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Clinical implications of molecular heterogeneity in triple negative breast cancer

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ABSTRACT

Triple negative breast cancer (TNBC) is a molecularly heterogeneous disease lacking recurrent targetable alterations and thus therapeutic advances have been challenging. The absence of ER, PR and HER2 amplifications, leaves combination chemotherapy as the standard of care treatment option in the adjuvant, neoadjuvant and metastatic settings. Recently, multiple studies have shed some light on the heterogeneity of TNBC and identified distinct transcriptional subtypes with unique biologies. Herein we review the molecular heterogeneity and the impact on previous and future clinical trials.

Introduction

Triple-negative breast cancer (TNBC) is inherently a heterogeneous disease defined by an absence of molecular markers. Lacking estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) amplifications, these tumors are insensitive to anti-hormonal and HER2 targeted therapies. Together TNBCs account for approximately 15% of all breast cancers, preferentially affect young women, and are more frequent in women of African and Hispanic descent [1,2]. With the lack of FDA approved targeted treatments available for TNBC, chemotherapy is the prominent treatment option for patients in the adjuvant, neoadjuvant, or metastatic settings.

Clinical heterogeneity

Consistent with being a diverse disease is the clinical finding that the majority of metastases of TNBC occur within the first three years following diagnosis, and patients who have not recurred during this time have similar survival rates as patients with ER-positive breast cancers [3,4]. Despite the rather aggressive clinical behavior of some TNBC tumors, approximately 30% of patients with TNBC benefit from neoadjuvant chemotherapy and patients with TNBC have better response to chemotherapy compared to other types of breast cancer. Patients treated with neoadjuvant chemotherapy who experience a pathological complete response (pCR) at the time of surgery have significant improvements in both disease-free and overall survival compared to patients with residual invasive disease [5]. Overall, patients with TNBC tend to have lower five-year survival rates compared to those with other types of breast cancer despite having a better response to chemotherapy. That latter difference in prognosis is likely driven by chemotherapy-resistant tumors that lead to residual disease after neoadjuvant chemotherapy in many TNBC patients.

Genetic susceptibility, genomic instability, and chemotherapy sensitivity

The earlier age of TNBC onset in select patients diagnosed with this type of breast cancer is consistent with a genetic predisposition syndrome and is supported by the finding that BRCA1 mutations occur with greater frequency in TNBC [6]. The BRCA1/2 genes

Abbreviations: TNBC, triple negative breast cancer; pCR, pathological complete response; HR, homologous recombination; EMT, epithelial–mesenchymal-transition; AR, androgen receptor; TILs, tumor-infiltrating lymphocytes; VEGFR, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; BL1, basal-like; BL2, basal-like 2; IM, immunomodulatory; M, mesenchymal; MSL, mesenchymal stem-like; LAR, luminal androgen receptor; BLIS, basal-like/immune-suppressed; BLIA, basal-like/immune activated group.

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encode E3 ubiquitin protein ligases essential for homologous recombination (HR) mediated-repair of DNA double-stand breaks [55]. Recently the germline DNA of 1824 TNBC patients, unselected for a family history of cancer, were analyzed for mutations in 17 genes associated with familial predisposition to cancer [7]. This study found 16.6% of TNBC carried germline mutations in 17 predisposition genes, 11.2% occurring in either BRCA1 (8.5%) and BRCA2 (2.7%) and the other 15 predisposition genes occurring in 3.7% of patients. Those non-BRCA1/2 mutations identified were enriched in genes involved in homologous recombination, such as PALB2 (1.2%) and BARD1, RAD51D, RAD51C, and BRIP1 (0.3%–0.5%). These findings suggest that defects in homologous recombination repair are an important early event in the development TNBC. Clearly BRAC1/2 mutant TNBC patients have a unique benefit from platinum agents, however further research is essential to determine the appropriate translation of non-BRCA1/2 breast cancer susceptibility genes to patient care. Retrospective analysis and previous trials have shown striking pathological complete response rates in *BRCA1* mutation carriers (72%–90%) with single-agent neoadjuvant DNA-crosslinking platinum salts (i.e. cisplatin) [8,9]. These data were recently confirmed in a phase III study of 376 metastatic TNBC patients (Triple Negative breast cancer Trial, TNT), in which BRCA mutant gene carriers who received carboplatin experienced significantly greater clinical response than those receiving docetaxel (68% versus 33.3%, 95% CI, 6.3–63.1) [10] The latter study also evaluated patients who had tumors that were molecularly similar to BRCA1- and BRCA2-mutant breast cancers, as determined by an homologous recombination deficiency (HRD) score, in which DNA patterns are used to identify defects in the homologous recombination [11]. While the HRD score was able to identify all of the tumors from women who were BRCA1/2 germline mutation carriers, there were no differences in objective response rates between carboplatin or docetaxel arms in patients with high HRD (38.2% vs. 42.6%) or low HRD (29.2% vs. 34.7%), respectively [10]. In addition to cisplatin, PARP inhibitors are showing efficacy in BRCA1/2 mutated metastatic breast cancer, with a response rate of 31% [60 of 193; (95% CI, 24.6 to 38.1)] compared to olaparib monotherapy, despite some of the patients have at least three prior chemotherapy regimens [12]. With exception to BRCA1/2 mutation carriers, there still remains a need to identify those TNBC patients that would benefit from chemotherapy.

