CANCER

A new strategy to ERADicate HER2-positive breast tumors?

Sanjeevani Arora and Erica A. Golemis*

HER2-positive breast cancers that have become resistant to HER2-targeting agents, such as trastuzumab (also known as Herceptin), have limited treatment options. In this issue of *Science Signaling*, Singh *et al.* have identified a characteristic increase in the endoplasmic reticulum (ER)–associated degradation (ERAD) system in HER2-positive tumors as a mechanism of relieving proteotoxic stress. Synthetic lethality arising from targeted disruption of ERAD signaling in conjunction with other HER2-dependent signaling may improve therapeutic management of this difficult class of breast tumors.

Defining the selective vulnerabilities of tumor cells versus normal cells is essential for effective treatment. First-generation targeted therapies typically focused on inhibition of driver oncogenes. For example, ~20% of breast cancers show amplification of *HER2* (*ERBB2*), which is associated with an aggressive clinical course and early metastasis. HER2-targeting agents, such as trastuzumab and lapatinib, are currently used to therapeutically target HER2-positive breast cancer. However, these agents typically show limited benefit, with many tumors characterized by intrinsic or acquired resistance (*1*). An increasingly promising complementary approach has been to leverage comprehensive data sets from The Cancer Genome Atlas (TCGA) and other resources in order to identify signaling dependencies of specific tumor classes that can be exploited to develop new therapeutic approaches. In this issue of *Science Signaling*, Singh *et al*. define such a "nononcogene addiction" in HER2-driven breast cancers, showing that these require endoplasmic reticulum (ER)–associated degradation (ERAD) for survival (*2*).

Integrating cell line and primary tumor data sets from TCGA, METABRIC, and the Cancer Cell Line Encyclopedia (CCLE), the authors first selected for genes and pathways that had increased expression in HER2-positive ($HER2^+$) breast cancers and that correlated with poor prognosis in $HER2^+$ patients. This analysis revealed that the transcriptional landscape remained remarkably conserved in $HER2^+$ cancers, whether in tumors or cultured cell lines. They then eliminated candidate genes that were directly dependent on HER2 activity—that is, those that either were induced by HER2 transformation of MCF10A primary breast cells or were overexpressed as part of the *HER2* amplicon on chromosome 17. For the remaining set of genes, they used the algorithm NetWalk (*3*) to perform a regression-coupled network analysis that identified the ERAD pathway as independently selected in HER^+ breast cancers.

Together with the unfolded protein response (UPR), the ERAD pathway (Fig. 1) maintains ER homeostasis and protein quality control and counters cytotoxic ER stress. Stress-inducing accumulation of unfolded proteins in the ER is linked to various pathologies (including, notably, cancer) in response to specific mutations, hypoxia, glucose deprivation, oxidative stress, and other stimuli. The inositol-requiring enzyme 1α (IRE1α), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6) are integral ER membrane proteins that serve as stress sensors; a fourth stress sensor, the transcription factor adenosine 3′,5′-monophosphate (cAMP) response element–binding protein 3 (CREB3), is tethered to the ER membrane when inactive. Activation of these proteins during persistent ER stress triggers a downstream effector response that includes a spliced form of XBP1, XBP1, the cleaved cytosolic domain of ATF6, and the nuclear translocation of CREB3 to influence transcription of genes that influence cell death (*4*, *5*). IRE1α also activates c-Jun N-terminal kinase (JNK) in a process involving phosphorylation of JNK by mitogen-activated protein kinase kinase 4 (MKK4), providing another stimulus to cell death. An important role of the ERAD system is to increase clearance of misfolded proteins by tagging them via deglycosylation and ubiquitination [by ligases including synoviolin-1 (SYVN1) and others], transporting them to the cytosol, and degrading them via the proteasome in a manner dependent on the ubiquitin segregase valosin-containing protein (VCP, also known as p97), which provides a structural platform for the assembly of the ERAD system (*5*).

In validation of their computational analysis, Singh *et al*. demonstrated greater abundance of VCP, SYVN1, and other ERAD-associated proteins in a panel of $HER2^+$ compared with HER2-negative ($HER2^-$) breast cancer cell lines. Expression of these proteins was unaffected by treatment with the HER2 inhibitor lapatinib or *HER2* copy number, confirming HER2-independence. In further messenger RNA analysis, they observed strong correlation of *VCP* expression with that of *CREB3* and noted that increased *CREB3* expression correlated with poor prognosis in patients with $HER2^+$ tumors. By then manipulating CREB3 abundance in cells, the authors demonstrated that overexpressed CREB3 induced *VCP* expression in nontransformed MCF10A mammary epithelial cells. Examining candidate death effectors operating downstream of the ER-stress sensors, the authors also found suppressed activity of the IRE1α effector JNK in $HER2^+$ cell lines. Mechanistically, this was mediated by a combination of loss of JNK activators (for instance, *MAP2K4*, encoding MKK4, was commonly deleted) and induction of endogenous JNK inhibitors such as the dual specificity phosphatase DUSP10, which was highly expressed in $HER2^+$ cell lines. Critically, the authors demonstrated the functional importance of increased ERAD activity in $HER2^+$ cells, showing that depletion of VCP or treatment with Eeyarestatin (*6*), NMS-873, or DBeQ—three drugs that specifically inhibit ERAD—was lethal in $HER2^+$ but not $HER2^-$ cells. This lethality depended on active HER2 signaling through the mammalian target of rapamycin (mTOR) pathway to JNK because treating $HER2^+$ cells with the mTOR inhibitor Torin, the HER2 inhibitor lapatinib, or IRE1α and JNK inhibitors reduced the cytotoxic effect of Eeyarestatin.

