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Triple Negative Breast Cancer Cell Lines: One Tool in the Search for Better Treatment of Triple Negative Breast Cancer

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"A man's got to know his limitations"

Clint Eastwood as Harry Callahan in Magnum Force (1973)

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in women and one of the leading causes of cancer death for women. Worldwide, over 1.3 million cases of invasive breast cancer are diagnosed, and more than 450,000 women die from breast cancer annually [1]. In the US, approximately 200,000 cases of invasive breast cancer and 50,000 cases of *in situ* breast cancer will be diagnosed annually, and more than 40,000 women die from breast cancer each year – second only to lung cancer [2]. The mortality due to breast cancer has been declining in the US since 1990 [2]. The continuing decrease in mortality from breast cancer has been attributed to early detection due to screening, improved adjuvant therapy, and more recently to decreases in the incidence due to lowered rates of usage of hormone replacement therapy [3, 4]. Despite the decreased incidence and mortality, breast cancer remains a major cause of cancer mortality for women and accounts for 15% of all cancer deaths in women in the US [2].

Clinically, breast cancer can be divided into distinct subtypes that have prognostic and therapeutic implications. Breast cancer patients routinely have the expression of estrogen receptor (ER), progesterone receptor (PR), and amplification of HER-2/Neu evaluated [5]. These markers allow classification of breast cancer tumors as hormone receptor positive tumors, HER-2/Neu amplified tumors, and those tumors which do not express ER, PR, and do not have HER-2/Neu amplification. The latter group is referred to as triple-negative breast cancer (TNBC) based on the lack of these three molecular markers. Generally, hormone receptor expressing breast cancers have a more favorable prognosis than either those with HER-2/Neu amplification or those that are triple-negative [5]. While all breast tumor types may be treated with chemotherapy, therapeutic options in both early and late stage breast cancer are affected significantly by the expression of these three markers. Tumors that express ER and PR are treated with agents that interfere with hormone production or action [5]. Tumors that have amplified HER-2/Neu are treated with agents that inhibit HER-2/Neu [5]. These targeted therapies are the mainstay of the successful outcomes seen in hormone receptor positive and HER-2/Neu amplified tumors. Both early stage and advanced TNBC tumors are treated with predominantly chemotherapy [5].

TNBC represents approximately 10–15% of all breast cancers and patients with TNBC have a poor outcome compared to the other subtypes of breast cancer [6]. Interestingly, the

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incidence of TNBC in African American women is two to three times higher than other ethnic groups, although the reason for this has not been elucidated [6, 7]. Given the lack of validated molecular targets and the poor outcome in patients with TNBC, there is a clear need for a greater understanding of TNBC at all levels and for the development of better therapies.

Cancer cell lines have proved useful in laboratory and preclinical investigations since the first cell line was established more than 50 years ago [8]. For example, the anti-HER-2/Neu 4D5 mouse monoclonal antibody (which was humanized to create trastuzumab) had anti-tumor effects as a single agent and acted synergistically with chemotherapeutic agents in breast cancer cell lines that had amplified HER-2/Neu but showed no effect in cell lines lacking amplified HER-2/Neu [9–12]. These results formed the basis for clinical trials of trastuzmab and predicted the outcomes of these clinical trials. Similarly, preclinical studies using xenografts of a hormone receptor expressing breast cancer cell line have accurately predicted the outcomes of clinical trials comparing aromatase inhibitors to tamoxifen and to the combination of tamoxifen and aromatase inhibitors [13]. In this review, we will describe triple negative breast cancer cell lines and discuss their utility and the limitations of these cell lines in the investigation of TNBC (mindful of Harry Callahan's admonition cited above).

THE PLAYERS

Review of the literature found 27 human cancer cell lines that can be classified as TNBC cell lines and their characteristics are summarized in Table 1 (and the references therein). While there may be other cell lines that are derived from TNBC, these 27 are the most commonly cited. The oldest TNBC cell line, BT20, was established from a pleural effusion in the 1950s and many of the most commonly studied cell lines (*e.g.*, MDA-MB-231) were established in the 1970s. These tumors were derived before the routine evaluation of hormone receptor expression and HER-2/Neu amplification in clinical samples. The identification of the older cell lines as TNBC cell lines is based primarily on characterization of the cells after they were established in culture. The HCC cell lines were derived most recently and thus the characterization of these cell lines and their primary tumors is more complete [14, 15]. Specifically, the histologic features (including evaluation of ER, PR and HER-2/Neu) and the grade of the cell lines were in good concordance with the tumors from which they were derived [15]. The SUM52PE cell line was derived from a pleural effusion and was found to be negative for ER and PR expression, however the original primary tumor from this patient was positive for both hormone receptors [16]. Sixteen of the tumors were derived from primary breast tumors, ten of the tumors were derived from metastatic pleural effusions, and one was derived from a metastatic skin lesion (Table 1). Interestingly, the SUM102PT cell line was derived from a patient with ductal carcinoma in situ (DCIS) with microinvasion [17, 18]. To our knowledge, this is the only breast cancer cell line derived from such an early stage breast tumor.

