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The PREX1/Rac signaling axis: Potential as a biomarker and therapeutic target in breast cancer

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PREX1 is a Rac guanine exchange factor that coordinates signaling inputs from G protein-coupled receptors and receptor tyrosine kinases (RTKs). PREX1 creates a positive feedback loop to drive RTK, phosphatidylinositol 3-kinase (PI3K)/AKT, and MEK/ERK signaling. High PREX1 levels predict sensitivity to PI3K inhibitors in breast cancer cells.

The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is the most frequently mutated pathway in cancer, promoting the growth, survival, and migration of cancer cells. Understanding PI3K pathway regulation will promote the development of more effective targeted therapies.

We identified the guanine exchange factor (GEF) phosphatidylinositol-3,4,5-trisphosphate (PIP_3)-dependent Rac exchange factor 1 (PREX1) in a mass spectrometry-based proteomic screen to detect proteins that are modulated by PI3K activation and inhibition.[1] PREX1 has been implicated in breast cancer cell migration and metastasis.[2] PREX1 protein levels were decreased by loss of PTEN (which activates PI3K signaling) and increased by pharmacologic inhibition of PI3K in breast cancer cells. In human breast tumors, PREX1 mRNA and protein levels were inversely correlated with PI3K pathway activation, suggesting negative feedback regulation of PREX1 expression. Inhibition of AKT did not alter PREX1 levels, suggesting that PREX1 is regulated downstream of PI3K but upstream of AKT. Interestingly, we also found that PREX1 protein and mRNA levels are higher in estrogen receptor-positive (ER+) breast tumors compared to ER− breast tumors and any other cancer type. Thus, PREX1 expression may be specific for ER+ breast cancer.

In a series of gain- and loss-of-function experiments we showed that PREX1 activates PI3K/AKT, MEK/ERK, and insulin/insulin-like growth factor-1 receptor (InsR/IGF-1R) signaling in ER+ breast cancer cells (**Fig. 1**).[1] PREX1 activates Rac GTPases[3], which in turn activate Pak/Raf/MEK/ERK signaling[4] and the

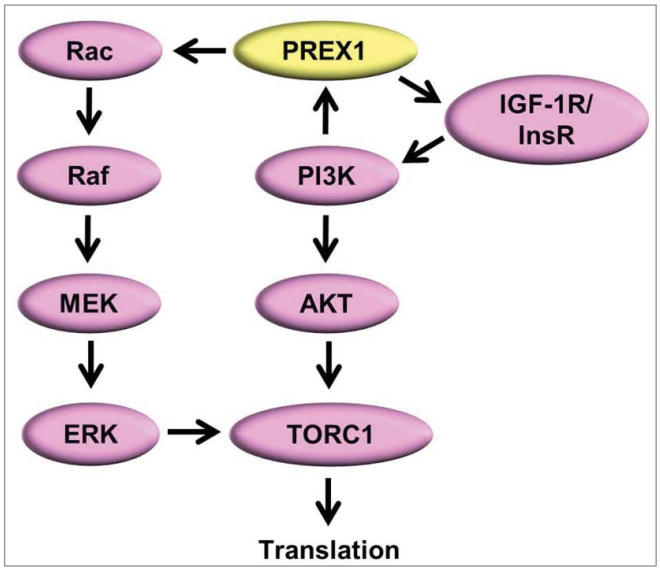

Figure 1. PREX1 signaling in ER+ breast cancer cells. Receptor tyrosine kinases activate PI3K, which produces the phospholipid PIP_3. G protein-coupled receptors generate Gβγ (not shown). PREX1 binds PIP_3 and Gβγ and activates Rac, which in turn activates Raf/MEK/ERK signaling. PREX1 also activates IGF-1R/InsR by an unknown mechanism. PIP_3 induces AKT activation, which ultimately drives activation of the mTOR-containing TORC1 complex and protein translation. There is crosstalk between the PI3K/AKT and Rac/Raf/MEK/ERK pathways at several levels, including convergence on TORC1. ER, estrogen receptor; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; IGF, insulin-like growth factor; mTOR, mechanistic target of rapamycin.

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p110β isoform of PI3K.[5] PREX1 increased the growth and viability of breast cancer cells *in vitro* and in mouse xenografts. Our results collectively show that PREX1 levels are regulated by PI3K activity and that PREX1 activates PI3K/AKT, MEK/ERK, and growth factor receptor signaling, placing PREX1 both upstream and downstream of PI3K in a positive feedback loop to activate oncogenic signaling in breast cancer.

The PREX1 homolog PREX2a binds and inhibits PTEN, which activates PI3K signaling.[6] In contrast, we found that PREX1 did not alter PTEN phosphatase activity;[1] in fact, overexpression of PREX1 in intrinsically PTEN-deficient breast cancer cells (ZR75-1 and MDA-MB-415) increased AKT and MEK/ERK phosphorylation, whereas PREX1 knockdown reduced levels of phospho-AKT in ZR75-1 cells. These findings show that PREX1 activates PI3K/AKT and MEK/ERK signaling independently of PTEN, and that PREX1 and P-REX2a serve non-redundant functions in breast cancer cells.

The PI3K/AKT and MEK/ERK pathways are major oncogenic drivers, and combined targeting of these pathways is a promising therapeutic strategy that is being tested in the clinic. PREX1 activated both of these pathways, and PI3K inhibition (which blocks PREX1 activation) suppressed Rac/MEK/ERK activation in PREX1-expressing cells. Similarly, pharmacologic inhibition of Rac suppressed PI3K/AKT/mTOR and MEK/ERK signaling.[1] Will et al. recently reported that transient inhibition of ERK signaling by treatment with a PI3K inhibitor is sufficient to trigger apoptosis.[7] We and others have found that PREX1 levels predict sensitivity to PI3K inhibition in cell lines.[1,8] We speculate that PREX1 might be a biomarker predicting sensitivity to PI3K inhibitors because PREX1 places ERK under the control of Rac, and PI3K inhibition disrupts ERK signaling. Thus, PREX1 expression may predict tumor sensitivity to PI3K inhibition in patients. Furthermore, Rac may be a viable therapeutic target in ER+ breast cancer.

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