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Editorial

The UPR as a survival factor of cancer cells: More than folding proteins?

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Organelle stress is a hallmark of several disease conditions including diabetes, neurodegeneration and cancer. Special attention has been given to the endoplasmic reticulum (ER) in cancer and tumor progression [1]. The ER expresses a complex system of protein chaperones, foldases and co-factors that assist protein folding in the secretory pathway. An oxidizing environment relative to the cytoplasm is maintained by these enzymes to catalyze crucial post-translational modifications and ensure proper protein function [2]. The ER also serves as the major calcium store, and where the lipid biosynthesis occur within. As a result, it plays a crucial role in organelle biogenesis, cellular differentiation and apoptosis. A number of physiological and pathological conditions interfere with the function of the ER and therefore lead to abnormal protein folding within the lumen, resulting in a condition termed 'ER stress' [2]. ER stress activates the unfolded protein response (UPR), an integrated intracellular signaling cascade that aims to restore ER homeostasis. In doing so, activation of the UPR attenuates the rate of protein synthesis, and upregulates genes encoding chaperones, foldases, ER-associated degradation (ERAD) proteins, autophagy regulators, and ER membrane biogenesis enzymes [5]. As a result, UPR signaling minimizes the accumulation and aggregation of unfolded proteins by increasing the functional capacity of the ER to facilitate folding and to degrade abnormal proteins.

Many ER chaperones and foldases display an abnormal expression pattern in human tumor samples. However, the actual contribution of the pathway to different types of cancer and disease stages remains speculative. Classical ER stress-inducible chaperones, such as GRP78 and GRP94, are increased in at least 10 different cancers, including lung, breast, liver and colon cancer (review in [1,3]). Besides catalyzing protein folding, these chaperones directly affect the ability of a cell to adapt to ER stress and thus their susceptibility to apoptosis under chronic stress. Similarly, disulfide isomerases (PDI), such as PDI and GRP58/ERp57, and their co-factor ERO1, are induced in some forms of cancer including skin carcinomas and breast carcinomas. Calnexin, an essential protein of the ER protein quality control system, is also induced in breast cancer (review in [1,3]). Together, these examples illustrate the possible application of UPR target gene profiling in cancer samples for diagnosis, prognosis and classification of cancer cells.

There are at least three classes of ER resident stress sensors in mammals to initiate UPR signaling cascades: double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring kinase 1 (IRE1) (see detailed reviews in [2,5]). Activation of PERK phosphorylates and inhibits the eukaryotic translation initiation factor 2α (eIF2α) decreasing ER protein synthesis. Alternatively, eIF2α phosphorylation augments the specific translation of transcription factor ATF4, which is essential for the upregulation of many UPR genes such as CHOP/GADD153 and GRP78/BiP. Activated ATF6 translocates from the ER to the Golgi apparatus where it undergoes proteolytic processing to release its cytosolic domain, which then moves to the nucleus to function as an active transcription factor [2]. This branch of the UPR became increasingly complex after recent studies identified a series of ATF6 homologues modulated by ER stress including OASIS, CREBH, LUMAN/CREB3, CREB4, and BBF2H7. IRE1 is a serine/threonine protein kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding the transcription factor XBP-1. A 26 nucleotide intron of XBP-1 mRNA is removed resulting in a shift of the mRNA reading frame and the expression of XBP-1s, a more stable and potent transcriptional activator. XBP-1s controls the upregulation of a subset of UPR genes related to folding, ERAD, protein quality control, ER translocation, lipid biogenesis and many others [5]. A recent study identified XBP-1 as a master regulator of cellular differentiation programs in different tissue contexts [4]. Besides, IRE1 is modulated by several co-factors and controls the activation of signaling pathways mediated by ERK, JNK and NF-kB through the binding of adaptor proteins. The complex IRE1 signaling platform has been referred as the *UPRosome* [5,6]. In summary, by controlling the expression of a set of specialized transcription factors, each of the stress sensors transmit information about protein folding status from the ER to the nucleus to initiate global changes in gene expression and potentially restore cellular homeostasis.

A defining characteristic of solid tumors is the capacity to divide aggressively and disseminate metastases under conditions of nutrient deprivation and limited oxygen availability. These microenvironmental stresses may affect protein folding at the ER by decreasing glucose supplies and lowering ATP production. It is

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well known that hypoxia causes a strong increase in the expression of several ER chaperones and foldases (reviewed in [7,8]). Under chronic or irreversible ER damage the UPR ultimately initiates apoptosis to eliminate damaged cells. Along this line, while tumor hypoxia is a physiologic barrier to cancer cell survival, it drives malignant progression by generating selective pressure on cells that can adapt to this stress and further proliferate. The functional link between ER stress responses and tumor growth was shown by targeting the expression of the ER stress chaperone GRP78 in cancer models (reviewed in [9]). Direct demonstration that the UPR is necessary for tumor growth came from studies examining the role of the IRE1α/XBP-1 pathway during hypoxia and in cancer models (reviewed in [7,8]). XBP-1-deficient mouse embryonic fibroblasts are severely impaired for survival following exposure to hypoxia [10]. Strikingly, XBP-$1^{-/-}$ cells do not grow into tumors. The IRE1α/XBP-1 pathway have been proposed to be a target of the chemotherapeutic agent PS-341, a proteasome inhibitor that blocks IRE1-mediated splicing [11]. *In vivo* analysis of XBP-1s activity has demonstrated that even relatively small tumors have detectable levels of XBP-1 splicing. Another study demonstrated the activation of PERK and its downstream target eIF2α during hypoxia. Mouse embryonic fibroblasts stably expressing a dominant negative PERK allele or PERK-deficient fibroblasts exhibited a remarkable attenuation of tumor growth in xenograft models [12,13]. Finally, a recent study demonstrated that IRE1 is a common determinant linking hypoxia- and hypoglycemia-dependent responses to the upregulation of vascular endothelial growth factor-A (VEGF-A), indicating an important role of this UPR branch in both angiogenic switch and tumor development [14]. These examples illustrate the essential role of the UPR as a survival pathway for solid tumor growth most likely due to adaptations to conditions of poor oxygen and nutrient supplies.

