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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.

Keywords

chemotherapy; TNBCtype; neoadjuvant; PIK3CA; androgen receptor

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.

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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 encode E3 ubiquitin protein ligases essential for homologous recombination (HR) mediated-repair of DNA double-stand breaks {DAndrea:2003kp}. Recently the germline DNA of 1,824 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% to 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% cribiform 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 form 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 androgen receptor (LAR) subtype[28]. The BL1 subtype is characterized by elevated cell cycle and DNA damage response gene expression, while the BL2 subtype is enriched in growth factor signaling and myoepithelial markers. Both M and MSL share elevated expression of genes involved in epithelial-mesenchymal-transition (EMT) and growth factor pathways, but the MSL subtype has decreased expression of genes involved in proliferation. Consistent with the de-differentiated mesenchymal gene expression pattern is the recent analysis of pathologically defined metaplastic breast cancers that show the majority of chondroid and spindle cells carcinomas to be of the MSL subtype[30]. The IM subtype is composed of immune antigens and genes involved in cytokine and core immune signal transduction pathways. The LAR subtype is characterized by luminal gene expression and is driven by the androgen receptor (AR). Comparison with the intrinsic PAM50 subtypes demonstrated that BL1, BL2, IM and M are largely composed of basal-like subtype, while MSL has a large fraction of normal-like and LAR mostly composed of luminal and HER2 subtypes[27]. In addition to the intrinsic subtypes, a claudin-low subtype has recently been described and

is enriched for EMT markers, immune response and cancer stem-cell like genes[31]. This claudin-low subtype mostly composed of M and MSL TNBC subtypes[27].

In addition to identifying distinct transcriptional subtypes of TNBC, representative cell lines with differential sensitivity to chemotherapy and targeted agents have also been identified[28]. BL1 cell lines are sensitive to genotoxic agents, LAR cell lines have differential sensitivity to the LAR antagonist bicalutamide and PI3K inhibitors, M and MSL cell lines are more sensitive to the multi-family tyrosine kinase inhibitor dasatinib and display some sensitivity to PI3K/mTOR inhibitors. Subtyping of breast tumors from The Cancer Genome Atlas (TCGA) resulted in identification of 163 tumors and analysis of the clinical data associated with TNBC tumors show that the median overall survival and disease-free survival time of patients with BL1, IM and MSL subtype tumors were nearly double that of patients with BL2, LAR and M tumors[32]. Further, patients with tumors of the IM subtype had the best outcome. Analysis of the gene expression data from the IM subtype and identification of transcripts associated with lymphocytes suggests that the IM subtype also contains tumor-infiltrating lymphocytes (TILs). The favorable outcome of TNBC patients with higher levels of stromal TILs has recently been demonstrated in two adjuvant phase III trials and may especially important in predicting sensitivity to neoadjuvant carboplatin. [33,34] The presence of immune cells and expression of immune checkpoint genes in the IM subtype may identify patient populations that benefit from immune checkpoint inhibitors, which is promising given the positive phase I results of anti-PD-L1 inhibitors[35].

A similar transcriptional analysis was recently performed on a smaller cohort (n=84) and investigators identified four stable TNBC subgroups associated with distinct clinical outcomes[29]. They defined these subtypes as “luminal / androgen receptor (LAR),” “mesenchymal (MES),” “basal-like / immune-suppressed (BLIS),” and “basal-like / immune activated (BLIA)” groups. Similar to the previous study, TNBC patients with tumors expressing immune component features had the best outcome. Between the two studies there is clearly evident overlap between MSL and MES, IM and BL1 with BLIA, M with BLIS and the two LAR subtypes. The combined data for the two studies show that reproducible and distinct transcriptional subtypes can be unmasked when TNBC samples are analyzed in the absence of ER- and HER2-expressing tumors and as sample size is increased there will likely be additional unique subtypes revealed[28,29].

Some of the differential gene expression could be due to distinct global methylation patterns. Recently, investigators have identified three distinct methylation clusters in TCGA that are associated with overall survival in TNBC patients[36]. Whether genetic or epigenetic mechanisms underlie the transcriptional differences, it is clear there are distinct subtypes of TNBC with similar biology and theses subtypes may dictate response to standard chemotherapy or targeted agents.

