Using gene expression data to direct breast cancer therapy: Evidence from a pre-clinical trial

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Introduction

One key characteristic of many cancers, including breast cancer, is substantial intertumor and intratumor heterogeneity since it confounds the diagnosis and treatment plan. Over the past several decades of research, the depth of knowledge has deepened from recognizing the presence of tumor evolution and heterogeneity (1) to characterizing it at the level of variation in mutational aberrations and gene expression patterns that result in altered cell signaling, growth factor deregulation, and distinct metabolic pathways (2). These diverse events impact an array of oncogenic networks within a single tumor. It has long been recognized that such networks provide the leverage to rely on redundant signaling arms necessary for cellular modification to survive and progress under various conditions (3). Therefore, the presence of heterogeneity poses challenges when choosing a therapeutic intervention to eradicate the tumor without development of drug-resistance, metastasis or tumor relapse. Indeed, it is essential to stratify breast cancer at a genomic and molecular level to decipher tumor heterogeneity and develop robust targeting strategies to improve patient outcome. By using a genomic strategy to predict therapy, personalized medicine can be tailored to individual tumors.

Breast Cancer Classification

To address tumor heterogeneity, breast cancer can be broken down into subgroups in terms of histology, molecular biomarkers, genomic and genetic profiles. Based on the status of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) expression in tumors, breast cancer is classified as hormone receptor positive (HR+), HER2+ or triple-negative in absence of all three receptors. Based on tumor morphology, growth and architecture patterns, breast cancer can be classified into distinct histological subtypes. The most common histological subtype is invasive ductal carcinoma, followed by classic lobular invasive carcinoma. The histopathological classification provides prognostic value. In general, tubular or mucinous carcinoma is associated with excellent prognosis while metaplastic carcinoma has an unfavorable outcome. To gain greater insight into the gene expression patterns that underlie the histological, ER/PR/HER status,

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prognostic and outcome features of breast cancer, a study was undertaken where global gene expression patterns were analyzed. This study characterized several so-called intrinsic subtypes of breast cancer based on high/low expression of specific sets of genes. This was refined through several critical manuscripts and evolved to intrinsic subtypes including luminal A, luminal B, HER2-enriched, basal-like, claudin-low and normal-like tumors (4– 10).

Sequencing the genome of breast cancer has revealed less than 100 driver mutations, most in genes that have previously been widely studied (11). This is consistent with prior reports with several genes that are frequently mutated with other supporting genes that are less commonly altered (12). However, the juxtaposition of sequence data with the intrinsic subtypes reinforces a critical need in breast cancer. That is to say, the ER/PR/HER2-ve (triple negative breast cancer or TNBC which largely overlaps with the basal subtype of breast cancer lacks oncogenic drivers at a high frequency. This is an urgent problem given that on average these tumors have the worst prognosis in breast cancer and typically affect younger women. However, it should be noted that when the subtypes of TNBC are stratified, there are clear distinctions in survival between the various subtypes of TNBC (13, 14).

Targeted therapy for HR+ breast cancer

Approximately 70% of breast cancers are identified as hormone receptor positive (HR+. +) and most of these tumors can be divided into Luminal A and B intrinsic subtypes. Endocrine therapy is the mainstay of treatment for all women with HR+ and is centered around targeting estrogen receptor to suppress its downstream functions. There are three main HR targeting strategies: inhibiting estrogen binding to ER by using selective estrogen receptor modulators such as tamoxifen; degrading ER by using selective estrogen receptor degraders such as fulvestrant; or depleting estrogen production by using aromatase inhibitor or ovarian suppression. The HR+ tumors can be further divided into luminal A and B intrinsic subtypes. Morphologically, luminal A tumors are well differentiated carcinomas and include classical lobular carcinoma, tubular carcinoma, and mucinous carcinoma(15). Tumors of luminal B subtype are less differentiated and consist mostly of invasive ductal carcinoma. Despite the effectiveness of endocrine therapy in HR+ breast tumors, treatment challenges remain in clinical practice. Clinical studies reported that luminal A tumors are more sensitive to therapy while luminal B tumors more often exhibit primary (intrinsic) resistance and may not respond to endocrine therapy alone (16). Conversely, luminal A tumors may respond to single endocrine therapy initially but may recur later due to secondary (acquired) resistance (17). To counteract primary and secondary endocrine resistance, combination strategies of endocrine therapy with cyclin-dependent kinase (CDK) 4/6 inhibitors or PI3K/ mTOR/Akt pathway inhibitors have been evaluated. These inhibitors in combination with endocrine therapies have improved progression free survival and disease free survival in women with metastatic breast cancer (18–21). The success of these clinical trials illustrate the need to develop selective targeted therapies based on tumor mechanisms to utilize combinatorial approaches to effectively treat tumors.

