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Determinants of Breast Cancer Progression

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Abstract

New therapeutic opportunities may arise as a result of discovering the determinants of breast cancer progression.

Breast cancer is the most common malignant disease in women; it begins as a local disease and later metastasizes to lymph nodes and other organs. Whereas surgery, chemotherapy, and radiotherapy can successfully control many primary tumors, a major barrier in eradicating the death and suffering from breast cancer is the shortage of clinically proven prognostic markers and therapeutic agents for metastasis. Another obstacle is the lack of targeted therapies for certain tumor subtypes, such as triple-negative breast cancer. Here, I describe the discovery of several determinants of breast cancer progression (Fig. 1A) and discuss how they advanced our understanding of breast cancer and hold promise for the development of future therapies (Fig. 1, B to D).

MICROMANAGERS OF BREAST CANCER METASTASIS

MicroRNAs (miRNAs) are endogenously expressed, small noncoding RNAs that negatively regulate the expression of their target genes. Over the past decade, numerous studies have revealed oncogenic and tumor-suppressing miRNAs, collectively termed “oncomirs.” Moreover, specific metastasis-regulating miRNAs, collectively termed “metastamirs,” have been found to be either positively or negatively associated with metastasis.

By comparing miRNA expression in normal mammary epithelial cells nonmetastatic breast cancer cells and metastatic cells, we identified miR-10b as a metastasis-regulating miRNA and provided functional evidence that overexpression of a specific miRNA can lead to the development of breast cancer metastasis (1). Mechanistically, expression of miR-10b is activated by the epithelial-to-mesenchymal transition (EMT)–inducing transcription factor Twist, and this miRNA down-regulates *HOXD10*, permitting expression of the prometastatic protein RhoC (1). Subsequently, a number of groups reported that miR-10b is overexpressed in highly metastatic or invasive human tumors (2). Furthermore, we reported the systemic delivery of miRNA inhibitors as antimetastatic therapeutics, showing that the miR-10b antagomirs can block metastasis in a mouse mammary tumor model (Fig. 1B) (3).

In addition, we identified miR-9 as an EMT-inducing miRNA that promotes metastasis by targeting E-cadherin (4). Together, these studies demonstrated the existence of metastasis-promoting miRNAs for the first time and represented the initial step in the development of miRNA-based, antimetastatic therapeutic agents.

A METASTASIS SUPPRESSOR THAT ACTIVATES HIPPO SIGNALING

Deregulation of signaling pathways that control organ size, such as the Hippo-YAP pathway, can lead to tumor formation and metastatic progression. As the core component of mammalian Hippo signaling, MST (the mammalian Hippo homolog) phosphorylates and activates LATS, and the LATS kinase in turn phosphorylates two mammalian Yorkie homologs, YAP and TAZ, leading to cytoplasmic retention and functional inactivation of these two transcriptional coactivators (5). Genetic deletion of *Mst1/2* or transgenic overexpression of *Yap* in mice increased the liver size and ultimately induced hepatocellular carcinoma, demonstrating a critical role of Hippo signaling in organ growth and tumorigenesis (5).

Several upstream regulators—including the cell polarity complex protein NF2 (Merlin), the tight junction-associated protein Angiomotin, the adherens junction protein α -catenin, and the scaffold protein Scribble—are known to provide inputs feeding into the core pathway (5). However, the cell membrane receptors that activate the Hippo kinase cascade are elusive. Although G-protein-coupled receptors have been found to regulate LATS and YAP phosphorylation, they do not regulate MST (6).

Combining high-throughput RNA sequencing, functional characterization, mechanistic studies, and clinical validation, we identified leukemia inhibitory factor receptor (LIFR) as a breast cancer metastasis suppressor downstream of miR-9 and upstream of Hippo signaling (7). Our data revealed that LIFR promotes cell membrane recruitment of the adaptor molecule Scribble, which in turn bridges MST, LATS, and YAP and facilitates this phosphorylation cascade (7). We also found that the LIFR co-receptor gp130 mediates LIFR-induced membrane localization of Scribble and activation of Hippo signaling (7).

In functional studies, restoring LIFR expression in highly malignant tumor cells suppressed metastasis by triggering the Hippo kinase cascade that leads to inactivation of YAP. Conversely, loss of LIFR in nonmetastatic breast cancer cells induced migration, invasion, and metastatic colonization through activation of YAP and the YAP target CTGF (connective tissue growth factor) (7). LIFR is down-regulated in human breast carcinomas and inversely correlates with metastasis. The loss of LIFR protein in nonmetastatic stage I to III breast tumors was highly associated with poor metastasis-free, recurrence-free, and overall survival outcomes in ~1000 patients (7). On the basis of these findings, we envision that by delineating the appropriate cutoffs, detection of LIFR could be implemented in the clinic as a prognostic marker of metastatic relapse, and that targeting miR-9–LIFR–Hippo–YAP signaling represents a potential therapeutic strategy (Fig. 1C).

Currently, we are investigating whether deletion of *Lifr* in the breast, liver, and other tissues will lead to enlarged organ size, tumorigenesis, and metastasis. This work will provide new insights into the role of LIFR in regulating Hippo signaling, tumor formation, and metastatic progression.

