Identifying Gaps and Relative Opportunities for Discovering Membrane Proteomic Biomarkers of Triple-negative Breast Cancer as a Translational Priority

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Triple-negative breast cancer (TNBC) remains a significant clinical and scientific challenge. The classification of TNBC is based on the lack of expression of the human epidermal growth factor 2, the estrogen receptor, and the progesterone receptor. TNBC accounts for more than 20% of all breast cancers (BCs), and TNBC patients experience a more aggressive clinical course, with high rates of disease recurrence, visceral metastasis, and low survival rates. A clinical biomarker is defined as a substance that indicates a biological or pathological process or a response to a particular therapeutic intervention. Known tumor biomarkers with established clinical utility include the prostate-specific antigen (prostate cancer), CA 19.9 (gastrointestinal), CA 125 (ovarian), CA 15.3 (breast) and α-fetoprotein (testicular). Their discovery has led to increased research into the discovery of proteomic biomarkers for patients with TNBC. Discovering new biomarkers for cancer prognosis, prediction, and monitoring through systems biology will provide valuable information about the patterns of disease development, will help inform therapeutic decisions, and allow for treatment monitoring. Table 1 lists emerging, clinically relevant, and recommended BC biomarkers by the American Society of Clinical Oncology (ASCO) and National Academy of Clinical Biochemistry. In this review, we seek to outline the current understanding of TNBC’s biology and discuss the potential of membrane proteins as biomarker for this disease.

Key words: Biomarkers and membrane proteins, breast cancer, metastasis, triple negative

INTRODUCTION

Assessment of HER2-positive BC follows standardized guidelines. However, hormone receptor measurements vary across countries. The lack of uniform assessment has been a major obstacle for understanding advanced BC such as TNBC. The analysis of TNBC patients needs to be reproducible to select appropriate treatment options that could directly influence and revamp translational research. Through extensive genetic profiling, it is clear that TNBC is a heterogeneous disease, with complex mutational profiles, varying cellular morphologies, and behavior. This complexity in genetic profile means that treatment of TNBC with molecular targeted therapies will prove to be difficult. Although much is known about TNBC, a number of critical limitations need to be addressed in the next few years to improve patient outcomes.

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DEFINING TRIPLE-NEGATIVE BREAST CANCER: MOLECULAR HETEROGENEITY

Kinship between basal-like breast cancer and triple-negative breast cancer

With the aid of complementary DNA microarrays, Perou et al. identified five distinct subgroups of BC that are biologically diverse and have different clinical outcomes: two luminal cell-related groups (luminal A and B), a myoepithelial cell-related group (basal like), a HER2-enriched group, and a normal breast-like group. Hierarchical classification of breast tumor has major, primary, and secondary divisions based on the ER and PR statuses, supported by the panelists of St Gallen Consensus 2009, also endorsed and approved by the expert panel of ASCO. From the primary division, the secondary division has been diversified considering the hormone receptor status which could be either positive or negative as well as optimal performance of HER2 status. Immunohistochemistry (IHC)-based proliferative index Ki67 has also been useful for classifying BC into luminal A (ER-positive, HER2-negative, and low Ki67) luminal B (ER-positive, HER2-negative, and high Ki67), and TNBC (any Ki67). As a consequence, Ki67 has become a routine clinical assay for BC treatment. The luminal subgroup of BCs is further classified as luminal A and B tumors which are histologically graded as low and high, respectively. Tertiary division classifies the nonluminal subgroup of BCs into HER2 overexpressing or enriched group and the TNBC group. The latter shows two core basal subtypes, in which the tumor expresses genes such as KRT5, KRT14, and KRT17 characteristic to normal basal myoepithelial cells with cytokeratin (CK) and epidermal growth factor receptor (EGFR) expression. These two TNBC basal subtypes are further subdivided into basal-like 1 and basal-like 2 subtypes based on keratin expression [Figure 1].

