Uncovering a Tumor Suppressor for Triple-Negative Breast Cancers

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"Triple-negative" breast cancers are aggressive malignancies that respond poorly to treatments. Now Sun et al. (2011) find that the activity of the protein tyrosine phosphatase PTPN12 is lost in a large percentage of this breast cancer subtype, offering molecular drivers and possible therapeutic targets for this heterogeneous and intractable cancer.

The molecular characterization of breast cancer subtypes has lead to significant progress in treating this common disease. Tumors that express the estrogen receptor (ER) or progesterone receptor (PR) respond well to ER antagonists or aromatase inhibitors (which block estrogen synthesis), whereas HER2-positive tumors are effectively treated with a HER2-blocking antibody (Trastuzumab) or a HER2 kinase inhibitor (Trikerb).

However, 15%–20% of breast cancers fall through the cracks of this classification scheme. Called "triple-negative" breast cancers, these cells lack detectable expression of the three key molecular signatures: ER, PR, and overexpressed HER2. Triple-negative breast cancers have the worst prognosis among all breast cancers and the fewest treatment options. Because these cancers are defined by what they lack, no common molecular "drivers" are available for developing targeted therapies. Moreover, triple-negative breast cancers are a highly heterogeneous group, and it has been difficult to identify common mutations underlying the disease. In this issue of *Cell*, Sun et al. (2011) uncover a new molecular feature common to a large fraction of triple-negative breast cancers: the loss of the protein tyrosine phosphatase PTPN12 (also known as PTP-PEST).

Sun et al. begin by performing a loss-of-function screen for human kinases and phosphatases that regulate anchorage-independent growth in human breast epithelial cells. The top hit was PTPN12. They then show that loss of PTPN12 expression enables these cells to grow in the absence of extracellular matrix and also leads to their hyperproliferation in a three-dimensional culture model that recapitulates structures of the mammary gland. These two assays are widely used to assess oncogenic transformation, and PTNP12's behavior in these assays establishes it as a candidate tumor suppressor gene in mammary epithelial cells. Importantly, the ability of PTPN12 to block growth in these assays depends on its tyrosine phosphatase activity.

Sun and colleagues then characterize the signaling events altered by disruption of PTPN12. The phosphorylation of specific tyrosines on both the EGFR and HER2 receptor tyrosine kinases increases, as does the activity of the downstream ERK/RSK (extracellular signal-regulated kinase/ribosomal S6 kinase) pathway. Together, these results indicate that PTPN12 acts as a growth suppressor by antagonizing key receptor tyrosine kinase pathways.

Further, an analysis of *PTPN12* gene sequences reveals a series of point mutations found only in triple-negative breast cancer cells. Using in vitro functional tests, Sun and colleagues confirm that these mutations represent loss-of-function alleles. Additionally, 20% of all breast cancers contain deletions encompassing *PTPN12*, although other genes within the deleted region may also be involved in tumorigenesis. Perhaps most strikingly, Sun and colleagues find that 60% of triple-negative breast cancer cells are histologically negative for PTPN12 in a panel of 185 breast cancers, whereas only 9% of HER2-positive cancers are PTPN12 negative.

Sun and colleagues also note that the PTPN12 mRNA contains three binding sites for miR-124, a microRNA repressed by the tumor suppressor REST (RE1-silencing transcription factor). The expression of REST correlates with that of PTPN12, which is consistent with miR-124 suppressing PTPN12. In addition, overexpressing miR-124 promotes anchorage-independent growth, as was observed when *PTPN12* was disrupted. The miR-124 locus is amplified in 20% of breast cancers, suggesting yet another mechanism by which PTPN12 expression may be lost in cancer. Together, these findings strongly suggest that PTPN12 loss plays a role in triple-negative breast cancers. Moreover, the near mutual exclusivity of HER2 overexpression and loss of PTPN12 suggests that disrupting PTPN12 represents an alternate route for activating survival and proliferation pathways known to drive cancer development.

Another intriguing finding by Sun and colleagues is that a common single-nucleotide polymorphism in the *PTPN12* locus (Thr573Ala) represents a loss-of-function variant. Although the correlation of this polymorphism with breast cancer falls below a strict threshold of statistical significance ($p = 0.2$), the trend hints that this variant may predispose an individual to breast cancer. Indeed, a previous study sequenced this polymorphic locus in 19 breast cancer cell lines (Streit et al., 2006), with the loss-of-function allele occurring more frequently in triple-negative breast cancer cell lines (4 out of 9) than in other types of breast cancer (2 out of 9). Interestingly, this previous study also found that the alanine substitution,

which resides outside the catalytic domain, enhanced phosphatase activity in vitro. Given that Sun et al. found loss of tumor suppressor function in cultured cell transformation assays, these results raise the possibility that the alanine substitution alters PTPN12 localization or its access to substrates inside the cell.