Targeted therapy for HER2 positive breast cancer

HER2 is a member of the epidermal growth factor receptor family and is well recognized for its role in cellular differentiation and proliferation. HER2/neu amplification or overexpression is noted in 20% of human breast cancer, and was first observed to be an independent prognostic factor in lymph node positive breast cancer patients (22). Historically it has been seen as a negative prognostic marker associated with increased risk or recurrent disease and worse prognosis. A key mechanism for studying the role of HER2 was the generation of transgenic mice expressing activated c-neu under the control of the mouse mammary tumor virus (MMTV) promoter enhancer. These mice developed multifocal mammary tumors after a brief latency (23).

The development of a humanized HER2/neu monoclonal antibody, trastuzumab, resulted in the first FDA approved targeted therapy for HER2 positive breast cancer patients(24). Multiple clinical trials demonstrated that trastuzumab treatment for the HER2+ breast cancer patients reduced the recurrence risk and improved overall survival (25–27). Currently, there are several HER2 targeting therapeutics utilized in clinical settings: monoclonal antibodies such as trastuzumab and pertuzumab, which bind to the extracellular domain of HER2; tyrosine kinase inhibitors such as lapatinib and neratinib, which bind to the intracellular portion of protein kinase domain of HER2; and toxic drug conjugates such as T-DM1, in which a cytotoxic molecule is linked to the HER2 antibody (28–30). Dual HER2 targeting therapy, a combination of chemotherapy with monoclonal HER2 antibodies, and a combination of endocrine therapy with HER2 targeted agents have been proven to be successful in overcoming breast cancer resistance. HER2+ breast cancer is an excellent example of how identification of a molecular target can lead to effective treatment and improved patient outcomes.

Triple Negative Breast Cancer

TNBC lacks expression of ER, PR, and HER2 receptors, accounts for 10–20% of all reported breast cancer, and largely overlaps with the basal intrinsic subtype. Epidemiologically, TNBC patients are over-represented for young (<50 years), African American and Hispanic women (31). Morphologically, most TNBCs are invasive ductal carcinoma; others include metaplastic carcinoma, carcinoma with medullary features, adenoid cystic carcinoma, and secretory carcinoma (B). Compared to other intrinsic subtypes, TNBC has been clinically observed to be aggressive in nature based on a high grade, advanced stage at diagnosis and a higher risk of metastasis. These tumors tend to recur within three years of diagnosis and are associated with increased mortality within 5 years, resulting in the worst prognosis of all subtypes (32). According to gene expression patterns, TNBC can be subdivided into basal-like subtype, claudin-low breast cancer subtype, and luminal androgen receptor subtype. From a therapeutic standpoint, the basal subtype is most sensitive to chemotherapy while the luminal androgen receptor subtype is least sensitive. Thus, the lack of targeted therapy in TNBC, coupled with the poor outcome and drawbacks of chemotherapy illustrate a critical need to identify the driving factors of TNBC and adapt a personalized medicine approach to effectively treat these patients.