Triple-negative breast cancer’s individuality within breast cancer heterogeneity

Gene expression profiling further classifies TNBCs into immunomodulatory cancers, which are enriched with lymphocytic infiltration. A subtype of TNBC also includes triple-negative cancers lacking expression of CK5/6 and EGFR, the so-called quintuple-negative phenotype. BC subtypes showing mesenchymal-like (M) and mesenchymal stem-like gene expression signatures which are enriched in epithelial-to-mesenchymal transition gene sets have also gained attention [Figure 1]. Finally, a subtype of triple-negative breast tumor classified as luminal AR contains all the ER tumors outside the basal group, which shows an androgen receptor signaling and a “molecular apocrine” gene expression signature. A significant overlap exists between TNBC and basal-like BCs (BLBC); approximately 85% of all TNBCs are basal-like tumors, which resemble the outer basal cells surrounding the mammary duct, and most BLBCs are

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ER: Estrogen receptor; PR: Progesterone receptor; ASCO: American Society of Clinical Oncology; NACB: National Academy of Clinical Biochemistry; HER2: Human epidermal growth factor receptor 2; CA: Carbonic anhydrase; TPA: Telapristone acetate; uPA: Urokinase plasminogen activator; PAI: Plasminogen activator inhibitor; CEA: Carcinoembryonic antigen

Table 1. Biomarkers of breast cancer diagnosis and prognosis

ER: Estrogen receptor; PR: Progesterone receptor; ASCO: American Society of Clinical Oncology; NACB: National Academy of Clinical Biochemistry; HER2: Human epidermal growth factor receptor 2; CA: Carbonic anhydrase; TPA: Telapristone acetate; uPA: Urokinase plasminogen activator; PAI: Plasminogen activator inhibitor; CEA: Carcinoembryonic antigen

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TNBC membrane protein biomarkers

Understanding the biology and genetic heterogeneity of BC in general, and TNBC as a subtype, continues to evolve and not all TNBCs are treated equally. Although the application of gene expression profiling is limited in clinical practice due to its complexity and high cost, transcriptome profiling has enabled researchers to identify molecular signaling cascades and targets which may prove useful in the development of novel therapeutic agents.33,34 Interestingly, BC associated with mutations in the BRCA1 tumor suppressor gene frequently lacks expression of ER, PR, and HER2. These BRCA1-mutant breast tumors cluster closely with TNBC/BLBC on microarrays and share many common molecular features including expression of CK5/6, CK14, CK17, p-Cadherin, and EGFR.35 Péro et al.26 characterized BLBC by its low expression of ER/PR/HER2 and high levels of CK5/6, CK14, CK17, p-Cadherin, p63, caveolin-1, carbonic anhydrase IX gene (CA IX), and EGFR. Thus, the TNBC/BLBC subgroups are associated with altered BRCA function and genomic instability, along with defective DNA damage repair, which may reflect their sensitivity to certain therapies that induce DNA damage.36

Clinically, TNBC receives a significant attention as it occurs more often in young women (under 40 years of age), particularly in African-American and Hispanic populations. A majority of tumors defined as triple negative have their genesis from the breast ducts and are associated with certain morphological characteristics including large tumor size, regions of central necrosis, pushing borders with lymphocytic stromal invasion, high nuclear expression, and histological grading with higher mitotic index. TNBCs are considered a special type of BC as they show some similarity with medullary and adenoid cystic carcinomas. Furthermore, TNBC is characterized by an early peak in recurrence between the 1st and 3rd year postdiagnosis followed by a sharp decrease in subsequent years with relapse seldom reported after 8–10 years. Unlike other subtypes of BC, TNBC outcome is not clearly related to clinical stage.37

TNBC’s rapid growth and disease frequency among younger women make mammographic detection difficult. In a nested case–control study carried out as part of National Mammographic Screening Program, TNBCs were predominantly seen among women having interval BCs and false alarm mammograms, especially within a period of 12 months.38 However, for BC patients with ER/PR-positive and HER2-negative profiles, mammograms reveal specific features on magnetic resonance imaging (MRI), such as rim enhancement and very high intratumoral signal intensity on $T_2$-weighed images of MRI. In addition, BCs with a core basal phenotype, unlike nonbasal triple negative, may be more likely than ER-positive BC to recur locally. The Kaplan–Meier survival curve for patients with triple-negative or BLBC differs drastically in relation to other subtypes. Women with luminal A subtype had prolonged survival compared to patients with luminal B- and HER2-positive BC ($P < 0.0001$). Women with BLBC showed early decline over the first couple of years after diagnosis, followed by gradual decline on follow-up with central nervous system spread.39 TNBCs tend to be more aggressive than all other BC subtypes and more likely to occur in the viscera, particularly lungs and brain, but less likely to spread to the bones.40