Pathological heterogeneity of TNBC

TNBCs show a remarkable diversity of histologic patterns and subtypes. While majority are high-grade invasive ductal carcinomas, there is a small subset with distinct pathological features and indolent clinical behavior. In an analysis of 426 TNBC tumors for histology, 82% were found to be ductal, 5% lobular, 4% metaplastic, 2.3% medullary, 1.6% apocrine, 0.9% neuroendocrine, 0.5% cribriform and 0.5% mucinous [13]. The 5-year overall survival rate for ductal TNBC was 62%, and was the better for patients with apocrine (100%), medullary (100%) and neuroendocrine (100%) histological types, while worse for papillary (50%) and lobular (68%). In addition, there are cases of adenoid cystic carcinomas and secretory carcinomas that share common recurrent chromosomal translocations, resulting in oncogenic chimeric fusions (MYB-NFIB and ETV6-NTRK3, respectively) [14,15]. Several TNBCs have atypical histologies such as medullary and metaplastic. Medullary carcinomas are characterized by infiltrating carcinomas with circumscribed pushing borders, dense peripheral lymphoid infiltrate and have favorable outcome, while metaplastic carcinomas display differentiation towards squamous epithelium with mesenchymal components and cells displaying spindle, chondroid, osseous or rhabdoid morphologies [16].

Mutational heterogeneity of TNBC

Apart from recurrent fusions in rare pathologic subsets, TNBCs display a diverse mutational pattern, with relatively few recurrently mutated genes outside of TP53 and PIK3CA and PTEN [17,18]. These mutations seem to be clonally dominant compared to other mutations and their frequencies are reflective of founder mutations in some tumors [17]. While mutations in cytoskeletal, cell shape and motility proteins occurred at lower clonal frequencies, they likely occurred later during tumor progression. Another study of targeted ultra-deep (3000×) sequencing of 104 TNBCs revealed similar conclusions with highly clonal TP53 mutations present in over 80% of samples and more sub-clonal mutations in the PI3K pathway (29.8%, mainly PIK3CA mutations), MAPK signaling pathway (8.7%) and cell-cycle regulators (14.4%) [19]. Recently, investigators have identified complex rearrangements and mutations within the PEST domains of NOTCH1, NOTCH2, and NOTCH3 genes enriched in TNBC tumors from The Cancer Genome Atlas (TCGA). These mutations may be biomarkers for Notch targeted therapy, as they are highly sensitive to with the γ-secretase inhibitor PF-03084014 in cell culture models [20]. The overwhelming lack of recurrent targetable mutations to date has limited treatment options for TNBC outside of standard chemotherapy.

While treatment naïve tumors are relatively heterogeneous, molecular analyses of the residual disease from 74 TNBCs after neoadjuvant chemotherapy showed an enrichment for *MCL1* (54%) and *MYC* (35%) gene amplifications [21]. Inhibition of MYC directly is currently not available, however a recent genetic chemical screen identified an unexpected a synthetic lethal sensitivity to dasatinib through LYN inhibition in an isogenic TNBC model overexpressing MYC [22]. Similarly, MCL1 inhibitors while currently unavailable, are under investigation and small molecules that competitively bind the BH3 domain have been identified [23]. Therefore, there may be more opportunities for targeted therapy in tumors with residual disease following neoadjuvant chemotherapy.

Despite great inter-tumoral heterogeneity, primary tumor and lymph node metastasis are highly clonal at the copy number level, at least prior to treatment, and suggest the use of primary tumor characteristics to guide adjuvant systemic chemotherapy in breast cancer patients [24]. However, differential mutation frequencies at primary and metastatic sites indicate that while the primary tumors may have considerable heterogeneity, mutation frequencies are decreased at metastatic sites, reflecting selection for a distinct subset of primary cells capable of metastatic transplantation [25]. Further investigations are needed to identify those alterations that confer a selective advantage for metastasis.

Transcriptional heterogeneity of TNBC

Given the diverse pathological classifications, one would predict that TNBCs have a diverse array of biological subtypes that could be revealed by transcriptional profiling. Initial global transcriptional studies showed TNBCs to largely display basal-like gene expression [26]. This observation led many investigators to consider basal-like breast tumors and TNBC to be relatively synonymous. The uniform basal-like gene expression pattern in TNBC is largely due to the significant transcriptional differences between hormonally driven cancers and TNBC [27]. However, when analyzed independent from ER and HER2 positive cancers, TNBCs have quite heterogeneous gene expression patterns that can be used to classify the tumors into distinct subtypes [28,29]. Using gene expression analyses from 386 tumors, we recently identified six distinct TNBC subtypes, each displaying unique biologies [28]. The TNBC molecular subtypes include two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal

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