CANCER

Focus Issue: Cell biology meets cancer therapy

Nancy R. Gough*

Cells are the targets of anticancer therapy, whether the therapy is directed at the tumor cells themselves or the cells of the immune system. Articles in this issue and in the 2015 *Science Signaling* archives provide insights into what makes a cell responsive to therapy and how understanding the cellular processes affected by the drugs (including endosomal trafficking and response to proteotoxic stress) can lead to personalized cancer therapies, thereby minimizing side effects and ineffective treatment strategies.

Growing and dividing cells, including cancer cells, have high metabolic requirements because they must build new proteins, membranes, and nucleic acids. Drugs that interfere with cellular metabolism or macromolecule biosynthesis can trigger various types of cell stress pathways, such as those involving proteotoxic stress. The accumulation of unfolded proteins in the endoplasmic reticulum (ER) causes ER stress and induces the unfolded protein response (UPR). The accumulation of unfolded or misfolded proteins in the cytosol also triggers proteotoxic stress. Another cellular response to proteotoxic stress, which can result from temperature stress, oxidative stress, or nutrient deprivation as well as ER stress, is the integrated stress response (ISR). Whereas the UPR increases the abundance of protein chaperones that reside in the ER, the ISR inhibits most protein translation, with the exception of specific genes, many of which are regulated by the stress-activated transcription factor ATF4, a key mediator of the ISR. Prolonged proteotoxic stress can induce cell death.

Research by Kline *et al.* and Ishizawa *et al.* (see the Focus by Greer and Lipkowitz) revealed that ONC201, a drug in clinical trials, triggers cell death in both hematological cancers and cells derived from solid tumors by inducing a stress response with characteristics of the ISR. The pair of papers demonstrated that ONC201 induced the activation of ATF4, which increased the expression of a gene encoding a death receptor and its ligand in solid tumor cells and a protein that inhibited the growth-promoting kinase complex mTORC1 in lymphoma and leukemia cells. Fortunately, ONC201-triggered apoptosis did not require the activity of the tumor suppressor and transcription factor p53, which is often mutated in many cancers.

One way that cells can adapt to ER stress is by increasing a process called ER-associated degradation (ERAD) that eliminates the unfolded proteins by transporting them out of the ER and targeting them for proteasomal degradation. Singh *et al.* found that breast cancers positive for the growth factor receptor HER2 had increased ER stress signaling and were "addicted" to ERAD for survival. Because HER2 signaling promoted protein synthesis, the cells experienced severe proteotoxic stress when ERAD was blocked. Pharmacologically blocking this pathway caused cell death in HER2-positive cells, including those that were resistant to clinically used HER2 inhibitors.

Growth factor signaling stimulates the phosphoinositide 3-kinase (PI3K)–AKT pathway. Activation of this pathway in the presence of abundant nutrients stimulates the kinase activity of mTOR, a kinase that as part of the mTORC1 complex promotes protein translation by phosphorylating the translation inhibitor 4EBP1. Intrinsic or acquired resistance of cancer cells limits the clinical usefulness of drugs that inhibit mTOR activity or signaling through the PI3K-AKT pathway. Hsieh *et al.* found that intrinsic differences in *4EBP1* expression and protein synthesis rates were associated with the propensity of the cells to become tumorigenic and with resistance to therapies targeting mTOR in mice and those targeting PI3K in patients. The luminal epithelial cells that tended to become cancerous had high 4EBP1 abundance and low protein synthesis rates. These data explain why drugs targeting this signaling axis have been ineffective in treating prostate cancer, despite data from experiments with cultured cells and xenografts showing drug resistance that was associated with loss of 4EBP1 and high protein synthesis. This study highlights the importance of understanding the cell-specific properties of particular cancers to optimize treatment approaches.

