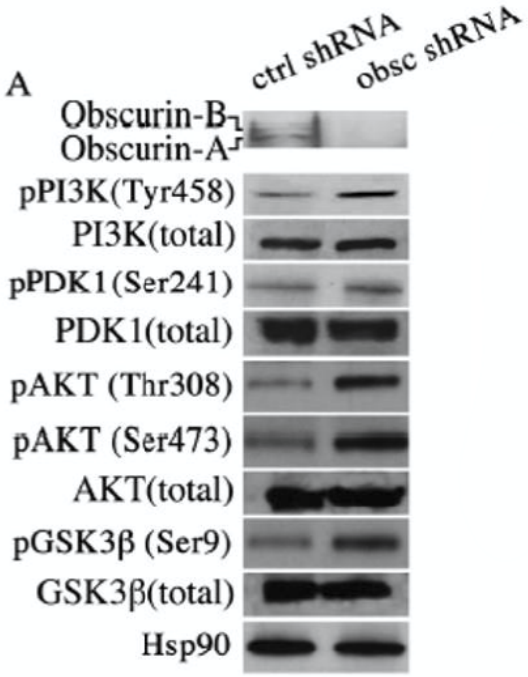
TECHNOLOGY

Generation of a Biomarker Kit with Prognostic Capability for Metastatic Breast Cancer

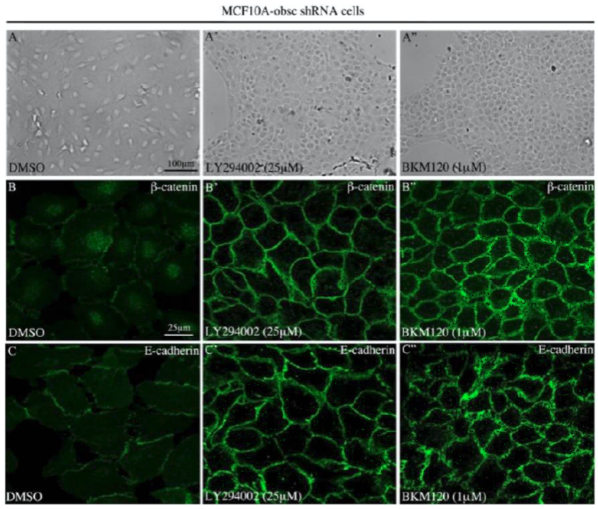
OVERVIEW

Obscurins are a family of giant cytoskeletal proteins primarily localized at cell-cell junctions of breast epithelial cells. While abundantly expressed in healthy breast epithelial tissue, their expression is dramatically decreased in tissue from advanced stage breast cancer biopsies (grade 2 or higher) compared to matched normal samples. Moreover, the subcellular distribution of residual obscurins is also altered from membrane to cytosolic, suggesting a role in tumor and metastasis suppression. In support of this, knockdown of obscurin A and B in non-tumorigenic MCF10A cells (via shRNA) induces epithelial to mesenchymal transition (EMT), and enhances cell survival, growth, migration, and invasion, as shown by the ability of the cells to form mammospheres enriched with markers of stemness, develop apoptosis resistance to common chemotherapies and anoikis, and develop primary tumors and lung metastases. These tumorigenic and metastatic effects of the loss of obscurins are associated with increased increased activation of the phosphatidyllnositol-4,5-biphosphate 3-kinase (PI3K) cascade. This signaling cascade is commonly overactivated in several types of cancer, including breast cancer.

PI3K is a lipid kinase that phosphorylates phosphatidyl inositol 4,5-biphosphate (PIP2) to generate phosphatidyl inositol 3,4,5-triphosphate (PIP3), a secondary messenger that activates AKT kinase. AKT kinase activation regulates many genes involved in cell proliferation, apoptosis, motility and invasion. Obscurins act upstreat of the PI3K pathway by directly interacting via their pleckstrin homology (PH) domain with the Src Homology 3 (SH3) domain present in the regulatory component of PI3K, therefore affecting the modulation of the enzymatic activity of its catalytic component. To assess the role of obscurin and PI3K signaling in breast cancer progression and metastasis, the Kontrogiani-Konstantopoulos team assayed the expression levels and phosphorylation state of major components of the PI3K pathway in MCF10A cells with obscurin knockdown. In summary, they found a considerable increase in: 1) phosphorylated PI3K at tyrosine-458, a phosphorylation site found to track with the activation levels of the enzyme; 2) phosphorylated PDK1 at serine-241, a downstream target of PI3K at a site that renders the enzyme catalytically active; 3) phosphorylated AKT, a direct target of PDK1, at threonine-308 and serine-473, indicating its maximal activation, and; 4) phosphorylated GSK3?, a downstream target of AKT, at serine-9, leading to its inactivation, therefore promoting cell cycle progression (Figure 1).


