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# MFN2 Stabilization: A Bridge for Endoplasmic Reticulum Stress Sensitivity in Melanoma

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**In a new article in the *Journal of Investigative Dermatology*, Wang et al. (2021) report that mitochondrial quality control modulates responses to endoplasmic reticulum (ER) stress in melanoma. They implicate a linear pathway of XBP1, MARCH5, and MFN2 that act together to regulate mitochondrial fission and mitophagy and ultimately mediate melanoma cell sensitivity to ER stress. This work informs therapeutic combinations and biomarker strategies for targeting melanoma organellar homeostasis as well as for life–death decisions.**

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Protein folding quality control and the initiation of glycosylation occur in the endoplasmic reticulum (ER) shortly after secreted and integral membrane proteins are translated into its lumen (Ron and Walter, 2007). Three parallel signaling apparatuses monitor protein processing in the ER and initiate a series of signaling events known as the unfolded protein response (UPR) on the disruption of ER proteostasis (Ron and Walter, 2007). In most contexts, UPR-driven transcriptional programs are prosurvival and include factors that slow the rate of protein synthesis and upregulate molecular chaperones (Hill et al., 2014; Ron and Walter, 2007). Critical to the findings of Wang et al. (2021), UPR activation also feeds into mitochondrial health and quality control because UPR induction rewires mitochondrial fission and fusion dynamics and initiates the clearance of dysfunctional mitochondria through mitophagy (Senft and Ronai, 2015). Continual cell division coupled with a tumor microenvironment containing limited nutrient and oxygen perfusion strains protein folding within cancer cells. The resulting high levels of ER stress in tumor cells distinguish them from most untransformed cells and provide a potential opportunity for a therapy capable of discriminating tumor selectivity (Ron and Walter, 2007). Whereas aggravating ER stress or inhibiting the UPR has been promising in some tumor types, melanoma cells appear to be relatively resistant. In their new article, Wang et al. (2021) investigate whether a particular cotargetable pathway could sensitize melanoma cells to ER stress–induced apoptosis (Figure 1).

> "Pharmacological inhibition of either mitochondrial fission or mitophagy effectively restored the sensitivity of melanoma cells to ER stress induction."

Wang et al. take a comparative approach, identifying a panel of cell lines that have different sensitivities to ER stress induction by both tunicamycin and thapsigargin treatment. Although chemical treatment of all profiled lines induced UPR, cell death responses stratified sensitive and resistant lines. Transcriptional analysis revealed that the genes encoding UPR-signaling proteins were anticorrelated with mitochondrial genes in melanoma, and staining of human melanoma tumors corroborated this. The authors hypothesized that mitochondrial health might buffer cells from ER stress and found that chemical ablation of mitochondrial ROS rescued sensitive cells from cell death on ER stress induction.

An analysis of mitochondrial morphology further implicated mitochondrial fission and fusion dynamics in determining sensitivity to ER stress induction. Cells that were more resistant

