Endoplasmic reticulum stress signaling: the microRNA connection

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The endoplasmic reticulum (ER) is the first compartment of the secretory pathway through which secreted and transmembrane proteins must travel (20). This organelle plays major roles in the maintenance of calcium and lipid homeostasis and is instrumental for protein folding and trafficking (20). All of these functions are achieved under conditions in which the homeostasis of this compartment is maintained. Different environmental and cell intrinsic challenges, such as nutrient or oxygen deprivation, toxic exposure, or oxidative stress, can affect the ER protein folding capacity. When the folding demand overwhelms the folding capacity of the ER, this compartment’s homeostasis is disrupted and its functions cannot be completed (79). This phenomenon is generally accompanied by the accumulation of improperly folded proteins in the lumen of the ER, leading to a configuration named ER stress (94). In response to this stress situation the cell has evolved an adaptive mechanism, first described in 1988, that aims at restoring homeostasis. The ERUPR is transduced by three major ER-resident stress sensors, namely PKR-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 (IRE1). Activation of these ER stress sensors leads to transcriptional reprogramming of the cells. Recently, microRNAs (miRNAs), small noncoding RNAs that generally repress gene expression, have emerged as key regulators of ER homeostasis and important players in ERUPR-dependent signaling. Moreover, the miRNAs biogenesis machinery appears to also be regulated upon ER stress. Herein we extensively review the relationships existing between “canonical” ERUPR signaling, control of ER homeostasis, and miRNAs. We reveal an intricate signaling network that might confer specificity and selectivity to the ERUPR in tissue- or stress-dependent fashion. We discuss these issues in the context of the physiological and pathophysiological roles of ERUPR signaling.

endoplasmic reticulum; ERUPR; microRNA; stress; cell fate; UPR

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**THE UNFOLDED PROTEIN RESPONSE SENSORS**

**IRE1**

IRE1α is ubiquitously expressed, whereas IRE1β expression is restricted to the intestine. Both isoforms are type I transmembrane proteins and reside in the ER. IRE1α is an essential gene in the mouse. Activation of IRE1α involves its dimerization, oligomerization, and trans-autophosphorylation. In turn, this leads to a conformational change that activates the RNAse domain. IRE1α excises a 26-nucleotide intron of the mRNA encoding the transcription factor X-box binding protein-1 (XBP1) (16, 55, 101), which is then ligated by a yet unknown ligase in mammals. This unconventional splicing event results in a coding reading phase frameshift in the mRNA and leads to the expression of a more stable and active transcription factor, termed XBP1spliced (XBP1s). XBP1s trans-activates a subset of target genes, which may vary depending on the tissue context and the stress stimuli (1). Among classical XBP1s targets are genes involved in protein folding, endoplasmic reticulum-associated degradation (ERAD), protein translocation to the ER, and protein secretion (54). In addition to control of gene expression through ER stress-dependent XBP1 mRNA splicing, IRE1α also signals through the assembly of many adapter proteins and regulators, referred to as the UPRoweome (41). For instance, IRE1α has been reported to interact with the adapter protein TNFR-associated factor-2 (TRAF2), instigating the downstream activation of apoptosis signal-regulating kinase 1 (ASK1) and c-Jun NH2-terminal kinase (JNK) (41). IRE1α also modulates other signaling pathways involving NF-κB and ERK (reviewed in ref. 42). Finally, IRE1 endoribonuclease activity also degrades a subset of mRNA through a process known as regulated IRE1-dependent decay (RIDD).
ATF4 also controls the expression of important genes involved in acid metabolism, in addition to ER chaperones and foldases. ATF4 controls the expression of genes involved in redox and amino acid metabolism, including the activating transcription factor 4 (ATF4). ATF4 - untranslated regions (UTRs) = open reading frames in their 5' UTRs. (39).

The ER stress-induced unfolded protein response (ERUPR) is mediated by three ER-resident sensors named PKR-like endoplasmic reticulum kinase (PERK; blue), activating transcription factor 6α (ATF6α; yellow), and inositol requiring enzyme 1α (IRE1α; orange). The ERUPR aims at restoring ER homeostasis by reprogramming the cell's transcriptional activity; if it fails, then a cell death program is triggered. ATF6α, eukaryotic translation-initiation factor 2α (eIF2α), regulated IRE1-dependent decay (RIDD), and growth arrest and DNA damage-inducible 34 (GADD34) (Fig. 1). GADD34 participates on a feedback loop that negatively controls eIF2α phosphorylation by interacting with protein phosphatase 1C (PP1C), restoring protein synthesis (41). Alternatively, another eIF2α kinase shown to be activated upon ER stress is the double-strand RNA activated kinase PKR (77). In particular, PKR activation in the context of the integrated stress response has been shown to link pathogen sensing to cell metabolism (69). ER stress-mediated activation of PKR could also represent an interesting link between the ERUPR and the interferon response (29).