Accumulation of damaged or improperly folded proteins in the cytosol can lead to the formation of protein aggregates, which can be eliminated through autophagy, a mechanism by which cellular membranes form around protein aggregates, forming autophagosomes that then fuse with lysosomes for degradation. However, if autophagy is compromised or insufficient, cells may experience proteotoxic stress that results in cell death. Indeed, Zhang *et al.* found that the clinically used drug verteporfin triggered the accumulation of toxic amounts of protein oligomers that selectively killed colorectal cancer cells in mice and cancer cells cultured under hypoxic and nutrient-deprived conditions. Normal cells in culture and in tumor-adjacent tissue sections from mice cleared these aggregates through autophagy and survived, indicating that in this model verteporfin produced tumor-selective proteotoxicity. These data not only suggest additional therapeutic applications for verteporfin but also indicate that drugs that can induce the rapid formation of protein aggregates may be effective when used in combination with drugs that inhibit autophagy or in cancers with limited capacity for autophagy.

Many proteins pass through the ER and Golgi before reaching the plasma membrane or being released from cells. This complex biosynthetic process enables cells to perform quality control on the newly synthesized proteins, control the rate of release or appearance of the proteins at the cell surface, and add posttranslational modifications (such as the addition of sugar moieties through the process of glycosylation). Lau *et al.* found that metastatic melanoma was associated with reduced abundance of the enzyme that mediated fucosylation. This type of sugar modification affects the interaction of growth factors and chemotactic molecules with their receptors, and attenuating fucosylation reduced melanoma cell adhesion and enhanced melanoma cell migration. Mice injected with melanoma tumor cells showed reduced tumor growth

Editor, *Science Signaling*, AAAS, Washington, DC 20005, USA.

***Corresponding author. E-mail: ngough@aaas.org**

and metastasis when fucose was added to their drinking water. Thus, understanding how protein glycosylation is altered in cancer cells may enable appropriate dietary or pharmacological strategies to restore proper glycosylation and cellular behavior.

Transmembrane receptors that reach the plasma membrane do not stay there permanently, but instead undergo dynamic endocytosis and recycling back to the cell surface or to the lysosome for degradation. Ben-Chetrit *et al*. found that many patients with aggressive breast cancer have tumors with increased expression of *SYNJ2*, which encodes the lipid phosphatase synaptojanin 2. In response to epidermal growth factor (EGF), SYNJ2 localized to lamellipodia and invadopodia, which are cellular protrusions associated with invasive behavior and are the sites where EGF stimulates its receptor to promote cell migration. Knocking down SYNJ2 inhibited recycling of the EGF receptor to the cell surface and decreased the invasive behavior of cultured breast cancer cells. Breast cancer cells expressing a phosphatase-deficient mutant of SYNJ2 produced smaller, less metastatic tumors when xenografted into mice. Encouragingly, inhibitors of SYNJ2 that reduce cell invasion in 3D culture were identified in a chemical screen, which suggests that it may be possible to target SYNJ2 and thereby prevent metastasis in breast cancer patients.

In addition to their role in receptor endocytosis and trafficking, endosomes also serve as organizing sites for signaling complexes. The kinase BRAF is normally activated by growth factor signaling, and activating mutations in this kinase cause colorectal cancer. Margalef *et al*. found that the endosome-associated kinase TAK1 phosphorylated a proteolytic fragment of another kinase, IKKα, and BRAF-mutant colorectal cells required TAK1-mediated phosphorylation of the IKKα cleavage product for transformation and proliferation. Endosomes are acidic compartments, and inhibition of endosomal acidification blocked the phosphorylation of the IKKα cleavage product and induced cell death. This study reveals an unexpected connection between endosomes, proteins involved in the nuclear factor κB (NF-κB) pathway, and a mitogen-activated protein kinase pathway activated by BRAF. Furthermore, the data suggest that drugs that block endosomal acidification and that are currently used for malaria prevention or as antifungals could be repurposed to treat cancers associated with active BRAF mutations.

The studies highlighted here underscore the importance of not only finding compounds that can kill cancer cells or stop their proliferation in culture but also understanding how they work to identify the patients with cancers most likely to respond to a particular treatment. Furthermore, these articles are examples of using a molecular understanding of the signaling events associated with cellular stress responses to leverage the development of novel therapeutic approaches or combination therapies for more effective treatment.

Related Resources

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