Most of the cell lines were derived from high grade tumors (reported in Table 1 as grade 3, anaplastic, carcinosarcoma, or poorly differentiated). This is consistent with the features of TNBC in general [6]. The pathological descriptions of the cell lines are heterogeneous. The majority of the cell lines are described as ductal carcinomas, but their pathological descriptions include poorly differentiated adenocarcinomas, anaplastic carcinomas, an infiltrating medullary carcinoma, an infiltrating lobular carcinoma, and an acantholytic squamous carcinoma (Table 1). One of the cell lines, SUM149PT, was derived from an inflammatory breast cancer [19]. The Hs578T and the SUM159PT cell lines are described as having carcinosarcomatous features and the HCC1806 cell line is described as an acantholytic squamous cancer [14, 18, 20]. This raises the possibility that these three cell

lines might be metaplastic cancers, which are a unique subset of breast cancer that is extremely refractory to treatment [21, 22].

The MDA-MB-435 cell line was derived from a pleural effusion from a patient with breast cancer, however the identity of the line has been questioned by recent results. The patient from whom the cell line was derived was a 31 year old Caucasian woman who underwent mastectomy and axillary lymph node dissection for breast cancer in October of 1975 at M.D. Anderson Cancer Center and was found to have extensive infiltrating carcinoma in the breast and two of eight axillary lymph nodes positive for breast cancer. She developed a malignant pleural effusion in December of 1975 as the first manifestation of metastatic disease and died from metastatic disease one year after her diagnosis [23, 24]. The cell line was derived from malignant cells in a pleural effusion in January of 1976 [23, 24]. In the course of doing cDNA expression analysis of the NCI 60 cell line panel, Ross *et al.* found that MDA-MB-435 clustered with melanoma cell lines and not with other breast cancer cell lines [25]. This analysis raised several possibilities. One is that the breast cancer cells are undifferentiated and express markers of melanocyte differentiation but are in fact breast cancer. Recent work has shown that primary breast tumors can express melanocyte related genes and found similar melanocyte related gene expression in the MDA-MB-435 cell line [26]. Also, this work found breast associated genes expressed by MDA-MB-435 cells. Consistent with this, Sellapan et *al.* found that the MDA-MB-435 cells express a number of breast and epithelial specific proteins (β-casein, α-lactalbumin, epithelial membrane antigen, keratin 19 and pankeratin), could be induced to express breast differentiation markers and secrete milk lipids, and expressed two melanoma specific antigens (tyrosinase and melan-A) [27]. A second possibility is that the MDA-MB-435 cell line is a melanoma cell line that has somehow contaminated and overgrown the original cell line. Consistent with this possibility, analysis using expression profiling, single nucleotide polymorphism (SNP) analysis and microsatellite analysis of the MDA-MB-435 cell line from multiple sources found that all clones of the cell line are identical to the M14 melanoma cell line [28, 29]. However, the M14 cell line was derived from a 33 year old male with melanoma at UCLA while all of the existing clones of the MDA-MB-435 and M14 cell lines are female [30, 31]. This raises the likely possibility that the existing cultures are all the MDA-MB-435 breast cancer cell line and not the M14 melanoma cell line [30]. Investigators should be aware of these issues when using this line for their investigations (caveat emptor).

There are six immortalized, but non-tumorigenic cell lines with features similar to TNBC cancer cell lines (highlighted in yellow on the table). These cell lines do not express hormone receptors nor do they have amplified HER-2/Neu. The MCF10A and MCF12A cell lines were spontaneously immortalized cells derived from benign breast tissue of women who had fibrocystic disease [32, 33]. HBL-100 is a spontaneously immortalized cell line derived from breast milk from a lactating, young, healthy woman [34]. However, these cells have SV40 sequences integrated within the genome and the DNA from these cells has transforming activity *in vitro* [35, 36]. The 184A1 and 184B5 cell lines were derived from cells from a reduction mammoplasty treated with the carcinogen benzo(a)pyrene and the hTERT-HME1 cells were derived from normal mammary epithelial cells immortalized by expression of human telomerase [37, 38]. As will be discussed below these non-transformed cells lines cluster with the TNBC cancer cell lines based on cDNA expression array analysis and thus can be considered non-transformed counterparts of the TNBC cancer cell lines [39–41]. A series of subclones of MCF10A have been developed that are progressively more transformed by transfection with activated HRas followed by serial passage through mice [42]. These cells are useful as models of malignant progression. KRas and HRas mutations are relatively rare events in breast cancer in general and TNBC specifically, so that generalization of results from the use of this series of cells to TNBC tumors needs to be circumspect [43].