Despite the rather aggressive clinical behavior of TNBC, approximately 30% of patients with TNBC benefit from chemotherapy. In a retrospective re-analysis of pretreatment biopsies, TNBC molecular subtypes were predictive of response to neoadjuvant anthracycline and cyclophosphamide followed by taxane[37]. This study showed BL1 had

highest pCR (50%) at time of surgery and BL2 and LAR the lowest (0 and 10%, respectively). Similar to the initial classification, patients with LAR subtype were significantly older at diagnosis and recent preclinical data suggest that these patients may benefit from anti-androgen or PI3K inhibitors[38]. We recently demonstrated that PIK3CA kinase domain mutations are a frequent event in AR-positive TNBC tumors relative to the other subtypes (40% vs. 4%); and, that targeting of AR in LAR cells increases sensitivity to PI3K inhibitors[38]. A recently completed phase II trial (TBCRC011) showed modest benefit (clinical benefit rate 19%) with bicalutamide in metastatic, AR-positive TNBC[39]. Several newer trials evaluating more-specific second generation AR antagonists are being evaluated in breast cancer including enzalutamide in patients with advanced, AR-positive TNBC (NCT01889238). While there are relatively few genomic alterations shared by TNBC as a whole, individual subtypes may be enriched in select somatic alterations, several of which may afford opportunities for preclinical discovery and translation to clinical investigation.

Lessons learned from targeted therapy in unselected TNBC

Currently, no specific targeted agent has US Food and Drug Administration (FDA) or European Medicines Agency (EMA) approval to treat TNBC in the adjuvant, neoadjuvant, or metastatic settings. While there are no common alterations in TNBC, several growth factor receptors are overexpressed in various subtypes of the disease., The results from phase II and III clinical trials targeting vascular endothelial growth factor (VEGFR) and epidermal growth factor receptor (EGFR) have been rather disappointing[40]. Initial studies have demonstrated that TNBC tumors have higher intratumoral VEGFA ligand levels compared to non-TNBC and these were associated with poorer prognosis[41]. This observation lead to several clinical trials for the anti-VEGFA drug bevacizumab which have thus far not shown an increased efficacy in triple-negative breast cancer[42-45]. The initial studies were limited to VEGFA ligand and more recently a reanalysis of a large microarray cohort identified high VEGFA expression in 60% of TNBC tumors and elevated VEGFC and KDR the gene encoding the VEGF receptor 2 (VEGFR2) unique to the MSL subtype of TNBC[46]. The presence of both ligand and receptor in the MSL subtype raises the possibility that this may be a therapeutically relevant subgroup, however the current inhibitors such as bevacizumab that target VEGFA are not likely to be successful in this subtype.

Similar to VEGFA, EGFR was found to be highly expressed in TNBC[47], with approximately half-of basal-like cancers positive for EGFR by immunohistochemistry[48], and sensitivity to EGFR inhibition demonstrated in a basal-like cell line[49]. These observations lead to several trials evaluating the anti-EGFR antibody cetuximab alone or in combination with chemotherapy. The addition of cetuximab to ixabepilone resulted in a similar progression-free survival of 4.1 months as ixabepilone alone[50]. Likewise, treatment with the irreversible EGFR/HER2 inhibitor afatinib had limited activity in HER2-negative breast cancer with no objects responses observed in TNBC[51]. In a trial conducted by the Translational Breast Cancer Research Consortium (TBCRC001), the combination of cetuximab plus carboplatin in metastatic TNBC produced responses in fewer than 20% of patients[52]. The addition of cetuximab only blocked the EGFR pathway in a minority of

patients, suggesting that most cancers had alternate mechanisms to activate the pathway. One possible mechanism may involve parallel activation by other growth factor receptors, such as MET activation by hepatocyte growth factor that is known to lead to resistance of EGFR tyrosine kinase inhibitors in non-small cell lung cancers[53]. Combined inhibition of EGFR and MET may be a potential strategy in TNBC and could be subtype specific, as BL2 tumors express high levels of both receptors[54]. Together, these studies demonstrate that targeted therapies evaluated in unselected TNBCs may delay the success of these agents in a given subtype and future studies should consider up front stratification by biomarkers.

Conclusions

Clearly, TNBC is a pathologically, molecularly and clinically diverse disease that will likely require multiple therapeutic approaches. Standard chemotherapy is quite effective in a subset of of patients with TNBC and identifying these patients and those least likely to respond prior to treatment could make a significant impact on treatment and outcomes. Previous trials have shown that targeted therapies are unlikely to benefit an unselected population of TNBC patients. The future success of clinical trials in patients with TNBC will likely require stratification of their tumors by molecular subtypes or specific genomic alterations such as those being done for Notch (NCT02299635) or FGFR (NCT02202746) and evaluations of targeted therapy in alteration positive and negative arms.

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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