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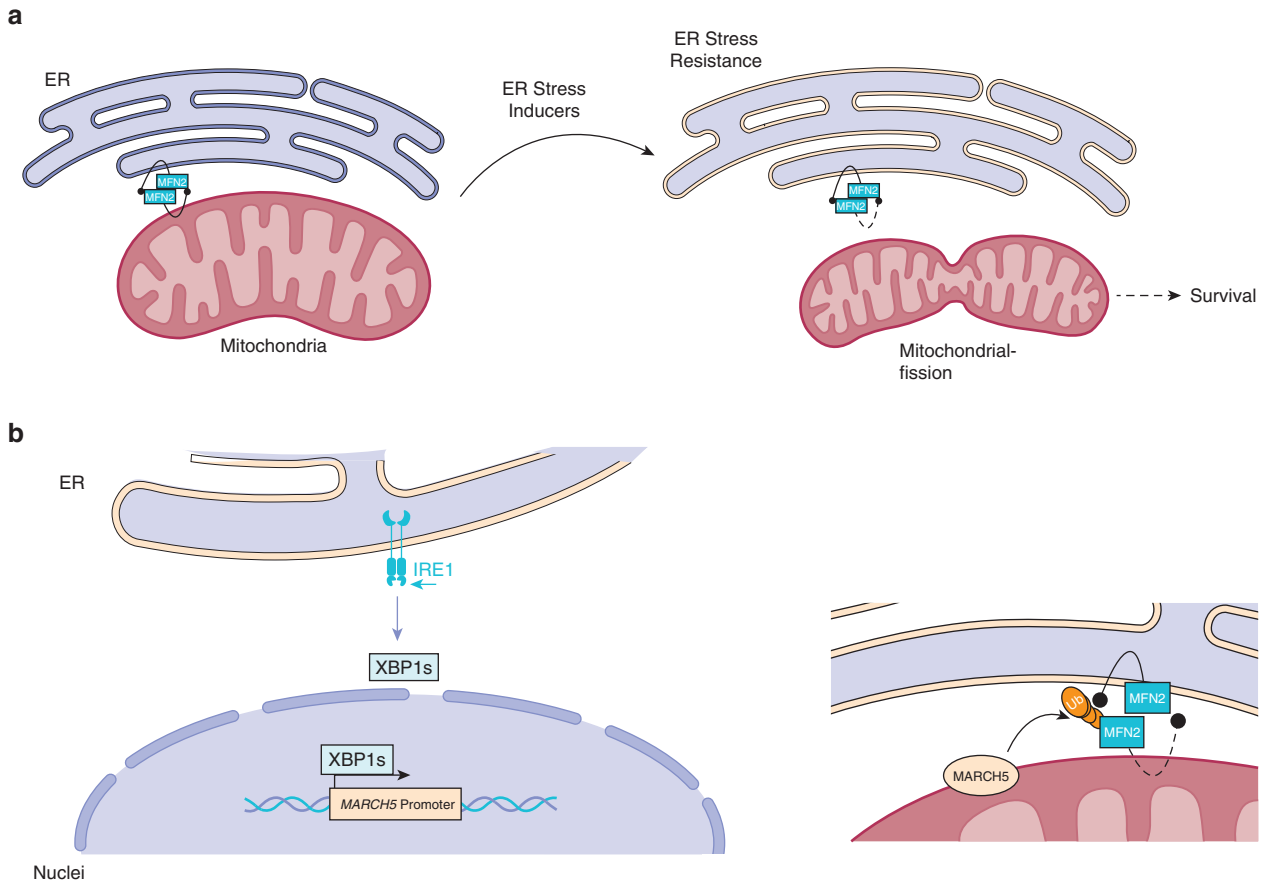


**Figure 1. The XBP1–MARCH5–MFN2 axis plays a pivotal role in resistance to ER stress–induced cell death.** (**a**) Treatment with ER stress inducers promotes mitochondrial fission, which rescues the cells from apoptosis. (**b**) The involvement of ER stress in the modulation of mitochondrial fission and survival is mediated in part by the activation of the UPR stress sensor IRE1. With the induction of ER stress, IRE1 licenses the translation of the XBP1 transcription factor, which in turn promotes the transcription of *MARCH5*. The MARCH5 E3 ligase ubiquitinates MFN2, promoting its degradation, which thereby triggers mitochondrial fission and mitophagy, a protective response in the context of ER stress. Images were created using BioRender.com. ER, endoplasmic reticulum; Ub, ubiquitin; UPR, unfolded protein response.

to the cytotoxic effects of ER stress induction had substantially greater mitochondrial fragmentation with treatment. It is well-appreciated that mitochondrial fission and fusion play key roles in sequestering damaged mitochondria for mitophagy, and this observation suggested that this mitochondrial quality control response might be a mediator of sensitivity to ER stress. Indeed, chemical inhibition of both mitochondrial fission and mitophagy potentiated the cytotoxic activities of ER stress induction. In a particularly promising demonstration of clinical tractability, Wang et al. (2021) show that chemically inhibiting either mitochondrial fission or mitophagy enhanced the activity of tunicamycin in a xenograft model.

