cytokines, TNF-α or IL-1, induces IκB phosphorylation and ubiquitin-dependent proteasomal degradation resulting in nuclear entry of NF-κB dimers to initiate target gene transcription. IκB phosphorylation is catalyzed by the IκB kinase (IKK) complex.[15] In the study described above,[14] numerous genes leading to activation of NF-κB were up-regulated in heterozygotes, as were many genes targeted by NF-κB including the proinflammatory chemokines IL8 and CFLAR (Table 1). Caspase 4 and caspase 8 were also up-regulated; however in the presence of induced CFLAR the latter was most likely inactive.[14]

Thus, these results suggest significantly higher activation of the NF-κB pathway in GC heterozygotes of miR-146a as compared to GG homozygotes. We speculate that this phenomenon might promote tumor progression and be responsible for the increased risk of PTC in heterozygotes.

A possible stimulus for an induction of the NF-κB pathway in thyroid might be ionizing radiation, the strongest environmental risk factor for thyroid cancer. Ionizing radiation is also used for the treatment of thyroid cancer. It induces DNA damage, e.g. double strand DNA breaks, and leads to significant activation of p53 and apoptosis. NF-κB is rapidly activated in the course of an immediate early response of thyroid cancer cells to ionizing radiation leading to increased thyroid cell survival. In an undifferentiated thyroid cancer cell line inhibition of NF-κB increased radiosensitivity and enhanced ionizing radiation-induced apoptosis.[16] Cells that do not undergo apoptosis after ionizing radiation are prone to genetic instability, increased rate of mutation, and accelerated cancer evolution. We suggest that thyroid cells heterozygous at miR-146a present higher activity of NF-κB and lower potential of inhibition of this pathway and therefore are more likely to survive after ionizing radiation.

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Cell Cycle Features:

Turning off the unfolded protein response

An interplay between the apoptosis machinery and ER stress signaling

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A number of conditions interfere with oxidative protein folding processes in the endoplasmic reticulum (ER) lumen,[1] resulting in a cellular condition referred to as "ER stress." Adaptation to ER stress is mediated by engagement of the unfolded protein response (UPR), an integrated signal transduction pathway that transmits information about protein folding status in the ER lumen to the cytosol and nucleus to increase protein folding capacity. Conversely, cells undergo apoptosis if these mechanisms of adaptation and survival are insufficient to handle the unfolded protein load.

The UPR signals through three distinct stress sensors including IRE1α, ATF6 and PERK.[1] Although PERK and IRE1α share functionally similar ER-luminal sensing domains and are both simultaneously activated in cellular paradigms of ER stress in vitro, they are selectively activated in vivo by the physiological stress of unfolded proteins. The differences in terms of tissue-specific regulation of the UPR may theoretically be explained by the formation of distinct regulatory protein complexes. This concept is supported by the recent identification of adaptor and modulator proteins that directly interact with IRE1α, a protein platform referred to as the UPRosome.[2] IRE1α is a Ser/Thr protein kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA encoding the transcriptional factor X-Box binding protein 1 (XBP-1).[3-5] A 26 nucleotide intron of xbp-1 mRNA is spliced out by activated IRE1α, leading to a shift in the codon reading frame and resulting in the expression of a potent transcriptional factor. Additionally, IRE1α binds the adaptor protein TRAF2, triggering the activation of c-Jun N-terminal kinase (JNK)[6] and other signaling cascades.[2]

Recent evidence indicates that IRE1α activation is specifically regulated by a set of proteins (co-factors and inhibitors), to assemble with different adaptor proteins. For example, the levels of IRE1α signaling were shown to be controlled by the expression of the pro-apoptotic

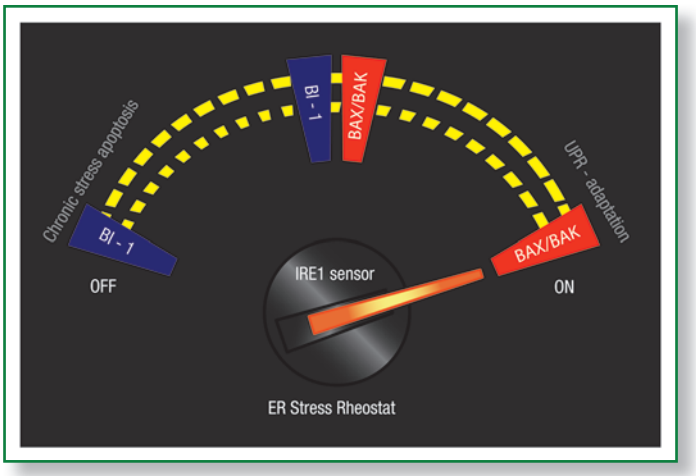

Figure1. The ER stress rheostat. The magnitude and kinetics of IRE1α signaling determines the ability of a cell to adapt to protein folding stress. Negative and positive regulators assemble to IRE1α to modulate its activity. The anti-apoptotic protein BI-1 negatively controls IRE1α activity possibly due to a direct physical association, turning off its activity. Alternatively, the expression of pro-apoptotic proteins such as BAX, BAK, PTP-1B and AIP1 enhances IRE1α activation. Thus, the expression of anti- and pro-apoptotic proteins at the ER membrane determines the amplitude and kinetics of UPR responses and the ability of a cell to adapt to ER injuries.