ATF6α is an ER located type-II protein that contains a bZIP transcription factor motif on its cytosolic domain. ATF6α belongs to a family of related proteins including ATF6β, old astrocyte specifically induced substance (OASIS), LUMAN (also known as CREB3), cyclic AMP-responsive element-binding protein hepatocyte (CREB3L3), and BBF2 human homologue on chromosome 7 (BBF2H7), and CREB4 (4); all contain a conserved bZIP domain, together with highly conserved defining features of an adjacent putative transmembrane domain and consensus cleavage site for S1P and S2P, indicating that they are membrane-anchored transcription factors subject to regulated intramembrane proteolysis. The binding of the ER chaperone BiP to the luminal domain of ATF6α retains this sensor at the ER due to the masking of a Golgi localization signal. Upon ER stress, BiP binds preferentially to misfolded proteins, releasing ATF6α, which then translocates to the Golgi where site-1 and -2 proteases cleave the protein at sites located on both sides of the ER membrane, thereby releasing a cytosolic fragment (ATF6c) (94). ATF6c is a transcription factor that regulates the expression of quality control genes of the ERAD pathway in addition to modulating BiP and XBP1 mRNA levels (99, 101). Other activation mechanisms have also been suggested for ATF6 activation depending on the reduction of disulfide bonds in the luminal domain of the protein (68). Although individual ATF6α or β mouse knockouts are normal, only the double is lethal (99).

The activation of the three arms of the ERUPR promotes cell adaptation to ER stress through proximal and distal signaling loops, leading, when necessary, to major cellular reprogramming. If the stress is not alleviated, death pathways activated in parallel become predominant and cells are triggered to die. The ERUPR is therefore a signaling pathway that can control cell fate in response to stress.

**THE ERUPR IN DISEASE**

The ERUPR has been traditionally linked to the maintenance of cellular homeostasis in specialized secretory cells (plasma B cells, salivary glands, hepatocytes, and pancreatic β-cells), where the high demand of protein synthesis and secretion constitutes a constant source of stress. In addition, specific transgenic mouse models have revealed unexpected functions of the ERUPR in many physiological processes such as lipid metabolism, energy control, inflammation, and cell differentiation (41). Collectively, the importance of the ERUPR in organ homeostasis is reflected by its prevalent roles in human dis-
Neurodegenerative Disorders

Specific signaling branches of the ER\textsuperscript{UPR} have distinct effects on Parkinson disease (PD). For instance, deletion of ATF6\textalpha{} enhances the susceptibility of dopaminergic neurons to PD-inducing neurotoxins (28), whereas CHOP deficiency has the opposite effect (85). In the case of Huntington’s disease, deletion of XBP1 in the nervous system triggers autophagy-mediated degradation of mutant huntingtin, whereas ATF4 does not (93). Moreover, both adaptive (XBP1 and ATF4-dependent) and proapoptotic (CHOP-dependent) ER\textsuperscript{UPR} signals have been linked to tissue damage in spinal cord injury (70, 90). In addition, ER stress has been linked to several myelin-related diseases including multiple sclerosis (reviewed in ref. 60). Finally, an amyotrophic lateral sclerosis (ALS) mouse model has revealed that PERK signaling alteration exacerbates disease progression and reduces mouse life span (95), whereas XBP1 conditional deletion leads to experimental ALS resistance. Surprisingly, there are no functional studies that have evaluated the impact of manipulating the ER\textsuperscript{UPR} in animal models of Alzheimer’s disease (reviewed in ref. 23).

Inflammatory and Metabolic Diseases

The ER\textsuperscript{UPR} has important functions in immune cells such as B cells and dendritic cells (reviewed in ref. 66). Toll-like receptor (TLR) 4 and 2 trigger the activation of IRE1 and the subsequent splicing of XBP1 mRNA (46) and attenuate ATF6 and PERK activity in macrophages (97, 98). ER stress is also involved in inflammatory diseases such as Crohn syndrome and ulcerative colitis, in which IRE1\beta{} (11), XBP1 (47, 48), and PERK (87) play key roles in disease progression. Similarly, ER stress enhances a variety of inflammatory and stress signaling pathways to aggravate metabolic dysfunction, leading to obesity, insulin resistance, fatty liver, and dyslipidemia (17).