The HCC cell lines and Hs578T have paired immortalized non-transformed cell lines derived from the same patient [14, 20]. The majority of these are Epstein-Barr virus transformed B-lymphoblastoid cell lines but in several instances, normal breast stromal or breast epithelial cell lines exist [14, 20]. These are of particular use for the determination of whether genetic variants found in the cell line represent somatic or germ line changes.

THE MOLECULAR CHARACTERIZATON OF TNBC CELL LINES

Over the past decade, cDNA expression profiling of human breast cancers has allowed classification of the tumors based on the similarity of gene expression patterns between normal breast cells and tumors [44, 45]. The hormone receptor expressing breast cancers resembled most closely the luminal cells of the breast ducts but could be further subdivided into several subgroups that have different prognoses and responses to hormonal therapy. The tumors with HER-2/Neu amplification clustered together and were found to have a poorer prognosis than the luminal subtype. These data were compiled prior to the introduction of trastuzumab. The TNBC tumors resembled most closely basal cells, cells found on the outside of the breast ducts, and had the worst prognosis [44, 45]. Subsequent analyses have suggested that the clinical TNBC classification and the array based basal classification significantly overlap but are not identical [6, 46, 47]. For example, array based classification of 172 TNBC found that 123 (71%) were basal-like breast cancer and of 160 basal cancers 123 (77%) were TNBC [46]. These distinctions were important as the clinical outcomes were worse for the basal breast cancers than for the non-basal TNBC [47].

Recently, several groups have performed array based cDNA expression analysis on breast cancer cell lines and classified them as either basal or luminal based on the intrinsic classification described above [39–41]. Twenty out of twenty three TNBC cell lines were classified as basal while only three out of twenty three were described as luminal [39, 40]. Thus most of the TNBC cell lines, as with the tumors, are basal-like. Whether the slightly higher frequency of basal-like cells seen in the cell lines (87%) compared to the primary tumors (70–80% described in several studies) represents a selection of these cells in culture or a sampling error due to the small number is not clear. All of the non-transformed cell lines clustered with the basal-like cancers suggesting that they represent non-tranformed cells derived from a similar cell of origin as the basal-like TNBC cell lines (Table 1, [39, 40]). An immunohistochemical (IHC) definition of basal-like tumors has been developed based on a triple-negative phenotype (ER, PR and amplified HER-2/Neu negative) and expression of either epidermal growth factor receptor (EGFR) or cytokeratin 5/6 [48, 49]. Most of the TNBC and non-transformed cell lines designated as basal express EGFR, consistent with a designation as basal based on this IHC method [40, 50].

The expression profiling of the cell lines revealed a subdivision of the basal cell lines into one subgroup which had epithelial features and was keratin 5 and 14 positive (Basal A) and a second subgroup which had mesenchymal features including vimentin expression (Basal B) [40]. Eight of the twenty cell lines classified as basal-like were Basal A and twelve were Basal B. These subcategories of basal-like tumors were not seen in the classification of the tumor specimens raising the possibility that they are a cell culture artifact [44, 45]. However, the expression profiling of tumors included both the tumor cells and the tumor stroma, while the profiling of the cell lines is looking only at the tumor cells. Thus the recognition of subsets of basal-like cancer cells may have been unmasked by the absence of other cell types contributing to the expression pattern. Supporting the argument that the Basal B subtype is not a cell culture artifact, several immunohistochemical studies of primary breast tumors have identified a subset of tumors in which the cancer cells express vimentin, consistent with the existence of mesenchymal tumors [49, 51, 52]. In the largest study, staining more than 2500 primary breast tumors demonstrated that approximately 14% of the tumors

expressed vimentin [52]. 35% of hormone receptor negative tumors expressed vimentin but only 7% of hormone receptor positive tumors expressed vimentin. The enrichment of vimentin positive tumors within the ER negative samples is consistent with enrichment within the triple-negative samples, but this study did not simultaneously evaluate HER-2/Neu amplification so that vimentin positive tumors cannot be classified as triple-negative [52]. Two small studies identified vimentin expression in 17 of 18 and 4 of 11 triple-negative breast cancer samples [49, 51]. Importantly, the study by Livasy *et al.* categorized tumors as luminal, basal, or HER-2/Neu amplified by cDNA microarray expression profiling and found that 17 of 18 triple-negative/basal-like tumors had strong and diffuse vimentin staining in the tumor cells [49]. Thus the Basal B phenotype seen in the cell lines is likely to reflect a subset of TNBC that has mesenchymal or Basal B-like features. Interestingly, all of the non-transformed cells that have been so characterized have a Basal B pattern of gene expression (Table 1, [39, 40]).

