Integrated Endoplasmic Reticulum Stress Responses in Cancer

Michel Moenner,1,2 Olivier Pluquet,3,4 Marion Bouchechareilh,1,2,3,4 and Eric Chevet5,4

1Institut National de la Sante et de la Recherche Medicale, E0113; 2Univ Bordeaux 1, Talence, France and 3Institut National de la Sante et de la Recherche Medicale, U889, Team Avenir; and 4Universite ´ Bordeaux 2, Bordeaux, France

Abstract

The endoplasmic reticulum (ER) has emerged as a major site of cellular homeostasis regulation, particularly in the unfolded protein response, which is being found to play a major role in cancer and many other diseases. Here, we address ER-mediated signaling and regulations in the context of environmental challenges in cancer, such as hypoxia, angiogenesis, and chemotherapeutic resistance, and we discuss how ER-resident molecular machines become deregulated and involved in cancer-related pathology. Further exploration of how the ER senses, signals, and adapts to stress may redefine and deepen our understanding of its functions in cancer pathobiology.

In many instances, deregulation of endoplasmic reticulum (ER) homeostasis has been correlated with pathologic states and particularly with cancer. These events can occur at several levels and are reflected by alterations of ER protein expression levels, posttranslational modifications, or abnormal secretion of ER-resident proteins. In addition, tumor microenvironments can lead to perturbation of ER functions. In particular, hypoxia, nutrient limitation, or low pH is known to promote the activation of specific signaling pathways, which emanate from the ER on accumulation of misfolded/unfolded proteins in its lumen (1). This adaptive response, named the unfolded protein response (UPR), tends to limit the de novo entry of proteins in the ER and facilitate both ER protein folding and degradation. The ability of the cells to handle these stresses may therefore condition their intrinsic capacity to adapt for cell survival or, alternatively, to enter apoptotic or senescence program through ER-associated machineries (2). We discuss the effect of these mechanisms on tumor plasticity and the clinical perspectives associated to the resistance they confer to chemotherapeutics. An integrated representation of these mechanisms is shown in Fig. 1.

Modulation of ER-Associated Functions in Cancer

Increased expression of ER stress-related genes and proteins. Many ER-resident proteins display an altered expression pattern in cancers. A consistent illustration for this is provided by the ER chaperones glucose-regulated protein (GRP) 78/BiP and GRP94 whose expression is increased in at least 10 different cancers, such as lung, breast, and colon cancers, or hepatocellular carcinoma (HCC). Both chaperones have been shown to play a role in the ability of the cells to resist to proapoptotic challenges. Another ATPase chaperone up-regulated in several cancers is GRP170/ORP150 (3), a protein whose function is associated to the extracellular release of vascular endothelial growth factor-A (VEGF-A) under hypoxia (3). Other ER-resident proteins that participate to secretory protein folding also display an altered expression in various cancers. This is the case for the protein disulfide isomerase (PDI)-like protein Erp29 and for the ER oxidase Ero1 in basal skin carcinomas and breast carcinomas, respectively (4, 5). The oligosaccharyltransferase complex component defender against death-1 and the lectin-like chaperone calreticulin (6) are overexpressed in HCC. The expression of the calreticulin orthologue calnexin is up-regulated in various cancer models, such as breast cancer, and this correlates with enhanced resistance to stress (7). In addition, two UPR-dependent transcription factors were either up-regulated at the mRNA and protein levels [activating transcription factor (ATF) 6] or underwent mRNA splicing [X-box binding protein-1 (XBP-1)] in HCC (8). Finally, the valosin-containing protein p97, which is involved in ER-associated degradation, has been identified as a prognostic factor for gingival squamous cell, lung, and esophageal carcinomas (9) as well as in HCC (10).

Posttranslational modifications. Similar to what has already been reported for the expression of ER-resident proteins, various posttranslational modifications occur under specific environmental conditions targeting the ER but have also been found constitutively present in cancers. For instance, various KDEL-containing proteins, such as GRP78/BiP, GRP94, or PDI (and related proteins), have been found to be secreted in cancer cells (6). This may only reflect a saturation of the KDEL receptor ER retrieval system but could also be functionally significant. This is well illustrated by calreticulin, which is found in the serum of cancer patients as revealed by the presence of autoantibodies (11) and protein fragments (6). In addition, proteolytic fragments specific of BiP and PDI have also been identified in the serum of HCC patients (6). In addition to proteolytic cleavages that may reflect the deregulation of proteases from the secretory pathway (12) and phosphorylation events (see below), glycosylation of secretory proteins is altered in many types of cancers (13).

