A Novel ER Stress-Independent Function of the UPR in Angiogenesis

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Tumors rely on the unfolded protein response (UPR) and angiogenesis to survive the metabolic stress of hypoxia. Karali et al. (2014) revealed that VEGF signaling engages UPR sensors in an unconventional manner that is independent of endoplasmic reticulum (ER) stress, mediated by mTOR signaling to promote endothelial cell survival and angiogenesis.

Cancer cells demand high metabolic rates to sustain proliferation and survival, especially in the hypoxic environment of solid tumors. This stressful condition engages distinct adaptive signaling pathways to maintain proteostasis and energy balance, including the hypoxia-inducible factor (HIF), the unfolded protein response (UPR), and macroautophagy. Coupled to these molecular changes, proangiogenic factors such as VEGF are required to remodel the microenvironment and induce neovascularization in solid tumors. In this issue of *Molecular Cell*, Karali et al. (2014) report a novel mechanism to activate the UPR by VEGF signaling in endothelial cells in an unconventional manner that is crucial to induce angiogenesis and cell survival.

The UPR consists of a dynamic homeostasis network essential to maintain the function of specialized secretory cells through the buffering of endoplasmic reticulum (ER) stress levels. In addition, accumulating observations indicate that the UPR has ER stress-independent roles in lipid metabolism, inflammation, and cell differentiation. Activation of the UPR is a salient feature of several pathological conditions, including cancer, where it can drive tumor growth, cell transformation, invasion, and tumor dormancy. The UPR is initiated by the activation of three main stress sensors, PERK, IRE1α, and ATF6, which control the expression of downstream transcription factors named ATF4, XBP1, and ATF6f, respectively. Recent advances have identified drugs that target and inhibit the enzymatic activity of PERK and IRE1α, having potent antitumor activities in several preclinical models of cancer (Hetz et al., 2013). Although most of the studies have linked the activity of the UPR to the survival of cancer cells under the hypoxic condition inside the tumor, accumulating evidence suggests that IRE1α may also contribute to metastasis and angiogenesis (Auf et al., 2010; Drogat et al., 2007).

Angiogenesis refers to the process of remodeling existing blood vessels, involving sprouting, migration, and proliferation of endothelial cells. Angiogenesis is regulated by several factors such as FGF, PDGF, IL-8, and VEGF. VEGF is the most important proangiogenic driver secreted by tumor cells, having an autocrine (tumor cells) and paracrine (endothelial cells) effect. The upregulation of VEGF in tumors is controlled in part by the induction of HIF-1α; however, recent studies have uncovered a relevant role of the UPR in modulating VEGF expression, observing that XBP1s and ATF4 can directly bind and transactivate its promoter (reviewed in Paridaens et al., 2014). In addition, characterization of IRE1α knockout animals revealed that a major phenotype explaining the lethality of the model is the reduction of VEGF levels in placenta (Iwawaki et al., 2009). Remarkably, recent reports suggest that HIF-1α and the UPR may potentiate to control VEGF and angiogenesis (Pereira et al., 2014). XBP1 was shown to drive breast cancer progression through the assembly of a transcriptional complex with HIF-1α that regulates the expression of its target genes, including VEGF (Chen et al., 2014), demonstrating a pivotal role of both stress pathways to tumor growth and angiogenesis.

An additional role of UPR as a downstream effector of VEGF signaling in the targeted cell has recently been suggested (Figure 1). VEGF was shown to induce endothelial cell proliferation in an IRE1α/XBP1- and ATF6-dependent manner in models of retinal vasculogenesis and ischemia (Liu et al., 2013; Zeng et al., 2013). These effects were specific since FGF did not activate XBP1. Karali et al. (2014) explored in detail the mechanisms connecting VEGF receptor signaling and UPR activation. Using a systematic approach, the authors identified an extremely rapid, robust, and transient activation of the three main ER stress sensors upon VEGF treatment in endothelial cells, involving the upregulation of classical UPR target genes. Surprisingly, UPR activation occurred in the absence of molecular signatures associated with protein misfolding in the ER. Using pharmacological and genetic approaches, the authors demonstrated that PLCγ and mTORC1 signaling were responsible for the activation of UPR sensors downstream of VEGF. This signaling crosstalk had a functional impact on endothelial cell survival and angiogenesis. The authors demonstrated that the prosurvival effects of VEGF were mediated by ATF6 and PERK, but not IRE1α, through the phosphorylation of AKT by mTORC2. Key mediators of ER stress-dependent apoptosis such

