Establishing Links between Endoplasmic Reticulum-Mediated Hormesis and Cancer

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The unfolded protein response (UPR) is an evolutionarily conserved adaptive response to disruptions of endoplasmic reticulum (ER) physiology. In response to the accumulation of misfolded proteins and ER stress, the UPR activates three specific pathways: the Ire1 (inositol-requiring enzyme 1), Atf6 (activating transcription factor 6), and Perk (protein kinase RNA-like endoplasmic reticulum kinase) pathways, which induce chaperone expression, suppress translation, and trigger ER-associated degradation (ERAD). Collectively, these responses enable the cell to deal with the accumulation of misfolded proteins. Recently, considerable attention has focused on the regulation of ER homeostasis and the pathological mechanisms that arise from disruption of ER homeostasis (1, 2). Depending on the level of UPR activation and the components of the pathway activated, ER stress can either lead to cell death or survival. A sustained UPR triggers the activation of the DDIT3/CHO transcription factor that directs UPR-induced apoptosis. This is particularly relevant in the context of cancers, where the UPR is now an attractive target for therapeutics. By contrast, mild ER stress (ER preconditioning) protects cells in models of neurodegenerative diseases (3). Thus, by fine-tuning the UPR, we may be able to improve treatments for both cancer and degenerative diseases. In this journal issue, [Ogden and co-workers describe their investigation](#) of the possible clinical relevance of ER stress and UPR induction in cancer treatment via deregulation of Hedgehog (Hh) signaling (4). Hh signaling is essential for patterning during embryonic development, but its deregulation is associated with cancer. Hh is a diffusible ligand and binds to its receptor, Patched (Ptc), which releases the inhibition of Smoothened (Smo) by Ptc. In turn, Smo provokes the cleavage and activation of Gli family transcription factors that induce expression of the Hh target genes that are normally required for proliferation, patterning, and survival. However, deregulated Hh signaling has been implicated in several pathological disorders, including birth defects and cancer. In particular, mutations of *PTC* and *SMO* manifest in medulloblastoma and basal cell carcinoma (5).

In a previous study, Ogden and coworkers showed that mutations affecting the conserved extracellular loops of Smo (EC1 and EC2) led to retention of Smo in the ER and constitutive Hh-independent Smo signaling (6). In this journal issue, Marada et al. now show that ER stress destabilizes active *smo* mutants and attenuates their signaling activities. Quite strikingly, those authors show that thapsigargin (a conventional inducer of ER stress) or heat treatment largely rescues the damaged wing phenotype provoked by the expression of *smoC320A* or *smoC339A* in the developing wing of *Drosophila melanogaster*. Further, those authors show that the ERAD pathway is required for UPR-directed destabilization of these Smo mutants and that this is mediated by the ERAD-specific ubiquitin Hrd1. Importantly, the UPR selectively targets the mu-

tant Smo proteins, with no effect on wild-type Smo protein and normal Hh signaling. These findings are particularly relevant for the treatment of cancers resistant to Smo inhibitors, especially since resistance to the Smo inhibitor Vismodegib has been observed in clinical practice (7). Thus, activation of the UPR may be an attractive Hh-independent approach to disable malignancies harboring activating Smo mutations. However, on a cautionary note, it should be borne in mind that several cancers, including multiple myeloma, make use of the cytoprotective functions of the UPR to promote tumor cell resistance and growth (8).

UPR cytoprotective mechanisms triggered by treatment with thapsigargin or heat can be seen as a preconditioning or adaptive stress response, otherwise known as hormesis. Hormesis is a cell-protecting response induced by exposure to a mild stress (e.g., a low drug dose or gentle heat treatment) that increases the resistance of the cell to a second, often stronger, challenge (9). In the above paradigm, the nonlethal activation of the UPR by thapsigargin or heat treatment leads to activation of the ERAD pathway, improving the ability of the ER to cope with the pathological production of misfolded proteins, such as activated Smo proteins. This is an example of ER-mediated hormesis, in which low levels of ER stress increase the resistance of the ER to the pathological production of misfolded proteins.