ER-located Protein-tyrosine phosphatase 1B (PTP-1B).[7] The absence of PTP-1B cause impaired XBP-1 splicing, JNK phosphorylation, and attenuated upregulation of XBP-1 target genes.[7] Remarkably, PTP-1B deficiency did not affect PERK signaling, suggesting a specific regulation of IRE1α. Besides, activation of IRE1α is instigated by the expression of pro-apoptotic BCL-2 family members such as BAX and BAK.[8] The BCL-2 family is a group of evolutionarily conserved regulators of cell death composed of both anti- and pro-apoptotic members that operate at the mitochondrial membrane to control caspase activation.[9] BAX and BAK deficient cells and animals were shown to have a specific attenuation of IRE1α signaling.[8] Interestingly, this regulation was mediated by a physical association between the cytosolic domain of IRE1α and BAX/BAK. This activity was mediated by the BCL-2 homology (BH) domains 1 and 3, essential domains in the regulation of apoptosis. At the molecular level, it was shown that BAX and BAK modulate the phosphorylation and oligomerization state of IRE1α. Likewise, the pro-apoptotic protein ASK1-interacting protein 1 (AIP1) specifically modulates IRE1α signaling. AIP1-deficient mice and cells presented impaired IRE1α signaling after exposure to ER stress agents. Similarly to the phenotype of PTP-1B or BAX/BAK deficient cells, the lack of AIP1 expression did not affect the PERK axis of the UPR.[10]

XBP-1 mRNA splicing levels decline after prolonged ER stress, whereas PERK signaling is sustained over time.[11] This mechanism may sensitize cells to apoptosis after chronic or irreversible ER stress, shutting down the pro-survival effects of IRE1α/XBP-1 signaling. However, it is not known how IRE1α is turned off. It has been suggested that the ER located protein BAX inhibitor-1 (BI-1) may negatively regulate IRE1α in models of ischemia reperfusion.[12] In a recent study from our laboratory we have further examined the role of BI-1 on ER stress.[13] BI-1 is an evolutionary conserved six transmembrane-containing protein that modulates ER calcium homeostasis and inhibits apoptosis by diverse death stimuli.[14] BI-1 expression suppressed IRE1α activity in animal and cellular models of ER stress.[13] Of note, BI-1 deficient cells displayed hyperactivation of the ER stress sensor IRE1α, associated with a sustained splicing of XBP-1 over time and thus abrogating the inactivation phase of the stress sensor. This phenotype was mediated with the formation of a stable protein complex between BI-1 and IRE1α, decreasing its ribonuclease activity. Our results, together with the aforementioned studies, suggest a model where the differential activation of ER stress sensors in different tissue contexts may be related to the engagement of specific regulatory complexes through the association of adaptor and direct binding of positive ad negative modulators.

We envision a model where a complex signaling platform is assembled at the level of IRE1α to modulate its activation status in terms of signaling intensity and kinetics of activation/inactivation. In the context of cell survival, the fine tuning of UPR signaling is particularly relevant in life to death transitions by controlling transcriptional programs that regulate adaptation to stress or by initiating apoptosis of irreversibly damaged cells. It remains to be determined if PERK and ATF6 are regulated in a similar manner by specific factors. The UPRosome concept may help to explain why each branch of the UPR differentially signals in vivo, despite of operating through similar protein misfolding-sensing mechanism.[2] It is interesting to notice that a set of apoptosis-related proteins (i.e. BAX, BAK, PTP-1B, AIP1 and BI-1) interact with IRE1α, regulating its activation status. This activity on early UPR responses (minutes after ER stress, defined as the adaptive phase) inversely correlates with the downstream role of this set of proteins on the apoptosis machinery (engaged >16h under chronic ER stress, death phase), depicting a new function beyond apoptosis (a dual role). These findings suggest a model wherein the expression of anti- and pro-apoptotic proteins at the ER membrane may determine the amplitude of UPR responses and the ability of a cell to adapt to ER injuries. Furthermore, this model may give ideas to define a molecular switch to the homeostatic transition between adaptation and cell death.

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