14]. Another study has shown that the c.1518C>T variant in *BARD1*, a *BRCA1* interactor, increases the risk of BC [15], while a possible role of *ZBRK1* variants/haplotypes and *ZNF350* promoter variants in intermediate BC risk has been suggested [16,17]. Other *BRCA1/2* interactors were also studied, many having no effect on BC predisposition [18,19].

Beside *BRCA1/2* mutations or interacting partners, there are other regulators of inherited BC. These are important to identify to differentiate the potential carriers and to identify biomarkers for such subgroups of patients. A thorough study by Pouliot *et al.* identified 67 transcripts differentiating BRCAX (i.e., non-*BRCA1/2*) individuals from *BRCA1/BRCA2* carriers (both affected and unaffected), and 28 transcripts that could differentiate BC affected women from their unaffected sisters among BRCAX individuals [11]. A follow-up of this study suggests that two isoforms of *AIF1*, namely, *AIF1v1* and *AIF1v3*, are major players in crosstalk between infiltrating immune cells and BC, resulting in increased tumorigenesis [20].

Epigenetics in BC

Another molecular aspect in cancer research receiving a lot of attention is epigenetic mechanisms involving DNA methylation, histone modification, chromosome structure and ncRNA. In BC, in particular, the promoters of more than a hundred genes have been reported to be hypermethylated, affecting cell proliferation, invasion, DNA repair and cell death [21]. Hypermethylation of genes regulating these processes results in reprogramming the cells toward carcinogenesis. In this context, Plourde *et al.* have shown that overexpression of DNA (cytosine-5-)-methyltransferase 3 β (DNMT3B) hypermethylates many CpG sites in breast cell lines involved in increased proliferation and in several other oncogenic processes [22].

The hypomethylation of genes is also present in BC, and this is highlighted in many primary tumors. It is allegedly a common phenomenon displaying considerable variability across patients, but without any concrete patterns to help in diagnosis [23].

Many questions such as BC recurrence and contralateral BC can be raised regarding the epigenetic-mediated reprogramming leading to cancer. The latter is important because data suggest that patients who have previously had BC are around six-times more at risk of having contralateral BC [24]. Also, in certain recurrences, the tumor changes its characteristics, for example, some tumors will express the HER2 biomarker, for which it was negative in primary tumors. To understand such events, epigenetic modulations could be very useful. Indeed, why or how a first cancer and the normal mammary tissue change the molecular background of patients to result into a recurrence or second cancer is still an unanswered question. The knowledge gained with this information might allow to explain the effectiveness or ineffectiveness of certain treatment regimens.

Triple negative breast cancer

Accounting for 10–15% of all BC, this subgroup is the most heterogeneous, usually of high grade and often diagnosed at later stages. The search for a biomarker for early detection or to guide a potential therapeutic option is a most important requirement for this specific subgroup, as it lacks any US FDA-approved therapy, with only one alternative chemotherapy [25]. Indeed, this subgroup cannot be treated with hormonal therapy as tumors do not express hormone receptors (estrogen and progesterone) in addition to an excess of HER2 expression/amplification. Hence, alternative approaches like targeting receptors (receptor tyrosine kinase, immune receptors and transmembrane receptors) and downstream signaling molecules for survival and proliferation pathways (such as MAPK, AKT, S6K, PTEN, PARP, HDAC, Wnt) are continuously emerging [25]. Several have proven effective either as single therapy, in combination with other targeted therapy, or as adjuvant therapy for a subset of

patients. A personalized therapy for each individual is yet to come, and research is required so that triple negative breast cancer is diagnosed at an early stage and can be differentiated from other BC subtypes. Early detection is key, with mean 5-year survival outcome for invasive BC at around 90%.

Conclusion

Along the path of finding gene signatures for BC development or progression, there are various milestones. The path divides in different directions due to heterogeneity of the disease and the different -omics datasets. Therefore, it becomes crucial to best define the questions to address, and in which population or breast samples, before extrapolating genetic targets to BC overall.

Author contributions

C Kothari drafted the manuscript. C Diorio and F Durocher critically revised the manuscript.

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