The carriers of BRCA1 germline mutation are more likely to develop TNBC (33). Tumors with BRCA mutations are deficient in homologous recombination repair (HRR). As such, these BRCA mutated TNBC rely on poly (ADP-ribose) polymerase 1 (PARP-1) for single strand DNA break repair, and therefore DNA damaging agents, such as platinum chemotherapy or PARP inhibitors, can induce synthetic lethality in HRR deficient cancer cells. Indeed, PARP inhibitor, olaparib, is currently FDA approved for the treatment for relapsed BRCA-mutated ovarian cancer (34). A recent phase III study reported increased progression free survival in metastatic breast cancer patients with a germline BRCA mutation treated with olaparib as compared to standard therapy (35). Clinical trials of PARP inhibitors in early stage breast cancer with germline BRCA mutation are ongoing.

TNBC is associated with a high number of tumor infiltrating lymphocytes (TILs) and indicate better sensitivity to chemotherapy (36). Furthermore, patients with high TILs may benefit from therapeutics that enhance antitumoral immune responses. Interest in the combination of chemotherapy with immune checkpoint inhibitors is growing and is reflected in a number of active clinical trials.

Luminal androgen receptor (LAR) breast cancer subtype is characterized by luminal gene expression. Preclinical models demonstrated that tumor growth is driven by androgen pathway activation and is estrogen independent (37). The androgen receptor (AR) is an emerging target in TNBC treatment. Androgen blockade in AR+ ER- PR- HER2- breast cancer is being evaluated in clinical trials.

The claudin-low breast cancer subtype is enriched with epithelial to mesenchymal transition (EMT) markers, stem-like features, and immune cell infiltration (38). Currently, there is no targeted treatment shown to be effective in this tumor subtype and preclinical studies are needed to identify actionable targets.

Current Directions

Models for breast cancer

To study breast cancer in a mammalian system, numerous murine models have been developed. The mouse mammary tumor virus (MMTV) promoter enhancer and the Whey Acidic promoter (WAP) have been widely used to drive the expression of specific gene in the mammary epithelium of transgenic mice. These promoters have been used to establish well-known oncogenic mouse models such as MMTV-Myc (39), MMTV-Ras (40), MMTV-Neu (23) and many others. Tumor suppressors have been studied in mouse models through mammary specific knockouts using MMTV-Cre, resulting in mammary specific loss of genes including BRCA and p53 (41, 42). Importantly, many models have now been compared to human breast cancer at a gene expression level, reflecting many of the broad patterns observed in human breast cancer (43–45). When gene expression data from these mouse models was used to predict chromosomal gain and loss, conserved events were noted in subtypes of cancer in specific models that mimicked human breast cancer (46).

Although these mouse models have played critical roles in establishing the mechanisms in breast cancer development and progression, their utility is tempered by limitations for

translation to clinical trials. For instance, oncogenes driven by MMTV alleviates the mutation selection pressure and alters natural pattern of genomic stability (46). In addition, human breast cancer routinely metastasizes to liver and bone while most mouse models demonstrate predominately lung metastasis (47). One approach to overcome these restrictions is the use of human breast cancer cell lines. Orthotopic injection of cultured human cell lines in immunocompromised mice allows for *in vivo* tumor growth and can be used for drug screening. However, cell lines maintained in culture accumulate additional mutations and have gene expression patterns divergent from human breast cancer.

Patient-derived xenograft (PDX) model systems use direct implantation of primary human tumors to the mammary fat pad of immunocompromised Nude/SCID mouse. PDX models eliminate *in vitro* culture and resulting tumors have been demonstrated to have strong similarities to the original primary tumor at both a histological and gene expression level (48, 49). Indeed, studies with PDX models have been argued to offer a translatable preclinical platform (50). The availability of gene expression data from the PDX models opens the realm of developing personalized medicine for testing potential drugs under preclinical settings, followed by clinical trials for an accelerated transition to develop individualized therapy.

Preclinical trials for personalized medicine

Given that human breast cancer, mouse model systems and PDX tumor samples have extensive gene expression data that has been deposited in public repositories, there is now an opportunity to use the various model systems in preclinical trials. Importantly, the gene expression data allows one to ensure that similar mechanisms to those used in human breast cancer can be tested. We have used a method that predicts pathway activation status from gene expression data to make predictions in both mouse and human. Essentially this method uses gene expression data from training datasets where pathways were activated in human mammary epithelial cells in comparison to non-activated controls. Once signaling pathway signatures were built and validated (13, 51, 52), they could be used to interrogate any breast cancer gene expression dataset. This method has previously been used to make cell signaling pathway predictions in both human breast cancer (13, 52–55) and mouse models (43, 56–62).