Two groups have performed comparative genomic hybridization (CGH) on a large panel of breast cancer cell lines, including the TNBC cell lines [39, 40]. In both studies, the pattern of copy number aberrations (CNA) is similar between the cell lines and primary tumors. However, there were generally more CNAs in the cell lines compared to tumors samples consistent with continued evolution of genetic aberrations in the cell lines [39]. Also, when genomic deregulation of gene expression (defined as correlation between DNA and gene expression) was evaluated there was again good concordance between cell lines and tumor samples [40]. However, these studies did not directly compare the cell lines to the tumors from which they were derived but rather compared the panel of cell lines to a representative panel of tumors. Wistuba *et al.* compared the ploidy and the retention or loss of heterozygosity across multiple loci for TNBC cell lines and the tumors from which they were derived [15]. There was a high concordance in all these parameters between the cell lines and the tumors [15]. Interestingly, the cell lines representing luminal, basal A, and basal B phenotypes had distinct patterns of CNA. Luminal cell lines displayed a greater frequency of high level DNA amplification and luminal and basal A cell lines generally had more CNA than basal B cells [39].

Loss or mutation of the p53 tumor suppressor gene is one of the most common recurrent genetic abnormalities observed in TNBC and most of the cell lines described in Table 1 contain mutant p53 [5, 7, 45]. Wistuba *et al.* found that two of three TNBC tumors had identical p53 mutations as seen in the cell lines derived from those tumors [15]. Tumors that are BRCA1 deficient are most commonly basal-like TNBC [53, 54]. Six of the TNBC cell lines (HCC1395, HCC1937, HCC3153, MDA-MB-436, SUM149PT, and SUM1315M02) have homozygous deleterious mutations in BRCA1 (Table 1) [55–57]. The HCC1937 cell line was derived from a 24 year old woman with a family history of breast cancer and a germ line mutation in BRCA1 [14, 57]. The BRCA1 mutation in HCC3153 was confirmed to be a germline mutation by sequencing the BRCA1 gene in the matched B-lymphoblastoid cell line derived from the same patient [39]. The HCC1395 line is reported to have a germline BRCA1 mutation [56]. The family history and the germline status of BRCA1 are not known for the other three BRCA1 mutant cell lines. All of the BRCA1 mutant cell lines are basal-like, consistent with the observations in BRCA1 mutant tumors [54].

Taken together, the genomic analyses above suggest that the TNBC cell lines are representative of TNBC tumors and where studied, closely resemble the tumors from which they were derived. Because of this similarity and the ability to harvest large amounts of DNA from essentially a pure tumor cell population, they are a useful tool in the further evaluation of genetic abnormalities in TNBC. In a dramatic demonstration of this, the Vogelstein group used a panel of breast and colon cancer cell lines to sequence the coding regions from all of the genes listed in the RefSeq database to identify somatic mutations in

the cancer samples [58, 59]. These studies did not focus on TNBC, however eight of the eleven breast cancer cell lines used for this study are TNBC cell lines. Using this sequence data and bioinformatic approaches, they identified approximately 140 genes that were likely to be “driver” mutations in the breast cancer cell lines. The same group utilized an expanded panel of cell lines to examine the presence of copy number alterations in all of the coding sequences within the entire genome and integrated this data with their sequencing data to identify dysregulated genes in breast cancers [60]. An important step in utilizing any genetic data generated from cell lines is confirmation of the findings in tumor samples. In the sequencing studies, the mutations found in the breast cancer cell lines were validated in a set of samples from microdissected primary breast tumors [58, 59]. Many, but not all, of the mutations described in the work cited above are found in tumor samples [58, 59]. The many genes found by these unbiased approaches will need to be studied further to validate their importance. Each mutation will need to be confirmed in tumor samples. Also, these genetic abnormalities represent pooled data from different subtypes of breast cancer and will need to be analyzed by subtype.

A study by Stephens *et al.* used a sequencing based approach to catalogue somatic gene rearrangements in breast cancer cell lines and primary tumors representing the major subclasses of breast cancer (*i.e.*, hormone receptor positive, HER-2/Neu amplified, and TNBC) [56]. They found that the TNBC cell lines and primary tumors had the greatest frequency of gene rearrangements compared to the other subtypes. The cancer cell lines and the primary tumors had similar patterns of different types of rearrangements, again supporting the idea that the cell lines reflect the primary tumors [56]. The average number of rearrangements was higher in the cell lines than in the primary tumors [56]. This could be due to the continued evolution of genomic aberrations in the cell lines in culture. On the other hand, this difference could be due to increased sensitivity of the assay in the cell line DNA samples compared to primary tumor DNA samples since the primary tumor samples contain an admixture of tumor cells and normal cells.