TRIPLE-NEGATIVE BREAST CANCER GENOMICS–MUTATIONS AFFECTING THE BRCA AND P53 GENES

Following the identification, mapping, and cloning of two major BC susceptibility genes such as $BRCA1$ (chromosome 17q21) and $BRCA2$ (chromosome 13q12.3), the biology and the molecular characteristics of BC have received greater attention. $BRCA1$ plays a significant role in repairing double-stranded DNA breaks and also acts as a regulator of the p53 pathway to maintain chromosome stability.41 Loss of $BRCA1$ function, therefore, results
in genomic instability, predisposing cells to transformation. Germ-line BRCA1 and BRCA2 mutations are inherited in an autosomal dominant fashion. More than 75% of BRCA1-mutant BCs exhibit a basal-like phenotype as assessed by gene expression microarray and IHC analysis. This is particularly true among younger patients who have a familial history of BC and who often present with p53 mutations.\textsuperscript{32} Cytogenetic aberrations identified in BRCA1-mutant BCs, including the deletion of chromosome arm 5q, are also common in basal-like breast tumors, but not in other BC subtypes.\textsuperscript{33} BRCA1-mutant BCs also resemble TNBC in having p53 loss of function mutations. The tumor suppressor p53 is involved in cell cycle checkpoint control and promotes cell cycle arrest or apoptosis in response to DNA damage. p53 is mutated in up to 82% of BLBCs by both gene and protein expression analysis.\textsuperscript{34} Mutated p53 leads to increased genetic instability, cytogenetic changes, and loss of heterozygosity (LOH), which is in accordance with the genetic profile of BLBC/TNBC subtypes. For instance, gain of 6p21-p25 and loss of 5q11 are common alterations in BLBC, with the latter region carrying several DNA repair and suppressor genes, including MSH3, RAD17, and APC. Interestingly, the spectrum of p53 mutations in BRCA1-mutated TNBC is distinct from the p53 alterations found in sporadic TNBC. The ataxia-telangiectasia-mutated kinase is underestimated with an expression of bi-allelic mutation in both BRCA1 mutant (33%) and BRCA2 mutant (30%) hereditary BC which is in contrast to sporadic TNBC (20%) that tends to be BRCA1/2 wild type.\textsuperscript{35}

Linkage analysis suggests that BRCA1/2-mutant breast tumors have unique pathological and gene expression profiles compared to high-risk non-BRCA1/2 (BRCaX) BCs. The majority of BRCaX breast tumors are believed to have originated from a distinct set of genetic alterations. Shah et al.\textsuperscript{36} reported that more than 20% of BLBCs carry genetic changes, such as BRAF\textsuperscript{VRQ15}, EGFR copy number gains, and ERBB2/3 mutations, which can be inhibited by currently clinically trials drugs. Integrative pathway analysis identified hyperactivated CTGF and FOXM1 transcriptional factors and increased MYC and HIF1-α/ARNT networks as key regulators of basal-like breast tumors.\textsuperscript{40} Furthermore, loss of RB1, cyclin E copy number gains, and BRCA1/2 loss of function mutations were confirmed as the common features of basal-like breast tumors. The loss of BRCA expression due to gene silencing by promoter methylation has been shown in TNBCs, and BRCA1 normally suppresses the expression of basal-like-related genes, which could provide an explanation for “BRCAness” of basal-like sporadic cancers.\textsuperscript{37} Studies report that sporadic TNBCs have similarities with BRCA1-linked BCs with their biological and histological features, both displaying genomic instability, lymphocytic infiltrate, central necrosis, and chromosomal loss. Moreover, BRCA1-linked BCs show typical molecular features of TNBC, such as EGFR overexpression, CK5/6 expression, ER/HER2 negativity, and p53 mutations. Finally, although the majority of BRCA1 mutant-BCs show the triple-negative/basal-like phenotype, it is important to recognize that majority of TNBCs are sporadic.\textsuperscript{38}