DEUBIQUITINATING ENZYMES AS ANTICANCER TARGETS

The addition of ubiquitin by ubiquitin ligases can target a protein for degradation via the proteasome, alter its subcellular localization, affect its activity, or modulate protein interactions. The human genome encodes 79 functional deubiquitinating enzymes (DUBs, or deubiquitinases), a group of proteases that regulate ubiquitin-dependent pathways by cleaving ubiquitin-protein bonds (8). U.S. Food and Drug Administration approval of the proteasome inhibitor bortezomib for treating multiple myeloma demonstrated that the proteasome can be targeted for cancer treatment (8). However, extended treatment with bortezomib is associated with drug resistance and toxicity. Therapeutic strategies targeting ubiquitin ligases and DUBs are predicted to be better tolerated (8).

Much attention has been paid to the role of ubiquitin ligases in tumorigenesis, whereas the functions of DUBs in cancer are just beginning to be revealed. As enzymes, DUBs are considered drug-targetable. However, no DUB inhibitors have entered clinical trials. A main challenge is to identify DUBs that represent key players and druggable targets in human cancer.

A full-coverage DUB open reading frame (ORF) library is lacking. To overcome this obstacle, we collected more than 90 DUB ORFs and cloned each ORF into a vector containing a triple-epitope tag, SFB (S-protein, FLAG tag, and streptavidin-binding peptide). So far, 68 DUBs have been validated by means of DNA sequencing and immunoblotting. Eventually, our library will contain all 79 functional human DUBs. The use of SFB triple-tagged protein allows the identification of its interactors with high fidelity.

The tumor suppressor PTEN (phosphatase and tensin homolog) is frequently lost in human cancer. Whereas ~5% of sporadic breast tumors harbor *PTEN* mutations, loss of PTEN protein is found in nearly 40%, indicating that posttranscriptional and post-translational regulation of PTEN may contribute substantially to the development of human breast cancer. Several ubiquitin ligases can target PTEN for degradation (9). On the other hand, reversal of the mono-ubiquitination of PTEN by USP7 regulates PTEN subcellular localization without affecting its protein level (9). However, the deubiquitinase that regulates PTEN polyubiquitination and protein stability was unknown.

We used two alternative screening approaches to identify the PTEN DUB. First, we cotransfected each of 30 SFB-tagged DUBs with PTEN into 293T cells, and then we pulled down the DUB and performed immunoblotting to detect PTEN. Second, we performed tandem affinity purification and mass spectrometric analysis of SFB-tagged PTEN-containing complexes. These two approaches identified the same set of five PTEN-associated deubiquitinases (10). Only one, USP13, stabilizes PTEN protein via direct binding and deubiquitination of PTEN (10). Loss of USP13 in breast cancer cells promotes AKT phosphorylation, glycolysis, and tumorigenesis through down-regulation of PTEN. Conversely, overexpression of USP13 suppresses tumor growth and glycolysis in PTEN-positive but not PTEN-null tumor cells (10). USP13 protein is down-regulated in human breast tumors and correlates with PTEN protein levels (10).

Thus, we identified USP13 as a DUB that regulates the polyubiquitination and protein stability of PTEN. Currently, we are investigating the physiologic functions of USP13 and how USP13 expression is lost in cancer. The likely keys to possible treatment include identifying druggable oncoproteins that are responsible for loss of USP13, or hitting targets controlled by PTEN (for example, by using an AKT inhibitor) (Fig. 1D).

The USP13 study paved the way for subsequent DUB research. We will use our DUB library to perform interaction screening for DUBs that deubiquitinate and stabilize key oncoproteins that are considered “non-druggable” and, in parallel, gain-of-function screening for DUBs that promote oncogenic transformation of human mammary epithelial cells. Once we identify oncogenic DUBs that represent therapeutic targets, especially in triple-negative breast cancer, we will develop small-molecule DUB inhibitors by using high-throughput, high-fidelity assays (Fig. 1D) (8). This will enable the rapid determination of selective inhibitors of DUBs and expedite the progress of lead compounds to the clinic. DUB inhibitors may emerge as new cancer drugs in the near future.

CONCLUSION

Cancer is a broad group of diseases, each with its own distinct, complex underlying mechanisms. Therefore, no single break-through will cure all cancers; instead, there will be many steps that will collectively lead to the reduction of cancer-related deaths. Identification of the determinants of tumor progression will facilitate implementation of innovative biomarker-driven clinical trials and personalized therapy for breast cancer and other cancer types.

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Fig. 1. Determinants of breast cancer progression and implications for therapeutics

(A) Functional involvement of USP13, miR-10b, miR-9, and LIFR in different stages of breast cancer progression. (B) Therapeutic silencing of miR-10b as an antimetastatic strategy. (C) Antimetastatic therapeutic strategies that target the miR-9–LIFR–Hippo–YAP signaling pathway. miR-9 antagonists and LIFR ligands (such as LIF) could be used for tumors with down-regulation but not complete loss of LIFR, whereas agents targeting the functional YAP targets (such as CTGF) may be useful for LIFR-negative tumors. LIF (rhLIF, AM424, emfilmermin) is a therapeutic agent tested in phase 1 and phase 2 clinical trials to treat neuropathy. A CTGF-neutralizing antibody (FG-3019) has been tested in phase 1 and phase 2 clinical trials to treat fibrosis and metastatic pancreatic cancer. (D) DUBs or their downstream signaling components as druggable targets.