These genomic studies support the idea that cell lines are useful tools for analyzing the genetic aberrations in TNBC.

USING TNBC CELL LINES TO STUDY THE BIOLOGY OF TNBC AND DEVELOP THERAPY – THE GOOD, THE BAD, AND THE UGLY

While the utility of TNBC cell lines for the discovery of genetic abnormalities is supported by the data, perhaps the greatest pitfalls arise in using TNBC cell lines to study the biology of TNBC and identify therapies for TNBC. However it should be recalled that hormone receptor positive and HER-2/Neu amplified cell lines and xenografts accurately predicted the response to targeted therapies [9–13].

As described above, TNBC/basal cancers and cell lines often express EGFR [40, 48–50]. EGFR expression in TNBC is associated with poor prognosis [48, 61]. Preclinical studies using TNBC cancer cell lines have demonstrated that inhibition of EGFR by small molecule tyrosine kinase inhibitors (TKIs) or the anti-EGFR monoclonal antibody cetuximab alone or in combination with cytotoxic drugs can inhibit the growth of TNBC cell lines [62, 63]. This has led to several clinical trials of EGFR inhibition alone or in combination with chemotherapy in TNBC (reviewed in [64]). Some trials suggest efficacy in TNBC. For example, preliminary results from one randomized phase II trial show that the addition of cetuximab to a carboplatin/irinotecan chemotherapy regimen increases the response rate from 30% to 49% [65]. However, the bulk of the data from clinical trials have not demonstrated a great deal of efficacy for EGFR inhibition in TNBC [64]. One possible reason for the lack of clear clinical efficacy is that these studies have generally included

patients who have been heavily pretreated [64]. However, it is likely that more information is needed about the role EGFR plays in TNBC and in the cell lines if EGFR is to be targeted for therapeutic benefit. This will be discussed further below.

Dasatinib is an orally available TKI that inhibits the abl and src family tyrosine kinases at sub-nanomolar concentrations and is currently used in the treatment of chronic myelogenous leukemia [66, 67]. Two recent studies characterized the response to dasatinib in breast cancer cell lines with different molecular phenotypes (*i.e.*, luminal or basal) and found the greatest sensitivity to dasatinib in the TNBC/basal cell lines [68, 69]. However, preliminary data from a phase II study showed a partial response in only two of forty four patients [70]. Again these results are disappointing in that the preclinical data with cell lines did not translate into significant clinical activity.

From these two examples, it is clear that preclinical data with cell lines must be used cautiously in the development of therapeutics. This raises questions about why the preclinical data do not translate into clinical efficacy and how to modify the use of cell lines so that they may be more informative.

One issue, not addressed by these studies, is that TNBC represents a heterogeneous group of tumors and that further study will be necessary to understand if there is a subset that may benefit from a particular therapy (*e.g.*, EGFR targeted agents or dasatinib). It is clear from successful targeted therapy that the best therapies are ones that affect pathways directly related to the pathogenesis of the tumors (*e.g.*, HER-2/Neu targeted therapies for HER-2/Neu in breast cancers containing HER-2/Neu amplification or EGFR TKIs in lung cancer patients with activating EGFR mutations [71, 72]). The majority of breast cancer cells expressing EGFR do not have activating mutations or amplifications suggesting that EGFR expression is a marker of the cell of origin of the TNBC but not necessarily a pathogenic driver of the transformation of the cells [73, 74]. Indeed, further evaluation of preclinical studies reveals that most TNBC cell lines are relatively resistant to EGFR inhibition as a single agent [75]. Two of the TNBC cancer cell lines (*i.e.*, BT20 and MDA-MB-468) used in the preclinical experiments cited above, have genetic amplification of the EGFR gene and very high expression of the EGFR protein [76, 77]. These are unusual cell lines as gene amplification of the EGFR is a rare event seen in 1–6% of breast cancers [73, 74]. The preclinical efficacy of EGFR inhibition in these cell lines is reminiscent of the preclinical results with HER-2/Neu targeted therapies in cell lines that have amplified HER-2/Neu [9–12]. This suggests that there may be a subgroup of TNBC which might be driven by EGFR amplification and for these tumors EGFR inhibitors may be efficacious.

For dasatinib, there is little or no data suggesting that the kinases targeted by dasatinib are mutated and might be driver mutations in TNBC. Further study will be necessary to determine if the expression signatures or other molecular markers developed in preclinical studies using the cell lines will allow the identification of a subset of patients in whom dasatinib is efficacious [68, 69].