Unfortunately, despite the prevalence of PTPN12 loss in triple-negative breast cancer cells, there is currently no feasible way to directly restore the function of this phosphatase therapeutically. Moreover, although PTPN12 appears to regulate the EGFR/HER2 pathway, inhibitors of EGFR and HER2 have failed to show clinical efficacy for triple-negative breast cancers, even though many of these breast cancers display high levels of EGFR. Therefore, reversing the effects of PTPN12 loss may require inhibition of multiple tyrosine kinases. This prompted Sun and colleagues to identify additional targets of PTPN12 using a bimolecular fluorescence complementation assay. These experiments pinpoint the platelet-derived growth factor receptor-β (PDGFR-β) as another tyrosine kinase that interacts with PTPN12. Indeed, inhibiting both EGFR/HER2 and PDGFR-β with two inhibitors, lapatinib and sunitinib, slows the growth of xenografted triple-negative breast cancer tumors.

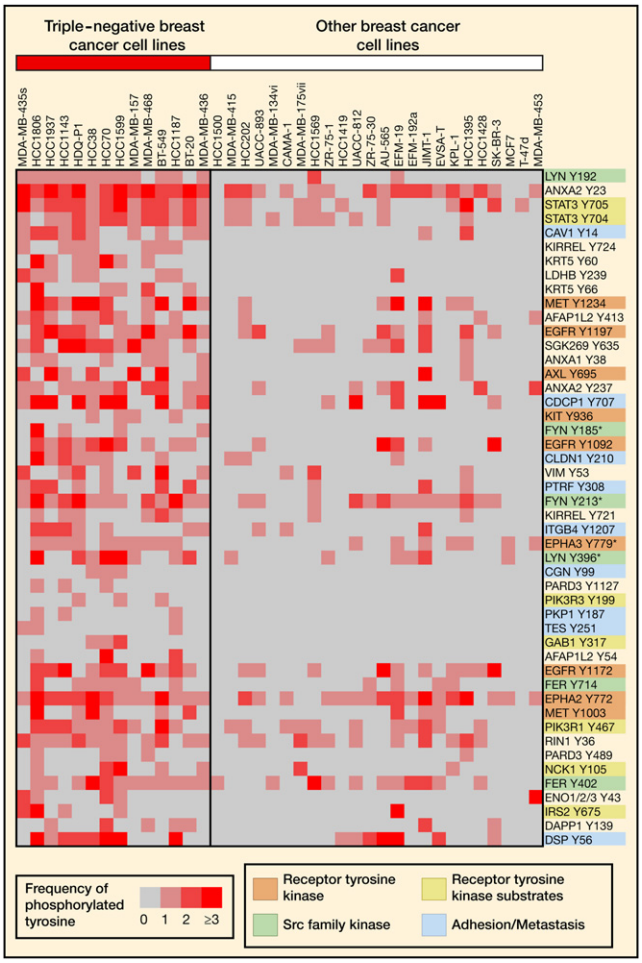

Figure 1. Triple-Negative Breast Cancer Cell Lines Are Enriched with Tyrosine Phosphorylation Sites

"Triple-negative" breast cancers, which do not express the three major molecular signatures of breast cancer cells (estrogen receptor, progesterone receptor, and HER2), are heterogeneous and difficult to treat. Sun et al. (2011) now find that the activity of the protein tyrosine phosphatase PTPN12 is lost in many of these cancers. Here a Wilcoxon rank sum test was used to identify tyrosine sites preferentially phosphorylated in triple-negative breast cancer cell lines versus other types of breast cancer cells (all sites shown are significant at the $p < 0.05$ level and are ranked from top to bottom in order of increasing p value). Asterisks indicate sites that may also be phosphorylated on closely related proteins. Data provided by Ting-lei Gu of Cell Signaling Technology, and the breast cancer dataset is downloadable from PhosphoSitePlus at http://www.phosphosite.org/downloads/breastcancerdataset.gz.

Importantly, the central concept advanced by Sun and colleagues is not that PDGFR-β is a key target in triple-negative breast cancer—indeed, datasets from Oncomine (http://oncomine.org) indicate that PDGF-α/β are not preferentially expressed in triple-negative breast cancer—but rather that the treatment of this obstinate cancer may require combinatorial inhibition of multiple tyrosine kinases. In support of this idea, we note that many sites of tyrosine phosphorylation are enriched in triple-negative breast cancer cell lines (Figure 1), based on mass spectrometry analysis of immunoaffinity-purified phosphopeptides (Ting-lei Gu, Cell Signaling Technology; http://www.phosphosite.org/downloads/breastcancerdataset.gz). Many of these sites are located on receptor tyrosine kinases (e.g., EGFR, MET, Axl, and EphAs), Src family kinases (SFKs), and proteins involved in cell adhesion and tight junctions (e.g., caveolin, desmoplakin, cingulin, and claudin-1). Together, these data are consistent both with Sun and colleagues' conclusion that loss of PTPN12 in triple-negative breast cancer cells stimulates a wide range of signaling programs and with other studies linking PTPN12 to the regulation of cell adhesion and motility (Angers-Loustau et al., 1999; Garton and Tonks, 1999).