Through profiling the known mediators of mitochondrial fission and fusion, the authors determined that MFN2, a positive regulator of mitochondrial fusion, exhibited decreased levels during ER stress induction. Strikingly, overexpression of MFN2 ablated ER stress–induced mitochondrial fission and sensitized otherwise resistant cells to death during ER stress induction. Proteosome inhibition rescued MFN2 degradation on ER stress induction, and the authors hypothesized that a UPR-dependent degradation pathway might lead to MFN2's degradation. Although the E3 ubiquitin ligase Parkin provides the canonical pathway for MFN2 degradation, the authors test several candidate E3 ligases and found that MARCH5 mediates much of the MFN2 degradation in the context of melanoma ER stress. In tumor models and human tumor samples, XBP1 and MARCH5 levels are correlated with each other and anticorrelated with MFN2. The authors posit that this is the linear pathway largely responsible for melanoma cells' resistance to ER stress–inducing treatments.

Although most untransformed cells do not experience ER stress, the demands of continual division within a nutrient-poor tumor microenvironment drive ER stress in most cancer cells (Hill et al., 2014; Ron and Walter, 2007). Several chemicals have been designed to inhibit the UPR-signaling machinery itself and show promise in other tumor types. In particular interest to this study, whereas the kinase inhibitors of IRE1 seem to lack some specificity, the inhibitor STF-083010 targets IRE1's ability to process XBP1 and has activity in preclinical models of myeloma (Hill et al., 2014). Other molecules for therapy act directly to aggravate ER stress, driving the dysregulation of ER proteostasis past a threshold where the UPR can compensate. Dose-limiting toxicities prevent the clinical use of the

inhibitors used as tools by Wang et al. (2021) in this study. Yet, efforts exist to develop chemical analogs of tunicamycin and thapsigargin that retain their ability to induce ER stress while potentially eliminating undesirable toxicities (Dong et al., 2018; Madden et al., 2019). In particular, the thapsigargin analog mipsagargin is under early-phase clinical trial evaluation for the treatment of multiple tumor types (Madden et al., 2019). Other strategies exist for aggravating ER stress and could be tested for their activity in melanoma clinically. Bortezomib and other proteasome inhibitors are routinely used clinically for the treatment of multiple myeloma, which exhibits exceptional levels of ER stress from the continuous production of immunoglobulins (Hill et al., 2014).

Cotreatment of the mitochondrial quality control response is a key feature of Wang et al.'s (2021) work. Whereas drugs that inhibit mitochondrial fission have not been extensively investigated in humans, drugs that inhibit the function of lysosomes and mitophagy have been used for decades (Ben-Zvi et al., 2012). In particular, chloroquine and hydroxychloroquine have been extensively tested in numerous tumor (as well as in other medical) contexts to inhibit the prosurvival effects of autophagy, and these inhibitors are readily available to test along ER stress induction (Ben-Zvi et al., 2012; Chude and Amaravadi, 2017). Testing the combination of chloroquine analogs with an approved ER stress inducer such as bortezomib would be informative.

A key remaining question is the precise underlying genomic (or epigenomic) explanation for how the XBP1, MARCH5, and MFN2 pathway becomes differentially regulated within melanoma subsets. Is the variation in this pathway's activity a fixed feature or fluctuating one? How does this pathway vary (if at all) within heterogeneous melanoma cell populations in vivo? Might it contribute to an outgrowth of resistant populations? The authors' mechanistic follow-up was guided by a transcriptional anti-correlation between mitochondrial genes and genes encoding UPR proteins. These gene expression signatures can be refined into biomarker assays for stratifying melanoma sensitivity to ER stress induction, particularly in contexts where combination therapy would be impractical. Whereas the promise of targeting ER stress and mitochondrial health remains unproven, Wang et al. (2021) provide a promising report worthy of further investigation.

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## CONFLICT OF INTEREST

DEF has a financial interest in Soltego, a company developing salt-inducible kinase inhibitors for topical skin-darkening treatments that might be used for a broad set of human applications. The interests of DEF were reviewed and are managed by Massachusetts General Hospital (Boston, MA) and Partners HealthCare in accordance with their conflict of interest policies. The remaining authors state no conflict of interest.

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