In medicine, hormesis is observed within the body during ischemic preconditioning, exercise, dieting, energy restriction, and exposure to low doses of certain phytochemicals. Indeed, surgeons frequently use ischemic preconditioning before heart surgery, where this is achieved through the application of rapid cycles of ischemia, which protects heart tissue. The mechanisms underlying the hormetic response are poorly understood and have been largely attributed to increases in the levels of reactive oxygen species (ROS) in mitochondria that are induced by the exposure to mild stress (9) and that are then thought to trigger an antioxidant response that protects the cell. Mitochondria are the principal organelles involved in the hormetic response, and hormesis mediated by mitochondria is known as mito-hormesis. ER stimulation can also result in preconditioning, and this promotes resistance to oxidative stress in renal epithelial cells and in the rat retina following ischemic reperfusion injury (12, 13).

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FIG 1 Perspectives of modulation of the UPR in therapeutics. Strong ER stress and activation of the UPR triggers apoptosis of cancer cells. In contrast, mild UPR activation induces a beneficial ER-mediated hormetic response that decreases the load of misfolded proteins and activates cellular protective mechanisms, such as an antioxidant response and autophagy.

ER-mediated hormesis was first used to describe cellular protection observed in the *ninaA* mutant, a genetic model of ER stress in the *Drosophila* retina (10). Specifically, flies carrying mutations in the gene encoding the ER-resident chaperone NinaA accumulated misfolded Rh1 protein in the ER. This activated the UPR, as demonstrated by the activation of an Xbp-green fluorescent protein reporter and Bip/hsc3 expression. The retinas of *ninaA* mutants displayed constitutive UPR activation but no signs of degeneration. In this model, ER stress was considered mild, as it did not lead to cell death. Indeed, in the *ninaA* mutant retina, mild ER stress leads to protect cells against various apoptotic proteins. Cellular protection in this scenario is not due to stimulation of ERAD activity, as in the *Smo* mutant. Instead, the UPR activates protective mechanisms inhibiting death triggered by external cues. Further, mild ER stress provoked by tunicamycin treatment induces an adaptive response, both *ex vivo* and *in vivo* (10, 11) that protects against cell death, and this is disabled by silencing *xbp1* expression. Together, these results suggest that mild ER stress activation via *xbp1* stimulates and confers resistance to cell death. The findings that ER-mediated hormesis protects against retinal degeneration led to studies of its roles in neurodegenerative diseases, where disruption of protein homeostasis appears to be involved in disease progression. The beneficial role of ER-mediated hormesis has been recently demonstrated in both *Drosophila* and mouse models of Parkinson's disease (11).

As underscored by the findings of Ogden and coworkers, ER-mediated hormesis plays important roles in at least some types of cancer, and it is exciting that ER-mediated hormesis may play beneficial roles in other pathological states that could be targeted by therapeutics (Fig. 1). These include protein-misfolding diseases, such as retinitis pigmentosa, an inherited form of blindness caused by misfolding mutations of rhodopsin; cataracts, in which

misfolded proteins accumulate in a highly oxidized lens; and type II diabetes, in which in β cells carrying insulin mutations or exposed to high concentrations of glucose produce large amounts of unfolded/misfolded proinsulin. Further, most neurodegenerative or muscle-degenerative diseases are caused by progressive or acute cell losses. In these models, cell-protecting mechanisms activated during ER-mediated hormesis, such as autophagy or the antioxidant response, help to prevent cell death. The appropriate therapeutic window will need to be defined for each disease if we are to consider the UPR to be a pathway that can be treated or changed with drugs. This will be particularly important for neurodegenerative diseases, in which patients are diagnosed late, after major cell damage has already occurred. It also remains unclear whether ER-mediated hormesis is potentially effective only for prophylaxis or whether it could also be used in curative approaches.

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