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A new strategy to ERADicate HER2-positive breast tumors?

Sanjeevani Arora and Erica A. Golemis*

Program in Molecular Therapeutics, Fox Chase Cancer Center, Philadelphia, PA 19111

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, approximately 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 typically show limited benefit, with many tumors characterized by intrinsic or acquired resistance (1). An increasingly promising complementary approach has been to leverage comprehensive datasets from The Cancer Genome Atlas (TCGA) and other resources 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 “non-oncogene addiction” in HER2-driven breast cancers, showing these require endoplasmic reticulum-associated degradation (ERAD) for survival (2).

Integrating cell line and primary tumor datasets from TCGA, METABRIC, and the Cancer Cell Line Encyclopedia (CCLE), the authors first selected for genes/pathways that had increased expression in HER2+ breast cancers, and which correlated with poor prognosis in HER2+ patients. This analysis revealed that transcriptional landscape remained remarkably conserved in HER2+ cancers, whether in tumors or cultured cell lines. They then removed genes that were directly dependent on HER2 activity, by eliminating genes that were induced by HER2 transformation of MCF10A primary breast cells, or which were overexpressed as part of the HER2 amplicon on chromosome 17. For the remaining set of genes, they used the algorithm NetWalker (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 (Figure 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 protein 1 α (IRE1 α), 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 CREB3, is tethered to the ER membrane when inactive. Activation of

*corresponding author: Erica Golemis, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111, (215) 728-2860, Erica.Golemis@fccc.edu.

these proteins following unrelieved ER stress triggers a downstream effector response including a spliced form of XBP1, ATF4, the cleaved cytosolic domain of ATF6, and, with these proteins CREB3 translocating to the nucleus to influence transcription of genes that influence cell death (4, 5). IRE1 α also activates Jun kinase (JNK) in a process involving phosphorylation of JNK by 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, ubiquitinating them by ligases including synoviolin (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)/p97, which provides a structural platform for the assembly of the ERAD system (5).

In validation of their computational analysis, Singh *et al.* demonstrated upregulation of VCP, SYVN1 and other ERAD-associated proteins in a panel of 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 mRNA analysis, they observed strong correlation of VCP expression with CREB3, and noted elevated CREB3 correlated with poor prognosis in HER2+ tumors; direct testing demonstrated that overexpressed CREB3 induced VCP in MCF10A mammary cells. Examining candidate death effectors operating downstream of the ER-stress sensors, the authors also found suppressed activity of the IRE1 α 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 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 elevated ERAD in HER2+ cells, showing that depletion of VCP or treatment with Eeyarestatin (6), NMS-873, or DBeQ - three drugs that specifically inhibit ERAD - was selectively lethal in HER2+ versus HER2- cells. This lethality depended on active HER2 signaling through the mTOR pathway to JNK, with treatment of HER2+ cells with the mTOR inhibitor Torin, the HER2 inhibitor lapatinib, or IRE1 α and JNK inhibitors reducing 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 combination with Eeyarestatin, the authors' demonstrated synergy of the two compounds in activating JNK and killing HER2+ but not HER2- breast cancer cell lines. Importantly, this synergy was equally observed in sensitive and resistant HER2-targeted therapy tumors. This observation of synthetic lethality suggests numerous promising new directions for research, and justifies further development of compounds selectively targeting DUSP10. Interestingly, another recent work has shown that ERBB2 deregulates the activity of 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 was recently shown to synergize with

34.5ENVE, an oncolytic herpes simplex virus-1, mediated by bortezomib-induced 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-significant 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 in which ERAD inhibition would be of highest benefit, has the potential to yield significant benefits with reduced toxicity. 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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Figure 1.

HER2 signals through mTOR to activate ER stress, and compensating 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 (red).