Recently we used signaling pathway signatures to predict signaling pathway activation status in MMTV-Myc transgenic mice. While we previously noted that Myc induced tumors with similar histological subtypes shared gene expression patterns (57) and chromosomal gain or deletion events (46), in our recent work we noted key differences in signaling pathways between papillary and epithelial to mesenchymal transition (EMT) tumors (63). Indeed, Myc, Stat3 and AKT signaling were predicted to be strongly activated in papillary tumors while EGFR, RAS and TGFβ activity was upregulated in the EMT tumors. Importantly, as a control we use immunoblotting to validate the gene expression based pathway predictions for each signaling pathway.

To begin to demonstrate the preclinical application of personalized medicine, we sought to use pathway signature predictions to direct therapeutic design, initially in the MMTV-Myc mouse model system (Figure 1). By selecting therapeutic compounds that are already FDA

approved or under various phases of trials for individual signaling pathway, we sought to accelerate the pace for potential transition to clinic. The proof-of-concept experiment demonstrated that combination therapy of three drugs simultaneously targeting the Myc, Stat3, and Akt pathways inhibited papillary tumor growth compared to vehicle controls in a dose dependent manner (63). Importantly, the same combination therapy that prevented papillary tumor failed to arrest growth of EMT tumors, emphasizing the importance of designing treatment regimen based on gene expression patterns for specificity.

In the described experiment, we noted the initial response of the papillary tumors to combination treatment. However, resistance quickly developed and tumors began to grow. Examining global patterns of gene expression from these resistant tumors with signaling pathway signatures, we predicted elevated probabilities of EGFR and Ras pathway activation. This was confirmed through phosphor-ERK immunohistochemistry, and we speculated that the cause of recurrence perhaps is not drug resistance but rather a clonal selection from tumors that were initially heterogenous due to the speed that resistance emerged. With the regrowth of the tumors with other pathways that were targetable, our findings suggest that a repeated biopsy in breast cancer patients to examine tumor cells for changes in characteristics following courses of treatments may provide critical information needed for subsequent treatment decisions.

In much the same manner as the papillary specific treatment, we designed a three drug combination to target EMT tumors arising in the transgenic mice. Targeting EGFR, Ras, and TGFB pathways in combination prevented EMT tumor growth compared to both vehicle control treatment in papillary tumors, again demonstrating that gene expression information could direct an effective and specific therapy. The EMT experiment was critical since prior comparisons to human cancer revealed a similarity of the EMT mouse tumors to the TNBC / Claudin low subtype (43).

To extend the signaling pathway probability method to treating human breast cancer, we applied the same signaling pathway predictions to a collection of human breast cancer samples from numerous datasets and included PDX samples. Limiting the predictions to the basal subset of breast cancer it was readily apparent that many PDX samples were highly similar from the standpoint of which cell signaling pathways were active (63). Choosing several PDX lines, including one that was similar to the MMTV-Myc EMT samples, we implanted the PDX samples into the mammary fat pad and initiated treatment once the tumors reached 6 mm. In a critical finding, we noted that cell signaling pathway inhibitors that were chosen based on gene expression data were capable of shrinking the PDX tumor samples. Together these data demonstrated that gene expression data from both mouse models and human breast cancer samples could be interrogated to predict the optimal therapy. Together these pre-clinical experiments demonstrated that a gene expression based approach could be used to direct effective and specific therapeutic interventions.