METASTASIS-RELATED PLASMA MEMBRANE PROTEINS OF BREAST CANCER

Systems biology approaches for discovering plasma membrane (PM) proteins in advanced BC are revealing exciting new potential biomarkers. TNBC metastases are established primarily when malignant cells disseminate to lymph nodes, and then start spreading preferentially into the organs such as the lungs, liver, and brain. The process of metastases is still not fully understood, but comprises a complex set of signaling networks. Metastasis involves the detachment of single cells from the original tumor, invasion into the tissue matrix, intravasation, survival in vasculature, and extravasation to distant locations and angiogenesis to enable survival and growth [Figure 2]. Why some disseminated cancer cells remain dormant for a lifespan and others get activated is a puzzle. However, it is likely that some dormant solitary cancer cells remain dormant and others get activated at distant organs by sensing environmental signals. For instance, inflammation, compromised immune system, changes to female hormonal levels, and hormone imbalances may all contribute to proliferation, differentiation, and metastatic growth. An experimental study on genetic signatures between metastatic tumors vs. primary tumors revealed that additional genomic changes gradually acquire during metastases. Reports claim frequent large chromosomal gains in 1q, 5p, 8q, 11q, and 20q along with overexpression of multiple genes (BRAF, NEK2A, ATAD2, DERL1, and DNMT4R) in metastatic tumors, supporting high genetic instability.\textsuperscript{39} Similarly, a panel of differentially expressed genes has been identified in the isogenic cell lines M-A44 and NM-2C5 derived from aggressive ductal carcinoma MDA-MB-435 studies.\textsuperscript{41} However, it is now important to turn to proteomic studies, which are much more complex than genomic studies. Focusing on membrane proteins is especially important as cell surface proteins in the PM which may add valuable biological insights for metastases. By validating both protein and RNA expression levels, we can gain a better understanding of the critical changes involved in tumor initiation and metastasis. It is important to select appropriate clinical cohorts with detailed and relevant clinical histories and to establish a comprehensive set of research standards and relevant in vitro assays to accurately explore the potential of membrane biomarkers.

Fidler’s classic study on the heterogeneous metastatic capacity of murine melanoma cell clones strongly suggests that some highly metastatic tumor cell variants preexist in the parental tumor population.\textsuperscript{51} In BC, two metalloproteinases, ADAMTS1 and MMP1, modulate the bone microenvironment to promote bone metastases. Similarly, lung metastases showed COX2, EREG, and MMP1 and MMP2 expression, which promotes angiogenesis and extravasation of metastatic cells from the capillaries of lung.\textsuperscript{53} There are several advantages in utilizing pathway-level analyses of membrane proteins in cancer. For instance, in vitro and in vivo studies support the conclusion that a number of ADAMs (a disintegrin and metalloproteinase), a family of transmembrane, and secreted proteins play a key role in cell adhesion, signaling, and cancer metastases. Considering their significant role in cancer progression and signaling, analysis of membrane proteins may provide a rich source of new prognostic and predictive biomarkers. Overexpression of the membrane-anchored protein urokinase plasminogen activator receptor (uPAR) has been reported in many cancers, including TNBCs. uPAR is an important regulator of extracellular matrix (ECM) proteolysis, cell adhesion, and also for signaling. uPAR interacts with the primary ligand uPA and several other proteins including the integrin family of membrane proteins, molecules of ECM, and transmembrane receptors to modulate intracellular signaling. The interaction of uPA/uPAR, integrins, and the uPAR inhibitor, PAI-1, has an important role in cancer, and its deregulation and expression are associated with a poor prognosis.\textsuperscript{54} Kischel et al.\textsuperscript{55} compared membrane protein
TNBC membrane protein biomarkers