Extending their results, the authors have begun to explore the therapeutic possibilities suggested by this new pathway dependence. Using BCI—a promiscuous inhibitor of dual-specificity kinases (*7*)—in combina-

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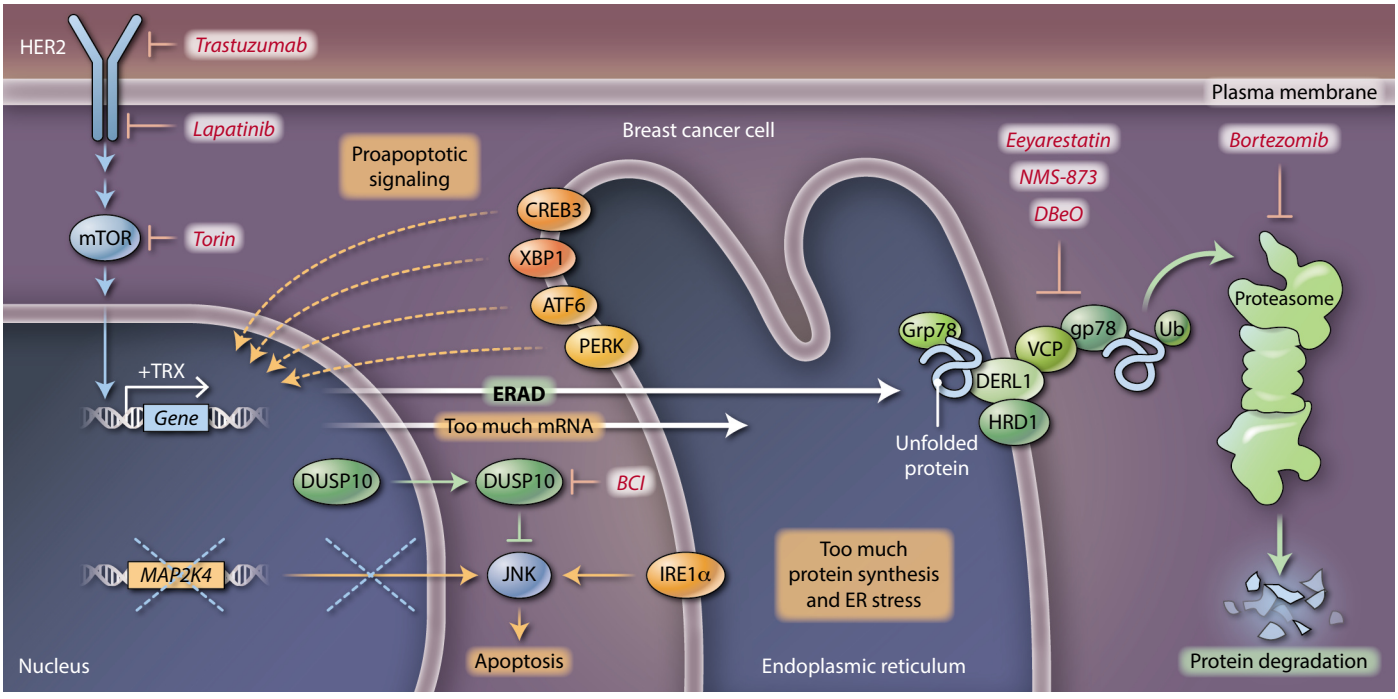

Fig. 1. ERAD dependence in HER2⁺ cancer. Shown is a schematic of how HER2 signals through mTOR to activate ER stress and thus trigger ERAD responses. Proteins contributing to ER stress and cell death are indicated in orange; proteins supporting ERAD and survival of transformed cells are shown in green; drugs targeting the pathway are indicated in red.

tion with Eeyarestatin, the authors demonstrated synergy between the two compounds in activating JNK and killing HER2⁺ but not HER2⁻ breast cancer cell lines. Importantly, this synergy was equally observed both in tumors that were sensitive to HER2-targeted therapy and those that were resistant. This observation of synthetic lethality provides new directions for research and justifies further development of compounds selectively targeting DUSP10. Interestingly, another study shows that HER2 deregulates the activity of the kinases extracellular signal–regulated kinase (ERK), AKT, and mTOR to mediate UPR signaling involving PERK, ATF4, and CHOP, leading to increased ER-stress and induction of cell death (*8*). This study parallels the work of Singh *et al*. in validating ER stress as a therapeutic target in HER2⁺ breast cancers.

This report is not the first to evaluate enhancing ER stress responses for cancer therapy. For example, the proteasomal inhibitor bortezomib synergizes with 34.5ENVE, a herpes simplex virus-1 (HSV-1)–derived oncolytic virus, to induce the UPR (*9*). Inhibition of the heat shock protein 90 (HSP90) chaperone complex induces protein unfolding, ER stress, and cell death in cancer cells (*10*). However, to date the use of proteasome inhibitors has been constrained by sometimes substantial dose-limiting toxicity, whereas HSP90 inhibitors are only now undergoing late-stage clinical assessment for effectiveness. A more narrow focus on inhibition of ERAD, coupled with identification of a defined subset of tumors that rely on ERAD for survival, has the potential to yield substantial benefits with reduced toxicity in patients. Future studies are warranted to increase our understanding of proteins in the ERAD network, particularly those that could be exploited by targeted therapy.

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