Cancer

The role of ER stress in cancer development, initially proposed by Ma and Hendershot (65), was experimentally documented in the following years. The three arms of the ER\textsuperscript{UPR} were described to participate in the different phases of oncogenesis including cell transformation, tumor cell dormancy, or tumor growth (63). For instance, ER stress signaling was shown to represent a barrier against oncogene-driven cell transformation (25, 45). As such, the PERK-ATF4-dependent induction of autophagy was demonstrated to favor cell transformation induced by myc (40, 76). ER stress signaling is also involved in the regulation of tumor cell dormancy through ATF6\textalpha{}, which is essential for the adaptation of dormant cells to chemotherapeutic, nutritional, and microenvironmental stresses (83). Finally, the IRE1 arm of the ER\textsuperscript{UPR} was shown to contribute to glioblastoma development through the regulation of angiogenic and inflammatory pathways (5, 26) and cell adhesion (24).

Collectively, this information demonstrates the critical impact of ER stress and the subsequent signaling pathways activated in pathophysiological processes. Besides undisputed roles in developmental and physiological events (80), the role of the ER\textsuperscript{UPR} in disease might confer to this pathway an interesting therapeutic application.

THE MICRONA PATHWAY

Biogenesis

MiRNAs are endogenous ~22 nt noncoding RNAs that regulate gene expression by controlling target miRNA translation and/or degradation. MiRNAs loci are intergenic (50%) or are located in introns encoded by protein-coding genes (40%) or noncoding RNA genes (10%) (35, 78, 82). MiRNAs loci are mostly transcribed by RNA polymerase II (13) (Fig. 2A). The primary transcript, defined as pri-miRNA, is cleaved by the microprocessor containing Drosha and DGCR8 to produce a second precursor, called pre-miRNA. Intronic miRNAs are cleaved by a microprocessor to generate pre-miRNA or bypass microprocessing and are directly recognized as pre-miRNA after splicing (50) (Fig. 2A). The pre-miRNA is exported into the cytoplasm through Exportin 5-Ran GTP (XPO5) and is processed by the RNase III Dicer to produce a mature miRNA (Fig. 2B). The mature miRNA is loaded within the RNA-induced silencing complex (RISC) containing argonaute protein (AGO) (50). Then, the miRNA recognizes a target mRNA thanks to a perfect pairing of miRNA nucleotides 2 to 7 with its target, called seed sequence, and a partial base pairing in the 3’-region of the miRNA (59) (Fig. 2B). Several unconventional target sites have been described previously (7). Based on the literature, miRNAs preferentially target the 3’-untranslated region (UTR) and more rarely, the 5’-UTR coding sequence and promoter.

Mechanisms of Action

The pairing miRNA:mRNA directs RISC to the target mRNA and allows its regulation. In most cases, miRNAs are considered as negative posttranscriptional regulators of gene expression (31). Molecular partners associated with RISC, such as AGO2, GW182, or eIF6, block translation initiation. AGO2 recognizes cap structure and represses cap recognition by eIF4F (52), GW182 interacts with PABP and inhibits circular conformation of mRNA (30, 102), and eIF6 suppresses 6OS subunit joining (19). RISC also interacts with deadenylation complex (CCR4-NOT) and decapping complex (DCP1, DCP2), leading to rapid degradation of target mRNA by exosome and 5’–3’ exonuclease (XRN1) (31). In a few studies, miRNAs are described as positive posttranscriptional regulators through a direct pairing depending on the cellular context or the miRNA binding site location (91). Other illustrations were provided by recent discoveries showing that some miRNAs attenuate some RNA binding proteins such as IRE1\alpha{} (67) or factors involved in nonsense-mediated mRNA decay (NMD) (14) and AU-rich-mediated decay (AMD) processes (64). A small number of studies have indicated that miRNAs are negative or positive transcriptional regulators of gene expression by targeting promoter sequence (49, 75).

MiRNA-Dependent Functional Networks

Since their discovery in the early 1990s, over 2,000 human miRNAs have been reported in miRBase (version 19; http://www.mirbase.org/). In mammals, the genome contains several copies of miRNAs paralogs, which have a lot of common
targets and constitute conserved miRNA families. Each family is able to control around 300 mRNAs (32). Because miRNA targeting usually requires partial sequence homology, a single miRNA recognizes numerous target mRNAs and a single mRNA is targeted by multiple miRNAs, providing an additional layer of regulation in the control of gene expression (74).

Globally, miRNAs constitute redoubling networks, increasing gene expression regulation complexity which itself depends on environmental factors, such as cellular context and respective expression of an miRNA and its target mRNA. As master coordinators, they are able to impact a variety of cellular pathways and functions. Moreover, by coordinating the expression of multiple genes, miRNAs are fine-tuning regulators, which efficiently modulate the most important physiological and pathophysiological processes including development, metabolism, immunity, cell death, and cell growth. Dysregulation of miRNAs also appears to play a fundamental role in a variety of human diseases.