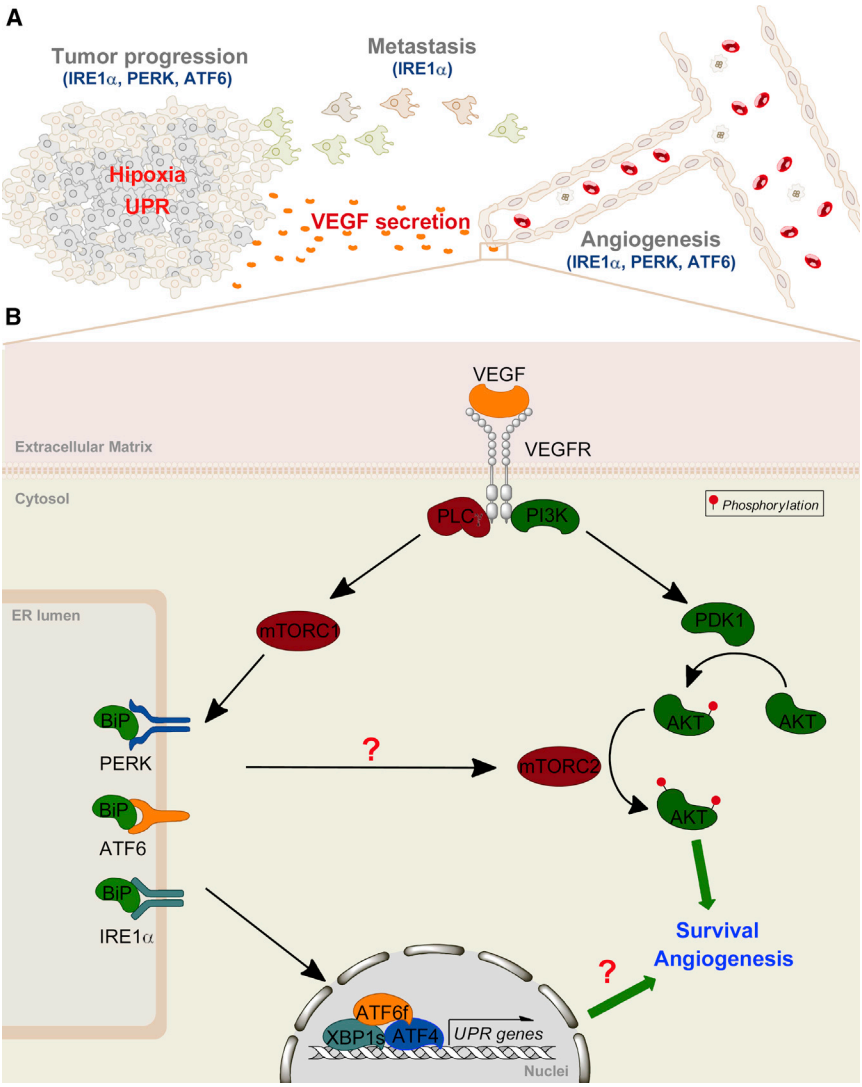

Figure 1. An Interconnection between VEGF Signaling and the Unfolded Protein Response in the Control of Endothelial Cell Survival and Angiogenesis

(A) ER stress sensors IRE1α, PERK, and ATF6 have distinct roles in cancer, mediating tumor growth and cell survival. The hypoxic conditions in solid tumors engage the UPR and HIF-1α, a phenomenon required for angiogenesis.

(B) VEGF signaling engages the activation of UPR stress sensors in endothelial cells through PLCγ and mTORC1. ATF6 and PERK are required to drive endothelial cell survival and angiogenesis, possibly through a mechanism involving phosphorylation of AKT by the mTORC2 complex and other effects.

as CHOP were not induced by VEGF. These observations were recapitulated in vivo on an animal model of angiogenesis. Thus, this study suggests that components of the UPR operate as downstream "VEGF signaling modules" that can amplify and control important physiological outputs beyond their classical role on the ER stress response.