Activation of the UPR. This adaptive response activated on accumulation of misfolded/unfolded proteins in the ER (1) is mediated by at least three ER proximal sensors, PKR-like ER kinase (PERK), inositol requiring enzyme-1 (IRE1), and ATF6. PERK is a serine threonine kinase that phosphorylates eIF2α on stress and consequently attenuates global protein translation. Interestingly, PERK+/− mouse embryonic fibroblast (MEF) cells or HT29 colon carcinoma cells expressing a dominant-negative PERK were more sensitive to hypoxic stress than parental MEFs and HT29 cells (14). These results were confirmed in vivo in a xenograft tumor model. Finally, PERK was found to functionally interact with the oncogenic protein NCK as part of its regulatory mechanisms (15). A similar interaction was also observed for IRE1 as a signaling intermediate event toward ERK1 activation (16). Besides its kinase

Note: We apologize to those whose work was not cited due to space limitation. Requests for reprints: Eric Chevet, Institut National de la Sante et de la Recherche Medicale, U889, Team Avenir, 33076 Bordeaux, France. E-mail: eric.chevet@ubordeaux2.fr or Michel Moenner, Institut National de la Sante et de la Recherche Medicale, E0113, Talence, France. E-mail: m.moenner@angio.u-bordeaux1.fr.
activity, IRE1 also displays a RNase domain that is involved in the unconventional splicing of XBP-1 mRNA (1) but also in the post-transcriptional regulation of a specific mRNA subset (17). XBP-1–deficient MEFs are sensitive to hypoxia compared with parental cells and these results were also confirmed in vivo in a xenograft model in severe combined immunodeficient mice (18). Recently, it was also shown that IRE1 signaling was enhanced by the protein tyrosine phosphatase 1B (19), also involved in breast cancer regulation in vivo. Finally, on ER stress, ATF6 translocates to the Golgi apparatus where it is cleaved by two proteases, S1P and S2P (1), thus releasing a transcription factor that promotes the transcription of ER stress-related genes (1). In HCC, compared with matched noncancerous liver tissues, an increase in ATF6 mRNA level and of the protein nuclear translocation can be observed (8).

**Figure 1.** Schematic representation of ER stress responses in cancer. This integrated mechanism involves the UPR to establish a communication axis between the ER and the nucleus, the ER-associated degradation machinery, the ER export machinery, and an interface with mitochondria involved in the activation of proapoptotic mechanisms. The integrated and balanced management of these various pathways on either genetic changes leading to uncontrolled cell proliferation or environmental challenges yields to enhanced tumor growth.

**ER stress responses and anticancer therapies.** The significance of the ER-dependent pathways to cancer development also extends to clinical applications. One of the most recent examples is illustrated by the discovery of the ER-mediated proapoptotic effects of sorafenib in leukemia cells (20). In addition, during tumor development, several ER stress activators (hypoxia and low glucose) are known to induce resistance to chemotherapy through processes that are suggestive of ER-dependent mechanisms (21). Moreover, in addition to proapoptotic activities, several anticancer agents also generate survival responses through activation of the UPR (22). Down-regulating the ability of tumor cells to activate these survival responses in association with the use of antitumor drugs provides new multitherapeutic perspectives against cancer development (22).

**ER-Dependent Tumor-Specific Signaling Alterations**

**ER-Associated Cell Death and Survival**

**ER-localized apoptosis/survival mediators.** Thus far, several ER-localized proteins are directly involved in the mediation of proapoptotic mechanisms in either UPR-dependent or UPR-independent fashions (2). Perturbation of ER homeostasis leads to the alteration of the resident molecular machineries and consequently affects the UPR (1). A strong link between ER stress signaling and apoptotic pathways has been established through the discovery of a physical interaction between Bak/Bax and the cytosolic domain of IRE1 (23). This interaction is necessary for IRE1 activation (23). In addition, the association of calnexin with the caspase-8 substrate BAP31 also regulates proapoptotic/antiapoptotic mechanisms in breast cancer cells (7). Besides the traditional BCL2 family members whose ER localization was shown to be an important apoptosis regulator, novel ER-localized
mediators were identified, such as BAK inhibitor-1 (2), which is involved in ischemia/reperfusion-mediated cell death processes, and the BCL2 binding protein ElF24/PiG8, which is involved in breast cancer regression. In addition, ER stress was recently reported to mediate cell death through the induction of Bim (24). Consequently, activation mode of these different pathways may be context (e.g., cell microenvironment) or cell/tissue specific and should also occur in tight conjunction with other ER-resident functional machineries.