profiles of the MDA-MB 231 BC cell line with bone metastatic subclones and showed upregulation of integrin αvβ3 in the bone metastatic cell variant. A comparison of uPAR expression in BC subtypes revealed that uPAR showed highest expression in TNBCs, and uPAR expression correlated with a poor prognosis and early disease recurrence. Co-immunoprecipitation studies of integrin αvβ3 with uPAR have been carried out in invasive BC, but there has been little insight gained into the interaction of uPAR with any integrin subtype in advanced BC. Reports suggest that the integrin αvβ6 subtype is the activator of transforming growth factor β (TGFβ), which is implicated in promoting and controlling multiple cancer types including ductal carcinoma in situ.

TRIPLE-NEGATIVE BREAST CANCER METASTASIS: MEMBRANE PROTEINS AND MOLECULAR SIGNALING

Genomic and proteomic studies need to focus on molecular signaling in TNBC to identify the drivers of proliferation and
metastasis. The RAS/mitogen-activated protein kinase (MAPK) pathway has been shown to be important for the initiation and progression of breast carcinoma. The RAS family of GTPases is activated by receptor tyrosine kinases and promotes the sequential activation of three tiers of kinases (i.e., the RAF, MEK1/2, and ERK1/2 proteins). ERK activation and nuclear localization promote the expression of many transcriptional factors, such as MYC, FOS, ELK-1, and ETS that support cell survival and proliferation. Alterations affecting the phosphatidylinositol-3-kinase (PI3K/AKT) pathway are frequent in luminal BCs whereas aberrant RAS activity has been identified in metastatic sites of BLBC/TNBC.68 ERK phosphorylation has both negative and positive prognostic implications for TNBC and this apparent discrepancy may be due to rapid loss of phosphorylation during handling procedures of tissue extraction and processing. Jing et al. reported that suppression of MAPK activity with the use of MEK inhibitors specifically blocks the proliferation of TNBC/BLBC cell lines. Role of microRNA (miRNA) as a noncoding RNA regulating mRNA stability and protein translation could potentially provide more insights about RAS/MAPK signaling within the TNBC/BLBC subtype.

Recent reports suggest that PI3K signaling, through the AKT and mTOR effectors, plays a central role in BC. Among the AKT isoforms, AKT1 mutations are found in ER positive breast tumors, and AKT3 alterations occur in ER negative breast tumors. Activated receptor tyrosine kinases bind the p85 subunit of PI3K, which then recruits the catalytic p110 subunit to form an active PI3K enzyme.60 Phosphatase and tensin homolog and inositol polyphosphate 4-phosphatase type II directly oppose PI3K activity achieving cell arrest. AKT activation modifies downstream proteins in TNBC and understanding their role provides an opportunity to inhibit PI3K signaling in advanced BC. MYC oncogene has been long known to be critical for breast tumor progression, but its exact role as a driver oncogene for promoting metastasis is unclear. Key molecular signatures for assessing MYC activity include Src, β-catenin, H-RAS, E2F3, Wnt and TGFβ signaling, which are analyzed with microarrays. Wnt receptor frizzled-7 and Wnt co-receptor, lipoprotein receptor-related protein-6, have been reported to be upregulated in TNBC.62 SMAD signaling is often initiated by TGFβ receptor activation, which promotes the phosphorylation and activation of receptor-regulated SMADs (R-SMADs). SMADs also enhance TGFβ signaling through sumoylation and ubiquitination in metastatic BC even though the functional consequences of these modifications are unclear.63 Rho family GTPase activity has also been demonstrated in invasive BC cells, and silencing RhoGDI-2 results in downregulation of the integrin β1, which is essential for cancer cell adherence to ECM. The role of RhoGDI-1 has been studied extensively in BC apoptosis, and findings by Muñiz Lino et al. have established that TNBC tissue-derived samples exhibit Rho family proteins having upregulated RhoGDI-2 associated with inhibition of caspase 3 and 9 and deregulation of COX5, MTPN, and DB1 proteins.