A
ctrl shRNA
obsc shRNA
Obscurin-B
Obscurin-A
pPI3K(Tyr458)
PI3K(total)
pPDK1(Ser241)
PDK1(total)
pAKT (Thr308)
pAKT (Ser473)
AKT(total)
pGSK3β (Ser9)
GSK3β(total)
Hsp90

Pharmacological inhibition of the PI3K cascade in MCF10A obscurin knockdown cells reverses EMT and restores adherens junctions (Fig. 2). Consistent with this, treatment of cells with the LY294002 (0-25 ?M) or BKM120 (0-1 ?M) PI3K inhibitors dose-dependently decreased the levels of the mesenchymal transcription factors Slug and Twist as well as their downstream target N-cadherin, a mesenchymal structural protein. The same treatment also induced a concomitant increase in the amounts of the epithelial proteins E-cadherin and ?- catenin. Moreover, PI3K signaling inhibition in obscurin knockdown MCF10A cells also decreases their migratory ability by affecting migration speed and directionality as well as their invasive potential. These data suggest that alterations in the expression profile of obscurins, PI3K, and E/N cadherins, four functionally related genes, in human tumor biopsies can be used as a set of biomarkers that can predict metastatic propensity.



APPLICATIONS

To date, the American Society of Clinical Oncology recommends testing for biomarkers to guide the treatment of metastatic breast cancer, specifically testing for estrogen and/or progesterone receptor (ER/PR) and human epidermal growth factor receptor 2 (HER2). These biomarkers are primarily used to guide treatment with targeted therapies. The discovery of four functionally-related proteins as a biomarker set with prognostic capability for the metastatic potential of breast cancer may serve to guide individualized therapeutic decision-making. For instance, obscurin-depleted breast epithelial cells survive taxane treatment and still exhibit reattachment capabilities. Thus, taxane treatment may not be optimal for patients that are lacking obscurins, instead PI3K inhibitors may be more effective. Currently, there are multiple inhibitors of the PI3K pathway being tested in clinical trials (Xa, Am J Hematol Oncol, 2015). This set of prognostic biomarkers could potentially be used alongside an approved therapy targeting this pathway. In addition, robust, well-validated clinical markers are increasingly needed at a time when more than 90% of oncological drugs that enter clinical development fail to demonstrate therapeutic benefit in clinical trials. A prognostic biomarker or set of biomarkers may assist the selection of the patient population of a clinical trial, minimizing the risk of failure. Furthermore, the identification of new molecules involved in EMT, such as obscurins, reveals potential new therapeutic targets for the treatment of breast cancer and preventing its progression.

Today, there are three major prognostic biomarkers for metastatic/invasive breast cancer in the market- Oncotype DX (Genomic Health Inc), Mammaprint (Agendia) and Prosigna, launched to market in 2004, 2007, and 2013, respectively. Together, these three tests assay 125 biomarkers, 12 of which are shared in two of them, and none shared across all three. All three tests are priced at approximately $4,000 and are reimbursed by Medicare. Genomic Health holds 55% of the US invasive breast cancer market share. They reported $325 million in revenue in 2016, for a yearly revenue growth of $40 million. With 246,660 new cases of invasive breast cancer diagnosed in the US in 2016, and three key players in the field with non-overlapping targets, the prognostic biomarker market is one with ample opportunity for growth.

ADVANTAGES

- Identifies novel targets for metastatic breast cancer therapies
- Identifies set of biomarkers that may guide treatment options

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Additional Information

INSTITUTION

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PATENT STATUS

US Patent, pending

LICENSE STATUS

Available for licensing and commercialization

CATEGORIES

- Biomarker

INVESTIGATOR(S)

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EXTERNAL RESOURCES

- Loss of giant obscurins alters breast epithelial cell mechanosensing of matrix stiffness.
- Giant obscurins regulate the PI3K cascade in breast epithelial cells via direct binding to the PI3K/p85 regulatory subunit.
- Loss of giant obscurins from breast epithelium promotes epithelial-to-mesenchymal transition, tumorigenicity and metastasis

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