P53. The tumor suppressor protein P53 is a transcription factor that mediates several biological effects, including growth arrest, senescence, and apoptosis, in response to a wide range of stress signals (25). The regulation of P53 in ER-stressed cells remains unclear. Some studies reported that ER stress stabilizes P53 in HCT116 cells and promotes P53-dependent apoptosis through the transcriptional activation of Puma and Noxa in MEFs. However, other findings reported that ER stress antagonizes drug-induced nuclear accumulation of P53 and inhibits P53-mediated apoptosis in response to 5-fluorouracil, suggesting an inhibitory role of ER stress on P53 functions. This idea is supported by data showing that ER stress (a) enhances P53 nuclear export through the coordinate action of HDM2 and glycogen synthase kinase-3β (26), a target of valproate, and (b) promotes P53 degradation in the cytoplasm by the ER-resident ubiquitin ligase synoviolin. Such discrepancy may reflect the differential capacity of cell lines to regulate P53 localization and degradation on ER stress. Given the protective role of ER stress in normal cells, P53 down-regulation on ER stress would prevent apoptosis. By contrast, in cancer cells, it is tempting to speculate that P53 destabilization on ER stress might provide growth advantage. Further experiments are required to determine whether ER stress might contribute to tumor progression through down-regulation of P53 in tumors that retain wild-type P53.

ER-Dependent Proangiogenic/Antiangiogenic Mechanisms

ER stress responses may also be beneficial for tumor development by contributing to the neovascularization process. Various activators of the ER stress response up-regulate the potent proangiogenic factor VEGF-A (27), whose secretion is dependent on the ER chaperone ORP150 (3). A functional link was also established between activation of the PERK/eIF2α/ATF4 pathway by ER stressors and the up-regulation of VEGF-A mRNA, although the protein expression of the growth factor remained low (14). A direct relationship was, however, established between activation of the PERK/eIF2α pathways and tumor neovascularization process in vivo through transcriptional or/and translational regulations of gene expression (28). The transcription factor nuclear factor-κB induced downstream of PERK/eIF2α activation under hypoxia may be associated to this effect through activation of JUNB in a hypoxia-inducible factor–independent manner. IRE1 branch of the UPR was also shown to be involved in tumor angiogenesis. Indeed, hypoxia/hypoglycemia-mediated VEGF-A up-regulation was impaired in tumor cells expressing a dominant-negative form of IRE1. This was confirmed in vivo as these cells formed poorly vascularized and more invasive tumors in an orthotopic glioma model (29). In addition to the reported unconventional splicing of XBP-1 mRNA, IRE1-associated RNase activity also selectively cleaves a subset of transcripts that includes SPARC mRNA in Drosophila S2 cells (17).

Although this effect has not yet been confirmed in vertebrates, it may be of general importance in angiogenic regulatory processes, as orthologous encoded SPARC proteins are involved in the neovascularization process. In a MEF-derived tumor model, XBP-1 was also shown to contribute to tumor growth in vivo while having little effect on VEGF-A expression (18). This further suggests that IRE1 mediates part of its angiogenic effect through XBP-1–independent pathways, although heterogeneous responses may also be considered according to the cell type. Finally, although no direct correlation has been yet established in vivo, calreticulin fragments showed antiangiogenic properties both in vitro and in vivo (30) and were also found in serum of HCC patients (6).

In conclusion, in this review, we have summarized several events linked to the ER, which are altered in various cancers. These integrated mechanisms, although they may reflect tumor cell specificities related to their metabolism or proliferation ability, could represent interesting avenue for therapeutic strategy as their inhibition/activation would promote cell death mechanisms or prevent tumor cell adaptation to environmental challenges.

Acknowledgments

Received 5/9/2007; revised 8/1/2007; accepted 8/8/2007.

Grant support: Institut National de la Sante et de la Recherche Medicale (Avenir) and E.U. Marie Curie International Reintegration Grant MIRG-044957 (E. Chevet) and Association de la Recherche sur le Cancer grant 3694 (E. Chevet and M. Moenner). O. Plaquet and M. Bouchecarell are supported by an Institut National de la Sante et de la Recherche Medicale postdoctoral fellowship and by a region Aquitaine doctoral studentship, respectively.

References

Cancer Research


Integrated Endoplasmic Reticulum Stress Responses in Cancer

Michel Moenner, Olivier Pluquet, Marion Bouchecareilh, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/22/10631

Cited articles
This article cites 30 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/22/10631.full.html#ref-list-1

Citing articles
This article has been cited by 47 HighWire-hosted articles. Access the articles at:
/content/67/22/10631.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.