One of the major observations of this study is the fact that activation of UPR stress sensors by VEGF did not involve dissociation of the repressive interaction with the ER chaperone BiP, a common factor driving activation of all ER stress sensors. Since PERK, IRE1α, and ATF6 are structurally dissimilar in their cytosolic regions, it remains to be determined if common mechanisms are involved in their activation by mTORC1 and PLCγ signaling. Interestingly, a few studies have indicated that alternative modes of activation of UPR sensors may exist independent of ER stress (reviewed in Hetz, 2012). For example, phosphorylation of IRE1α at serine 72 upon high-glucose stimulation results in its unconventional activation, regulating insulin biosynthesis in pancreatic β cells. Similarly, IRE1α is rapidly activated by Toll-like receptors through the adaptor protein TRAF6 and reactive oxygen species signaling, triggering the production of proinflammatory cytokines. Finally, posttranslational modifications of IRE1α and PERK by ADP-ribosylation were recently proposed to induce their activities in the absence of ER stress. A deeper biochemical characterization is needed to define at which molecular stage VEGF signaling engages the UPR. Accumulating evidence indicates that distinct protein complexes are assembled at the level of IRE1α, PERK, and ATF6, regulating the amplitude and kinetic behavior of their signaling through the assembly of a protein platform referred to as the UPRosome (Hetz, 2012). It remains to be established if the engagement of UPR signaling by VEGF is mediated by the interaction with a novel common regulator. However, it is still possible that VEGF signaling may activate a subpopulation of UPR stress sensors that is not detected with classical biochemical approaches and may involve higher rates of physiological ER stress due to secretion of metalloproteases and extracellular matrix proteins. Strategies to attenuate ER stress (i.e., chemical chaperones) could be employed to discard the contribution of protein folding stress to the activation of the UPR by VEGF. In summary, novel functions of the UPR are emerging in different pathological and physiological contexts that may dissociate from the classical role in alleviating ER stress. These novel activities of the UPR involve a complex signaling crosstalk with other important stress pathways, having global implications to fundamental cellular processes, as highlighted here for endothelial cell survival and angiogenesis.

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Ubiquitin Puts Actin in Its Place

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In this issue of *Molecular Cell*, Yuan et al. (2014) report that the Cul3-KLHL20 E3 ubiquitin ligase regulates protein anterograde transport from the trans-Golgi network (TGN) by facilitating localized actin assembly at the TGN through K33-linked ubiquitination of coronin 7.

Eukaryotic cells are spatially and functionally divided by membrane expansion and compartmentalization. Thus, intracellular trafficking is critical for proper protein distribution and function, and its regulation is a central issue in cell biology. Ubiquitination, a posttranslational modification in which a small 76 amino acid protein is covalently conjugated to lysine residues on substrate proteins by a three-step enzymatic cascade (E1, E2, E3), plays important roles in protein trafficking. It is well established that ubiquitination of membrane proteins facilitates their endocytosis and endosome-to-lysosome transport through the function of many ubiquitin-binding adaptor proteins (Hicke and Dunn, 2003). Recently, we reported a new role for ubiquitin in the regulation of Retromer-mediated retrograde transport (endosome-to-TGN) and protein recycling (Figure 1A) (Hao, et al., 2013). In this issue of *Molecular Cell*, Yuan et al. (2014) elucidate a role for atypical K33-linked ubiquitin in the regulation of protein anterograde transport from the trans-Golgi network (TGN) by promoting localized actin assembly by coronin 7 (Crn7) (Figure 1B).

Yuan et al. (2014) observed that the Cul3 E3 ubiquitin ligase substrate adaptor KLHL20 localized to the TGN. The precise mechanism regulating its localization is unclear but is sensitive to Brefeldin A, suggesting a role for Arf or Arf-like GTPases. Given that KLHL20 localized to the TGN, the authors anticipated that KLHL20 and the associated Cul3 E3 ubiquitin ligase may regulate anterograde transport from the TGN. Indeed, they found that KLHL20-depleted cells are defective in anterograde transport of several (but not all) proteins, and the primary cause for this deficiency is the inability to assemble post-Golgi carrier tubules. To understand the molecular mechanism(s) behind Cul3-KLHL20 function in assembly of post-Golgi carrier tubules and anterograde transport, Yuan et al. performed a yeast two-hybrid screen for possible KLHL20 interactors that could be substrates for ubiquitination. Through this approach, they identified Crn7 as a substrate of the Cul3-KLHL20 ubiquitin ligase. Crn7 is a member of the coronin family of proteins that bind actin to stabilize actin filaments and regulate actin dynamics (Uetrecht and Bear, 2006). Consistent with Yuan et al., Crn7 has previously been shown to localize to the TGN and regulate anterograde transport (Rybakin et al., 2006). Interestingly, Yuan et al. found that Cul3-KLHL20 does not promote Crn7 degradation, but rather promotes atypical K33-linked ubiquitination of Crn7. The authors go on to show that K33-linked ubiquitination of Crn7 results in its translocation to the TGN through the interaction with the ubiquitin-interacting motifs (UIMs) of Esp15, a clathrin adaptor protein. Importantly, Crn7 TGN localization facilitates the generation and elongation of post-Golgi carrier tubules through the stabilization of F-actin. Significantly, artificial targeting of Crn7 to the TGN rescued TGN actin assembly and post-Golgi transport in KLHL20-RNAi cells, suggesting that the localization of Crn7 to the TGN is the critical function of KLHL20-mediated Crn7 ubiquitination.

The findings of Yuan et al. (2014) add to the growing body of literature that