Clearly the identification of driver oncogenic pathways in TNBC is a priority. Advances in sequencing technology now allow the rapid sequencing of the entire genome of cancer cells. For example, a recent study sequenced the entire genome from the primary and a metastasis from one TNBC/basal cancer and identified multiple mutations in the primary tumor and additional mutations in the metastasis that were not present in the primary tumor [78]. As oncogenic mutations are identified in tumors, relevant data about therapy may then be obtained from cell lines containing those mutations. This would be similar to studies in HER-2/Neu amplified cell lines or lung cancer cell lines harboring EGFR mutations where data about therapies in cell lines did parallel the data in the clinic. It is important to note that

cell lines acquire additional genetic abnormalities after they are cultured and these abnormalities may make the cells susceptible to therapeutic interventions that would be irrelevant in the original tumors [39].

Another important issue is the lack of proper microenvironment in cultured cells. It is clear that tumors represent a complex interaction between the tumor cell and the surrounding stroma [79]. Three dimensional (3D) culture systems have been developed in which cells are embedded in extracellular matrix (ECM) [79]. Non-transformed mammary epithelial cells form monolayers and are indistinguishable from transformed cell lines when gown on tissue culture dishes (2D culture) [79]. When grown in 3D culture systems non-transformed mammary epithelial cells develop polarized spherical structures containing lumens resembling a normal breast acinar structure [79]. In contrast, cancer cells grow in a disorganized mass of cells in such culture systems [79]. Furthermore, the different subtypes of breast cancer grow with distinct patterns when grown in 3D cultures [80]. For example, the basal B TNBC cell lines MDA-MB-231 and HS578t grow with a stellate pattern while the basal A TNBC cell line MDA-MB-468 TNBC grows with a grape cluster like pattern [80]. Because these 3D cultures allow for interaction between the cell and the ECM, they are a more physiological system in which to study the cell lines and their response to therapies. The interaction with ECM can affect the sensitivity to therapy of the cells. For example, work using the MCF10A series described above, Li *et al.* found that non-transformed and transformed cells were equally sensitive to MEK inhibition when grown in 2D culture but that the non-transformed cells were resistant to MEK inhibition when grown in 3D culture while the transformed cells remained as sensitive or more so to MEK inhibition in 3D culture [81]. Interestingly, work with cancer cell lines grown in 2D culture found that many TNBC/basal cell lines are sensitive to MEK inhibition but the MDA-MB-231 cell line was relatively resistant [82]. When grown in 3D cultures, the MDA-MB-231 cells were significantly more sensitive to MEK inhibition [81]. In contrast to the results with MEK inhibition, the transformed cells became more resistant to doxorubicin when cultured in 3D culture [81]. Other investigators have also found resistance to chemotherapeutic drugs and TNF family ligands when breast cancer cells are grown in 3D culture [83–86]. While these data need to be investigated further, the use of 3D cultures for the assessment of therapies may increase the likelihood that the preclinical data will translate into the clinic.

Utilizing the cell lines for animal xenograft studies is the most physiological means to use cell lines in the study of cancer. There have been numerous reports utilizing TNBC cell lines in mouse xenograft studies to assess efficacy of antitumor treatments. More recently, molecularly targeted agents have been evaluated in xenografts to confirm their *in vitro* effects in an *in vivo* model. For example, a recent paper demonstrated that the anti-diabetes drug metformin induces G1 cell cycle arrest, caspase activation, and apoptosis in triple negative breast cancer cell lines *in vitro* and decreased tumor growth in xenograft studies [87].

Xenografts can also be used to identify genes or pathways that regulate tumor behavior such as metastasis. For example, stable expression of the putative metastasis suppressor gene BRMS1 in the MDA-MB-231 and MDA-MB-435 cell lines results in significantly fewer metastases to the regional lymph nodes and lungs [88]. Recently, MDA-MB-231 cell line was used to identify genes and pathways that regulate metastasis to different sites [89–92]. In these experiments subclones of the MDA-MB-231 cell line that preferentially metastasize either to the bones, brain, or lungs of mice after intraventricular injection were isolated. This has allowed the use of expression profiling to identify genes that are potential mediators of metastasis to specific sites [89–92]. Again, a critical step in evaluating the importance of any gene or gene set in metastasis generated by the use of the cell line is the demonstration that these genes are expressed in tumors in a pattern consistent with the metastasis models. Using

the genes identified for each site of metastasis to create gene signatures, the investigators were able to identify tumors that preferentially metastasized to each site from human tumor samples [89, 91, 92]. These experiments have allowed the investigators to begin to evaluate the function of the genes and the effects of disrupting them. For example, global expression analysis identified high expression of cyclooxygenase 2, heparin-binding epidermal growth factor, and the α2,6 sialotransferase ST6GALNAC5 in the subclones of MDA-MB-231 that preferentially metastasize to the brain [89]. Inhibition or RNAi mediated silencing of these genes decreased the ability of the cell lines to metastasize to the brain in the xenograft model [89]. These experiments are hypothesis generating and provide useful insight into pathways that may mediate metastasis and suggest potential treatments that might be effective in preventing the spread of the TNBC to specific sites. However, their importance must be confirmed in the clinical setting.