Although a number of studies have reported UPR activation in a variety of solid tumors, the role of the UPR in different forms of cancer or metastasis remains poorly characterized. It is not clear whether UPR activation in cancer is solely due to microenvironmental stress or other mechanisms. In contrast to this prevailing view, a recent bioinformatic analysis of gene expression profiling reported that targets of the three UPR branches appear selectively downregulated in mouse models of prostate tumorigenesis [15]. UPR repression was observed at different disease stages, from low-grade prostatic intraepithelial neoplasia to high-grade prostatic intraepithelial neoplasia and then invasive adenocarcinoma with metastatic potential (cancer) [15]. These data suggest that the initial prediction about the role of ER stress in cancer is much more complex than anticipated. Recently, Carrasco et al. demonstrated that a phenotype similar to multiple myeloma is generated spontaneously in transgenic mice with Em-directed expression of the active XBP-1s form [16]. This mouse presented elevated serum Ig and skin alterations related to the disease in addition to other disease parameters. Remarkably, Em-xbp-1s transgenics developed aberrant expression of known human multiple myeloma genes, indicating that some cancer-related genes may be directly regulated by the UPR. These findings suggest that enhanced expression of XBP-1 may confer a survival potential to aberrant cells of the immune system, promoting cancer progression. In this line, XBP-1 was shown to be essential for the survival and development of dendritic cells and plasma B cells [5,17] *in vivo*. The influence of the UPR on leukemogenesis remains largely uninvestigated. In this volume of *Leukemia Research*, Tanimura et al. investigated for the first time the expression of UPR makers in cells expressing the oncogene Bcr-Abl [25]. The Bcr-Abl fusion protein plays a central role in chronic myeloid leukemia and Philadelphia chromosome-positive acute leukemia. The expression of XBP-1 and the chaperone GRP78/BiP was increased in these leukemia cells. Targeting IRE1 and ATF6 demonstrated that these stress sensors modulate the ability of Bcr-Abl to protect leukemia cells from apoptosis triggered by anti-cancer drugs. More importantly, high expression of multiple UPR-related genes was observed in primary leukemia cells from Philadelphia chromosome-positive cells derived from human patients [25]. The primary source of ER stress in leukemia cells remains to be established. Overall, this article suggests an important role of the UPR in promoting leukemia cell survival, a disease context that remarkably differs from the conditions for solid tumor formation. Whether or not the effects of the UPR in leukemia are related to protein folding stress is not known. Interestingly, recent data indicates that ER stress sensors physically interact with proteins of the apoptosis machinery (reviewed in [6,18]) suggesting a direct link between the UPR and apoptosis. It remains to be determined if these interactions contribute to anti-apoptotic effects of the UPR in leukemia that are beyond classical ER stress-related functions.

Analysis of the phenotype of knockout mice for essential UPR components revealed unexpected results that, in many cases, were not directly related to the predicted function of the UPR. Although PERK and IRE1α share functionally similar luminal sensing domains and are both activated in cells treated with ER stress inducers *in vitro*, they are selectively activated *in vivo* by the physiological stress of unfolded proteins. These differences were particularly observed when secretory cells such as B lymphocytes and pancreatic β-cells were studied (reviewed in [5]). The differences in terms of tissue-specific regulation of the UPR *in vivo* may be explained by the formation of tissue/context-specific regulatory protein platforms (i.e. distinct *UPRosome* complexes). It remains to be determined if differential UPR signaling occurs in specific types of cancer and disease stages. Finally, non-UPR roles for XBP-1 have been recently shown in different organs including control of inflammatory responses in intestine paneth cells [19], and lipid biogenesis in the liver [20]. In the line of Tanimura's study [25], these data may be considered when the role of the UPR is investigated in cancer.

The data discussed so far suggests that compounds inhibiting targets such as PERK and IRE1α could be considered for cancer therapy, as long as their therapeutic index is acceptable and the expected secondary effects are low (reviewed in [21]). Proteasome inhibitors such as Velcade have been shown to induce ER stress and activate the pro-apoptotic effects of PERK, contributing to their cytotoxic activity against cancer cells. These anti-cancer activity is enhanced by RNA-interference-mediated reductions in GRP78/BiP [1,3]. Derivates of brefeldin A, another ER-stress-inducing agent, have been explored for cancer therapy. Recent studies indicate that PDI inhibitors have therapeutic effects in cancer models [22]. Another way of exploiting ER and UPR components was recently described by Kroemer's group. A set of anti-cancer drugs triggered the expression and redistribution of GRP58 and calreticulin into the cell surface, a phenomena that was exploited for immunotherapy against cancer *in vivo* [23,24]. Overall, the data discussed here and the current study from Tanimura et al. [25] gives clues about the multiple roles of the UPR and ER stress in cancer, identifying a new series of potential therapeutic avenues to examine as anti-cancer agents.

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