The promiscuity of tyrosine kinase signaling networks has also been observed in lung cancer, in which signaling behaves as a highly interconnected web of substrates that collapses dramatically when key "hub" tyrosine kinases are inhibited (Guo et al., 2008; Rikova et al., 2007). These webs appear to be highly flexible, resulting in cells with variable sensitivity to tyrosine kinase inhibitors, depending on the cell's profile of kinases and phosphatase levels. Given that a number of tyrosine kinase inhibitors are currently approved for treatment of other cancers, each of which targets three or more different tyrosine kinases, it will be of great interest to determine whether combinations of these drugs are effective against triple-negative breast cancer, and whether PTPN12 status can predict sensitivity.

Although the work of Sun and colleagues opens a new window on a poorly understood and aggressive malignancy, it also underscores the inherent difficulties in targeting complex cancers like triple-negative breast cancers. The signaling changes that accompany the loss of PTPN12 or overexpression are modest, in the range of 2- to 3-fold. This suggests that PTPN12 exerts its potent phenotypic effects not through a single main effector but rather by many small changes distributed throughout a signaling network.

Lastly, it is important to note that a sizable fraction of the general population (2.5%) harbors single-nucleotide polymorphisms that inactivate the tumor suppressor activity of PTPN12, but the majority of these individuals do not develop cancer. Therefore, loss of PTPN12 alone is not sufficient to drive tumor formation. Interestingly, the effect of PTPN12 appears confined to the ERK/RSK pathway, without affecting the important PI3K/Akt pathway. This observation suggests that PTPN12 loss cooperates with mutations in other pathways, including the PI3K/Akt pathway, which is hyperactivated in nearly 50% of breast cancers. These considerations together lead us to conclude that, although PTPN12 may be an important piece of the triple-negative breast cancer puzzle, it is still a member of an even larger constellation of alterations that together drive this recalcitrant malignancy.

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The Ribosome and TORC2: Collaborators for Cell Growth

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The target of rapamycin complex 2 (TORC2) is a key regulator of cell growth. Zinzalla et al. (2011) now provide evidence that TORC2 is activated by direct association with the ribosome, which may ensure that TORC2 activity is calibrated to match the cell's intrinsic growth capacity.

TOR is an atypical serine/threonine protein kinase conserved from yeast to mammals that forms two distinct physical and functional complexes termed TORC1 and TORC2 (Figure 1) (Wullschleger et al., 2006). TORC1, a rapamycin-sensitive complex, regulates translation, autophagy, cell growth, and cell size. In contrast, TORC2 is not directly inhibited by rapamycin and controls cell survival and morphology. The frequent dysregulation of mammalian TOR (mTOR) signaling observed in human cancer is thought to contribute to tumorigenesis (Zoncu et al., 2011). Extensive studies have revealed the molecular mechanisms of mTORC1 regulation in response to signals, such as growth factors, cellular energy status, nutrient availability, and stress. Although compelling evidence has placed TORC2 downstream of phosphatidylinositol 3-kinase (PI3K) and upstream of the serine/threonine kinase AKT, it has been unknown how TORC2 is regulated (Wullschleger et al., 2006). Using a combination of elegant genetic screening and sophisticated biochemical studies, Zinzalla et al. (2011) significantly advance our understanding of TOR biology by identifying the ribosome as a missing link between PI3K and mTORC2 (Figure 1).

In yeast, TORC2 phosphorylates and activates YPK2, the ortholog of mammalian kinase SGK1, a known substrate of mammalian TORC2. Loss of TORC2 function is lethal in yeast; however, overexpression of a constitutively active YPK2 suppresses the lethality caused by a loss-of-function TORC2 mutation (Kamada et al., 2005). Zinzalla et al. designed a clever reverse suppressor screen in search of yeast mutants that require the expression of constitutively active YPK2 for survival. This strategy was aimed at uncovering mutations in TORC2 upstream activators. Perhaps not surprisingly, many mutations isolated were found in genes encoding components of TORC2. Interestingly, the only non-TORC2 component isolated was NIP7, which encodes a protein involved in the maturation of rRNA and ribosome biogenesis. Survival of NIP7 mutant yeast requires overexpression of the constitutively active YPK2. Many scientists would abstain from studying ribosomal proteins because their inactivation may disrupt protein translation and lead to pleiotropic effects. Undeterred, Hall and colleagues