Another useful resource for xenograft studies is the MCF10A series of cell lines [33, 42]. MCF10A is a spontaneously immortalized non-tumorigenic cell line derived from a benign breast tissue from a mastectomy specimen from a 36 year old woman with no evidence of breast cancer who had fibrocystic disease [33]. A series of cell lines were derived from MCF10A cells expressing activated H-Ras which are progressively more tumorigenic [42, 93]. These cell lines can be used to study the effects of therapeutics or molecular pathways at different stages of transformation in a relatively constant genetic background.

CONCLUSIONS

Human cancer cell lines have been a useful tool for the study of the genetics, molecular biology, biology, and therapy of cancer in many tumor types, including breast cancer. The TNBC cell lines mirror the original tumors from which they were derived morphologically and molecularly. Thus, they are useful for the study of molecular aberrations in TNBC and the study of the pathways affected by those aberrations. However therapeutic studies in TNBC have not readily translated into clinical results. TNBC and the cell lines derived from them represent a heterogeneous group of tumors. The challenge for the future is to understand the molecular pathways that drive transformation in different subsets of TNBC and then, using cell lines that are driven by the same pathways, to study how to manipulate them.

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Table 1

Human TNBC Cell Lines

Cell line[1]	Site of Origin[2]	Pathology[3]	Grade[4]	Age	Ethnicity	Molecular Classification	P53	BRCA1	PI3K Pathway	Other features
BT20 [94]	PT	IDC	NA[5]	74	Caucasian	Basal A [39, 40]	Mut [95]	Wt [55]	PI3CA mutation [95]	Amplified EGFR [77].
BT-549 [40]	PT	IDC	NA	72	Caucasian	Basal B [39, 40]	Mut [95, 96]	Wt [39, 55]	PTEN homo deletion [95]	
Cal51 [97]	PE	AC	NA	45	NA	Basal B [39]	Wt [98]	NA	PTEN protein not expressed [99]	These cells have been described as luminal in some reports but this classification is not based on global gene expression [100, 101].
HCC 38 [14]	PT	IDC	3	50	Caucasian	Basal B [39, 40]	Mut [15, 40]	Wt [39]	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 1143 [14]	PT	IDC	3	52	Caucasian	Basal A [39, 40]	Mut [40]	Wt [39]	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 1187 [14]	PT	IDC	3	41	Caucasian	Basal A [39, 40]	Mut [40]	Wt [39]	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 1395 [14]	PT	IDC	3	43	Caucasian	Basal B [39]	Mut [15]	Homo Mut [56]	NA	Paired normal cell lines derived from blood leukocytes and breast stroma established [14].
HCC 1599 [14]	PT	IDC	3	44	Caucasian	Basal A [39]	NA	NA	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 1739 [14]	PT	IDC	3	51	Caucasian	NA	NA	NA	NA	Paired normal cell lines derived from blood leukocytes, breast stroma, and breast epithelial cells established [14].
HCC 1806 [14]	PT	ASq	2	60	Black	NA	Mut [15]	Wt [39]	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 1937 [14]	PT	IDC	3	24	Caucasian	Basal A [39, 40]	Mut [15, 95]	Homo Mut [55, 57]	PTEN homo deletion [95]	Paired normal cell line derived from blood leukocytes established [14].