TRIPLE-NEGATIVE BREAST CANCER METASTASIS AND CIRCULATING TUMOR CELLS

Circulating tumor cells (CTCs) are thought to be the metastatic seeds, which can break away from the primary site of cancer and spread to other parts of the body. Cells corresponding to the blood-borne tumor of BC patients could be tested, but their ability to disseminate is unclear. It has been suggested that the presence of putative BC stem cells with CD44(+) CD24(−/low) phenotype constitutes a population in primary BC having self-renewal and tumorigenic potential in bone marrow.65 It remains unclear whether CTCs originate from the primary site of tumor, as micrometastases or from multiple tumor sites. Researchers at the University of Texas measured CTCs in 151 women with metastatic BC stage 3.66 Blood-based prognostic biomarkers for hormone receptor status CA 27.29 were tested to measure metastatic activity, with results confirming that patients having five or more CTCs had a median overall survival of 13.5 months. The median overall survival increased to 29 months if CTCs were <5 and their findings showed that five or more CTCs had the highest predictive value compared to all other tumor markers. Current methodologies rely only on the analysis of the parental tumor using specific markers, such as ER, PR, and HER2 to justify clinical decisions and treatment. However, analysis of a single primary or metastatic tumor may not provide sufficient details especially on the heterogeneity of cancer and the genetic variability of CTCs. Cell-free tumor nucleic acid, both DNA and RNA, can also have prognostic value. For instance, circulating telomerase mRNA was increased in serum and plasma samples derived from patients with advanced BC.68 Research is now focused on identifying differentially expressed blood-based markers, their role as prognostic markers, their malignancy potential, and their characteristics and relationship with primary tumors.

As explained previously, even though BRCA1 gene mutant BCs are phenotypically similar to TNBC, TNBCs have unique characteristics including the absence of ER, PR, and HER2 expression. The homologous recombination deficiency status of TNBCs has also made them a poor predictor of the outcome. As already discussed, it is important to define new prognostic and predictive biomarkers for TNBC, complemented by a detailed characterization of TNBC compared to other subtypes of BC.69 Biomarker-led membrane protein characterization and cell signaling will help with patient stratification, therapy selection, and will provide a tool to improve the response to treatment. Biological markers of radiosensitivity for tumor and normal tissue require functional characterization so that personalized treatment would be highly efficacious, while minimizing radiotherapy-induced toxicity. A combination of nuclear magnetic resonance spectroscopy and liquid chromatography-mass spectrometry (LC-MS) was used to measure serum metabolites which absolutely identified 80% of BC patients who failed to show a complete response to chemotherapy.70 The role of lymphangiogenesis in metastasis, enlarged lymph nodes due to BC, needs more research. An understanding about the vascular endothelial growth factor-driven angiogenic signaling pathways and identification of specific biomarkers is required as there are no validated biomarkers currently available that address angiogenesis. Lymphedema-based research may be useful to validate responses in vasculature of anatomically dispersed TNBC metastases, especially the organ dissemination of lungs, liver, bone marrow, and brain.72

TRIPLE-NEGATIVE BREAST CANCER BIO-FLUIDS: PLASMA AND SERUM PROTEOMICS

Human plasma and serum are the most commonly evaluated biofluids for diagnosis and prognosis. These biofluids are quickly
TNBC membrane protein biomarkers

accessible, have cellular versatility with the number of peptides with a mass ranging between 1 and 15 kD, and comprise ~ 40% of all detected molecular signaling. Changes in the expression of plasma protein reflect the state of originating tissues at the molecular level as the tissues are constantly getting bathed in plasma. To study plasma as a source of clinically relevant biomarkers, high abundant proteins may require specific depletion to enrich and reveal specific low-abundant biomarkers. Currently, many proteomic techniques and methods have been applied in the analysis of human plasma. Sample collection and processing is critical to ensure safe and secure handling that includes stabilization, processing, and storage to carefully avoid protein denaturation. It is also important to consider the plasma and serum yield which is generally very low, ranging from 0.2 to 1 µg RNA/mL. Importantly, one has to understand that plasma protein biomarker research and human plasma peptide research depend on the quality of processing and the sensitivity of the detection techniques.

