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PERK-ing up autophagy during MYC-induced tumorigenesis

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Stress in the tumor microenvironment in the form of hypoxia and low glucose/amino acid levels activates the evolutionarily conserved cellular adaptation program called the unfolded protein response (UPR) promoting cell survival in such conditions. Our recent studies showed that cell autonomous stress such as activation of the proto-oncogene *MYC/c-Myc*, can also trigger the UPR and induce endoplasmic reticulum (ER) stress-mediated autophagy. Amelioration of ER stress or autophagy enhances cancer cell death in vitro and attenuates tumor growth in vivo. Here we will discuss the role of the UPR and autophagy in MYC-induced transformation. Our findings demonstrate that the EIF2AK3/PERK-EIF2S1/eIF2α-ATF4 arm of the UPR promotes tumorigenesis by activating autophagy and enhancing tumor formation. Therefore, the UPR is an attractive target in MYC-driven cancers.

Keywords: unfolded protein response, EIF2AK3/PERK, c-Myc, ULK1, ATF4, Burkitt lymphoma

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Accumulation of misfolded/unfolded proteins in the ER or an imbalance between client proteins and chaperones activates the UPR, a tightly regulated gene expression program directed to restore ER homeostasis, alleviate cellular stress damage and prevent cell death. Activation of the UPR primarily involves ERN1/IRE1α, ATF6 and EIF2AK3/PERK, a trio of ER resident transmembrane proteins that act as sensors for recognizing cellular ER stress and subsequently upregulate genes such as ER chaperones accompanied by attenuation of global protein synthesis (**Fig. 1**). EIF2AK3/PERK regulates the translation control arm of the UPR by phosphorylating the EIF2S1/eIF2α initiation factor subunit, inhibiting cap-mediated eukaryotic translation initiation. The resultant block of protein synthesis prevents further influx of nascent unfolded proteins to the ER and subsequent protein overloading. However, translation of a select set of mRNAs, such as the stress inducible transcription factor ATF4, is preferentially upregulated upon EIF2S1/eIF2α phosphorylation (**Fig. 1**). ATF4 sequentially activates a cascade of gene expression known as the integrated stress response that alleviates the cellular stress, or induces apoptosis in the case of prolonged ER stress. We and other groups (including those of Alan Diehl and Celeste Simon at the University of Pennsylvania, and Brad Wouters at the University of Toronto) previously reported that the EIF2AK3/PERK arm of the UPR is hyperactivated in tumors and contributes to cancer cell survival in response to ER stress induced by genetic and tumor microenvironmental conditions such as hypoxia and DNA damage.

Cellular transformation into the malignant phenotype is characterized by hyperactivation of oncogenes leading to unregulated cell cycle progression, proliferation and protein synthesis. As a result, the process of oncogenic activation and transformation can be regarded as a form of cell-autonomous, intrinsic stress. The *MYC* oncogene undergoes chromosomal translocation and gene amplification in many human cancers, especially in Burkitt lymphoma. Activated MYC upregulates genes involved in ribosome biogenesis resulting in substantial enhancement of protein translation and protein content, particularly in B-cells. Thus, we hypothesized that elevated protein synthesis due to increased MYC expression in tumor cells could lead to activation of UPR signaling, and asked what effect the induction of

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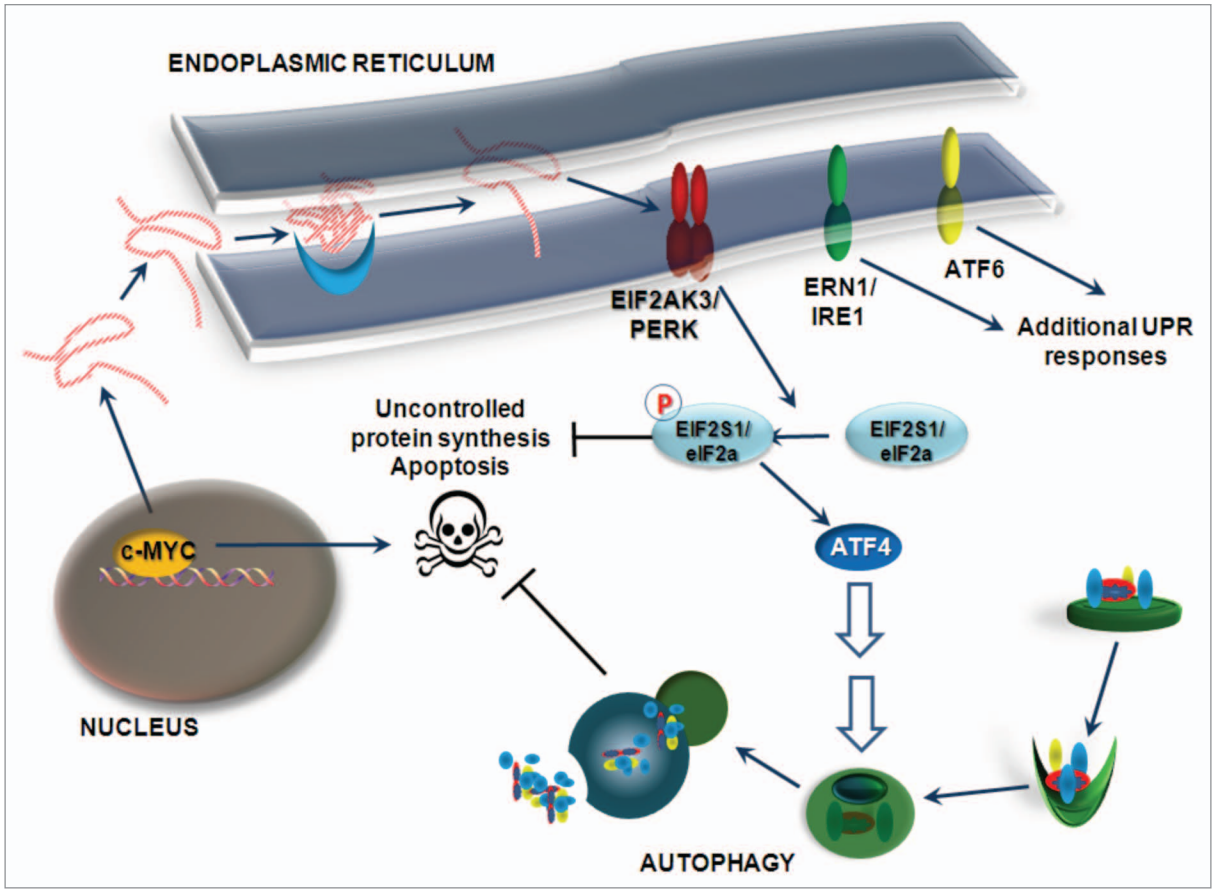