Cell line[1]	Site of Origin[2]	Pathology[3]	Grade[4]	Age	Ethnicity	Molecular Classification	P53	BRCA1	PI3K Pathway	Other features
HCC 2157 [14]	PT	IDC	2	48	Black	Basal A [39, 40]	NA	NA	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 2185 [14]	PE	ILC	2	49	Cauc/Hisp	Luminal [39, 40]	NA	Wt [39]	NA	Paired normal cell line derived from blood leukocytes established [14].
HCC 3153 [14]	PT	IDC	NA	NA	NA	Basal A [39, 40]	NA	Homo Mut [55]	NA	.
Hs578T [20]	PT	IDC	CS	74	Caucasian	Basal B [39, 40]	Mut [95, 102, 103]	Wt [55]	Wt [95]	Paired normal cell lines derived from breast stroma established [20]. HRas mutation [95].
MDA-MB-157 [24, 104]	PE	IMC	NA	44	Black	Basal B [39, 40]	Mut [95]	Wt [39, 55]	Wt [95]	
MDA-MB-231 [24, 105]	PE	AC	PD	51	Caucasian	Basal B [39, 40]	Mut [95, 102]	Wt [39, 55]	Wt [95]	KRas mutation [95].
MDA-MB-435 [23, 24]	PE	IDC	NA	31	Caucasian	Basal B [40]	Mut [95, 102]	Wt [55]	Wt [95]	The original cell line was derived from a patient with breast cancer but recent array based analysis have questioned whether some of the current clones have been contaminated with a melanoma cell line (see discussion) [25, 28, 29, 106].
MDA-MB-436 [23, 24]	PE	IDC	NA	43	Caucasian	Basal B [39, 40]	Mut [95]	Homo Mt [55]	Wt [95]	
MDA-MB-468 [23, 24]	PE	AC	NA	51	Black	Basal A [39, 40]	Mut [95, 107]	Wt [55]	PTEN homo deletion [95]	Amplified EGFR [76]
SUM52PE [16, 19]	PE	IDC	NA		NA	Luminal [39–41]	Mut [95]	Wt [55]	Wt [95]	The primary tumor was ER + and PR+ but the cell line, derived from cells isolated from a pleural effusion after metastatic recurrence, is triple negative [16].
SUM102PT [17, 19]	PT	IDC	NA	56	NA	Basal B [39]	Wt [95]	Wt [55]	PI3CA mutation [95]	Cell line derived from Ductal Carcinma in situ (DCIS) with microinvasion [17, 19]. Chk2 mutation [95].
SUM149PT [19]	PT	IBC	NA		NA	Basal B [39, 40]	Mut [95]	Homo Mt [55]	Wt [95]	Derived from Inflammatory Breast Cancer [19].
SUM159PT [19, 108]	PT	IDC	AP		NA	Basal B [40]	Mut [95]	Wt [55]	PI3CA mutation [95]	Carcinosarcoma features [18]. HRas mutation and PI3CA mutation [95].

Cell line[1]	Site of Origin[2]	Pathology[3]	Grade[4]	Age	Ethnicity	Molecular Classification	P53	BRCA1	PI3K Pathway	Other features
SUM185PE [19]	PE	IDC	AP		NA	Luminal [40]	Mut [95]	Wt [55]	PI3CA mutation [95]	
SUM229PE [19]	PE	IDC	NA		NA	NA	Mut [95]	Wt [95]	Wt [95]	KRas mutation [95]
SUM1315M02 [19]	Sk	IDC	NA		NA	Basal B [40]	Mut [95]	Homo Mt [55]	PI3CA mutation [95]	Myc amplification [95].
184A1 [38]	NB	Normal		21	Caucasian	Basal B [39]	Wt [109]	NA	NA	Derived from cells from reduction mammoplasty exposed to benzo(a)pyrene [38].
184B5 [38]	NB	Normal		21	Caucasian	Basal [41]	Wt [109]	NA	NA	Derived from cells from reduction mammoplasty exposed to benzo(a)pyrene [38].
HBL-100 [34]	NB	Normal		27	NA	Basal B [40]	Wt [40]	NA	NA	Derived from milk from lactating healthy woman [34]. Contains SV40 sequences in genome of cell line [35].
hTERT-HME1 [110]	NB	Normal		53	NA	Basal B [39]	Wt [37]	NA	NA	Mammary epithelial cells from reduction mammoplasty immortalilzed with human telomerase [110].
MCF10A [33]	NB	Fibrocystic		36	Caucasian	Basal B [39, 40]	Wt [40, 111]	NA	NA	Derived from mastectomy for benign disease [33].
MCF12A [32]	NB	Fibrocystic		60	Caucasian	Basal B [40]	Wt [40]	NA	NA	Derived from a reduction mammoplasty [32].

[1] Cell lines shown in white are derived from tumors while those in the yellow highlighted area are cell lines derived from non-malignant tissue. These later cell lines are immortalized but non-transformed.

[2] Site of origin: NB, normal breast; PT, primary tumor; PE, pleural effusion; Sk, skin

[3] Pathology: ASq, acantholytic squamous carcinoma; AC, adenocarcinoma; IBC, inflammatory breast cancer; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; IMC, infiltrating medullary carcinoma.

[4] Where available we have included the grading of the tumor, using the current grading 3 point grading system. Otherwise the grading is as described in the references. AP, anaplastic; CS, carcinosarcoma; PD, poorly differentiated.

[5] NA, not available