5. Future Directions

While there are numerous ongoing studies where molecular profiles are used to direct therapy, the future of personalized medicine in cancer will be tied to information gathered from individual patient tumors. Indeed, the transition to the clinic in the NCI-MATCH trial

(https://www.cancer.gov/about-cancer/treatment/clinical-trials/nci-supported/nci-match) has already begun and is reliant upon genomic sequencing of individual tumors to choose drugs, many of which are already FDA approved. In the future, a combination of standard biomarkers (ER/PR/HER2), as well as genomic sequence and gene expression information will provide an opportunity to tailor treatment for each individual breast cancer patient. A future challenge is to develop effective combination strategies. Further preclinical studies may identify resistance mechanisms of targeted therapies and inform clinical trial design of rationale combination strategies.

Acknowledgments

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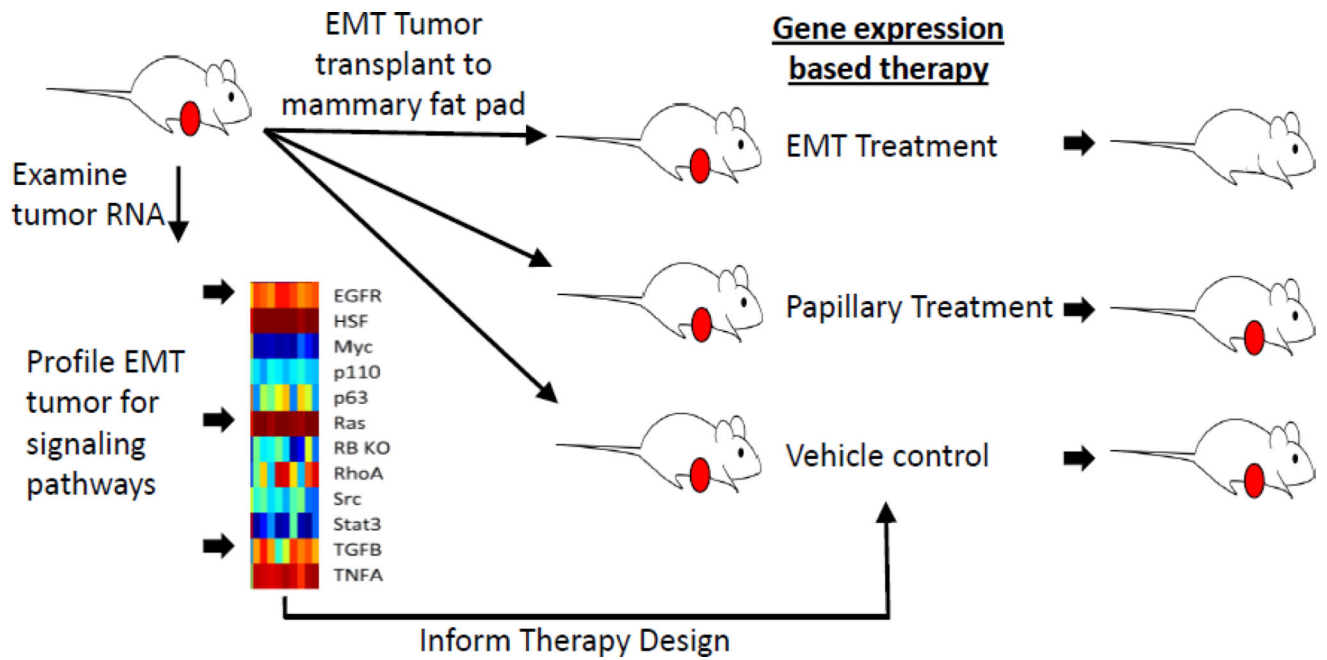
Figure 1. Summary of personalized therapy design
Using the MMTV-Myc mouse model, EMT and Papillary tumors were used in a proof of principle experiment to predict therapy. For all tumors RNA was collected and gene expression was surveyed. Signaling pathway predictions (red on, blue off) for key signaling pathways were calculated based on gene expression signatures. EMT tumors were then transplanted to the mammary glands of syngenic mice. Once tumors reached 6 mm in the largest dimension, therapy for EMT, control therapy based on papillary tumors and vehicle control therapy was initiated in each cohort of mice. EMT treatment was found to be effective and specific for EMT tumors. Papillary treatment was also shown to be effective and specific for papillary tumors (not shown).