Comprehensive data on plasma research have revealed many potential biomarkers and some insights into the mechanisms of tissue-specific diseases in cancer, Alzheimer’s disease, and myocardial infarction. Circulating tumor cells and disseminated tumor cells have an impact on the levels of cell-free DNA in the blood of patients with BC. Circulating DNA can exist as naked (unbound) DNA, DNA-associated histones which could either be mononucleosomes or oligonucleosomes, DNA bound with plasma proteins, or DNA packed as apoptotic bodies. Enrichment and detection of CTCs in blood samples include the cell search system, positive or negative immunoselection or both, and molecular approaches. In BC patients independent of histological cancer type, elevated levels of circulating nucleosomes in blood have been detected. In general for BC, the proliferation marker Ki67 is the blood-based serum/plasma biomarker which is under surveillance, and uPA/PAI-1 is often assayed in combination as tissue-based biomarker for prognosis. Circulating microRNAs have recently attracted a great deal of attention in the advanced stages of the breast disease, in which higher levels of miR-34a were observed having changes in miR-10b, miR-34a, and miR-155 levels which get correlated with metastases. Circulating miR-155 induces EMT which, in turn, activates transforming growth factor signaling leading to cell invasion. The differential expression of the proteins in the serum showing hormone sensitivity compared to nonhormone-sensitive BC has a direct correlation to many factors such as clinical stage, p53 status, and also on the biomarkers. Experiments performed to analyze differences in protein expressions among various BC cell lines identified groups of protein which are being associated with invasive phenotype. A comprehensive review of genome-wide circulating miR data has been created for BC detection and the same could get attempted with circulating miRs that could be useful for detection and application. An experimental technique using SELDI-based serum analysis in advanced BC metastasis accurately predicted the outcome of 83% of early BC patients. Another report with SELDI compared the serum profiles of patients with benign breast disease with serum healthy controls and led to the identification of potential biomarkers that separated healthy controls from cancer patients. To date, the development of a reliable, reproducible, and noninvasive clinical test using circulating biomarkers is still in its infancy, and extensive research is required to standardize and validate them prior to routine clinical use.

NEWLY IDENTIFIED BIOMARKERS OF BREAST CANCER AND FUTURE DIRECTIONS

Although there has been substantial progress in subtyping BC, there is only limited information available on robust prognostic biomarkers exclusively for BC subtypes. HER2 amplification occurs in 30% of BCs, and HER2-amplified BCs commonly show amplification of the topoisomerase IIα, gene which has decreased sensitivity toward anthracyclines. TNBC usually expresses the EGFR protein, and high levels of Ki67 positivity are seen in luminal B BC subtype. FOXP3, a marker for immunosuppressive CD25 + TRegs, has been identified as a marker of larger tumor size. BC stem cell research has identified and recognized aldehyde dehydrogenase 1 as an independent prognostic marker of TNBC in African patient cohorts. Ohi et al. reported ALDH1 expression in 49% TNBC cases without any other correlating clinical parameters. Specific biomarkers of BC subtypes could help clinically in disease prognosis, optimizing therapies, and in predicting responses. Culmination of data gathered about specific biomarker subtype helps in differentiating specifically from other subtypes which could eventually support better patient outcomes.

CONCLUSION

In summary, TNBC is a molecular heterogeneous BC subtype that is, highly aggressive, with poor prognosis and with no effective therapy. Through this review on TNBC membrane protein biomarker discovery, important gaps and relative opportunities has been identified. It has been suggested, discovering newer biomarkers of TNBC through membrane research could map important functional biomarkers that are involved in adhesion, invasion, and metastasis corresponding with extracellular matrix. Newer protein discoveries could potentially evolve as a therapeutic target. However, current proteomic technologies require careful standardization across different sites to achieve sensitive, specific, and reproducible results. Research validation is also highly critical. Engagement with the HUPO Proteomic Standards Initiative will lead to significant advances in this area. Finally, it is important that the research community consider systems that can effectively integrate proteomics data with next-generation transcriptomic, genetic, epigenetic, and metabolomics data.

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Conflicts of interest

There are no conflicts of interest.
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