Figure 1. Model of how EIF2AK3/PERK-mediated autophagy promotes tumorigenesis by MYC. A hyperactive MYC increases protein synthesis and leads to accumulation of unfolded proteins in the ER, which activates the ER kinase EIF2AK3/PERK. EIF2AK3/PERK in turn phosphorylates EIF2S1/eIF2α, leading to attenuation of global protein synthesis. Conversely, phosphorylated EIF2S1/eIF2α promotes preferential translation of the transcription factor ATF4, which induces cytoprotective autophagy. Therefore, EIF2AK3/PERK-mediated reduction of protein synthesis, selective translation of certain mRNAs and induction of autophagy, work together to block cancer cell death.

such a stress response would have on cell survival.

Utilizing MYC-inducible cell lines, we observed that overexpression of MYC upregulated UPR signaling with increased EIF2AK3/PERK-mediated EIF2S1/eIF2α phosphorylation and *XBP1* mRNA splicing, a well-characterized target of ERN1/IRE1α. Analysis of publicly available gene expression databases from human lymphomas shows a similar induction of the UPR. Interestingly, the MYC-mediated UPR is critical for cell survival, as genetic ablation of EIF2AK3/PERK or blocking of EIF2S1/eIF2α phosphorylation significantly increases apoptosis. Such sensitivity of EIF2AK3/PERK knockout cells toward MYC-induced ER stress is also observed in vivo, as the absence of EIF2AK3/PERK in MYC-overexpressing cells fails to induce palpable tumors when injected in immunodeficient mice.

Multiple groups have now shown that UPR activation promotes cytoprotective autophagy in cancer cells under conditions such as hypoxia, nutrient limitation and oxidative stress. Of the three arms of the UPR, the EIF2AK3/PERK-ATF4 arm has been systematically linked to increased autophagosome formation and upregulation of autophagy genes such as *ATG5* and *MAP1LC3B* via ATF4 upregulation. We observed a similar EIF2AK3/PERK-dependent induction of cytoprotective autophagy in MYC-overexpressing cells. Isolated B cell lymphocytes from Eμ-myc transgenic mice, which are characterized by enhanced MYC expression, display increased UPR signaling with a concomitant increase in autophagy, further validating our observation in vivo. Conversely, blocking ER stress by introducing haplo-deficiency of *RPL24,* which encodes a ribosomal protein, in Eμ-myc transgenic mice (Eμ-myc; $L24^{+/-}$) restores normal levels of protein synthesis and consequently reduces autophagy; thereby demonstrating a causal role of increased protein synthesis for both ER stress and autophagy in the context of MYC upregulation.

Although chronic activation of the UPR can lead to cell death in conditions such as diabetes and neurological disorders, and following acute activation by pharmacological means (e.g., treatment with the glycosylation inhibitor tunicamycin, or the SERCA pump inhibitor thapsigargin), tumors have co-opted this pathway as a mechanism for adaptation to hypoxic and nutrient stress, which ultimately promotes cell survival. Interplay between various stress signaling pathways with the UPR allows cells to tailor the signaling toward specific stress conditions. Is autophagy the only mechanism by which the UPR

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can confer a survival advantage? This is unlikely, as even near complete ablation of critical autophagy mediators, such as ULK1 and ATG5, does not completely block cell survival in these systems. It should be noted that ATF4, via upregulation of a large number of mRNAs involved in antioxidant responses and nutrient biosynthetic pathways, can potentially confer stress adaptation by other means, not involving autophagy induction.

Another possible mechanism by which EIF2AK3/PERK regulates autophagy might involve the underlying translational control of mRNAs associated with phosphorylation of EIF2S1/eIF2α by EIF2AK3/PERK, providing cells a faster and energy-efficient way to express important regulators required for cellular stress adaptation. It will be quite informative to determine if EIF2AK3/PERK differentially upregulates translation of autophagy-related mRNAs following ER stress. Ultimately, it is formally possible that multiple autophagy genes and proteins may be modulated by MYC upregulation, combining transcriptional, translational and post-translational modifications.

Even though several critical questions remain to be answered, our findings suggest a key link between MYC-mediated oncogenic transformation and cancer cell survival via the EIF2AK3/PERK-mediated UPR and activation of autophagy. Thus, specific inhibitors of EIF2AK3/PERK could provide a new potential therapeutic target in MYC-overexpressing lymphomas as alternative or complementary strategies to the pleiotropic response of existing, less specific autophagy inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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