Early studies have indicated that miRNAs might be involved in the control of ER$_{UPR}$. In this review, we will focus on miRNA effectors and modulators of the ER$_{UPR}$ and their role in regulation of the survival/death balance during ER stress.

**ER$_{UPR}$ REGULATORY CIRCUITS TOWARDS miRNA**

In recent years, the discovery of a functional connection between ER$_{UPR}$ signaling and the expression of miRNAs has revealed another layer of complexity in the regulation of protein homeostasis. Not only has the miRNA biogenesis machinery been found to localize at proximity of the endoplasmic reticulum but also miRNA expression was shown to regulate and be regulated by ER$_{UPR}$ signaling.

**MicroRNA Silencing Is Involved in ER Stress Signaling**

Converging lines of evidence have suggested a role of miRNAs in ER$_{UPR}$. As such, the relocation of AGO in stress granules upon ER stress and the alteration of miRNAs expression pattern when cells are subjected to ER stress represent a strong argument in favor of an ER$_{UPR}$/miRNA connection. The majority of AGO protein is distributed diffusely in the cytoplasm under basal conditions, whereas, upon ER stress, AGO is accumulated in a punctuate manner within structures known as stress granules (57, 58). Stress granules are dynamic cytoplasmic macrostructures that aggregate translationally stalled mRNA/protein complexes and are specifically formed in response to stress conditions (3). Upon ER stress, PERK activation leads to the phosphorylation of eIF2$\alpha$, thereby inducing translational arrest and inactivating mRNA release from polysomes and nucleation of stress granules (51). The complete structure/composition of stress granules is not well known; nevertheless, they are enriched in stalled preinitiation complexes, small ribosomal subunits, various inhibitors of mRNA stability/translation, miRNAs, and AGO proteins (2, 3). Furthermore, miRNAs are required for AGO localization to the stress granules, thus suggesting a miRNA-mediated repression role during ER stress (57, 58). Moreover, recently it was also shown that the recruitment of DICER to the ER, as
previously observed in postmitotic neurons (6), could occur through an interaction with the cytoskeleton-linking endoplasmic reticulum membrane protein of 63 kDa (CLIMP-63) (73). This interaction is presumed to impact on DICER function.

Global approaches in different cellular contexts revealed that ER stress modifies the expression of many miRNAs. Geslain et al. (34) developed chimeric tRNA to induce proteome damage, leading to proteostasis imbalance and consequently to ER stress. They applied their strategy to identify human miRNAs modulated upon stress in HEK293T cells. The strongest effect was obtained using tRNASer (Ile), which modulates the expression of ~250 miRNAs (34). Moreover Behrman et al. (9) profiled miRNAs expression in wild-type mouse embryonic fibroblasts treated with the ER stress inducers tunicamycin or thapsigargin and found 11 miRNAs differentially expressed upon ER stress. Following ER stress induction, Bartoszewski et al. (8) performed miRNA microarray profiling in human airway epithelial cells (Calu-3) and identified 47 and 39 miRNAs enhanced by tunicamycin or the proteasome inhibitor ALLN, respectively (8). Finally, Belmont et al. (10) performed miRNA array analysis of RNA from the hearts of ATF6 transgenic mice and identified 13 ATF6-regulated miRNAs. Altogether, these studies demonstrate the existence of an ER stress-dependent regulation of miRNA expression.

**MiRNAs Modulators of the ERUPR**

Several miRNAs have been described to positively or negatively impact on the ER stress response (Fig. 3) either through specific targets or through yet unclear mechanisms. For instance, overexpression of the miR-23a–27a–24–2 cluster in HEK293T cells led to induction of components of CHOP, TRIB3, ATF3, and ATF4 and subsequent cell death (21). Although this effect might be indirect, ER stress-mediated apoptosis was indeed activated upon overexpression of this miRNA cluster. It was also shown that overexpression of miR-122 in hepatocellular cancers leads to the repression of ERUPR signaling through a CDK4-PSMD10 pathway and increases cisplatin-mediated apoptosis (100). Other miRNAs have been reported to directly affect the expression of specific components of ER molecular machines.

The trafficking machinery. MiRNA-dependent regulation of the trafficking machinery is well illustrated by the miR-490–3p-mediated targeting of endoplasmic reticulum-Golgi intermediate compartment protein 3 (ERGIC3) (40). MiR-490–3p is upregulated in hepatocellular carcinoma, directly targets ERGIC3 3′-UTR, and increases its expression. This mechanism may play a role in tumor cell growth through the regulation of critical components of the export/trafficking machinery (Fig. 3).

The calcium homeostasis machinery. In metastatic brain cancer, miR-708 expression is downregulated, leading to accumulation of its target neuronatin (NNAT), an ER protein that controls intracellular calcium levels, thereby leading to metastasis (81). Interestingly, miR-708 is also induced by CHOP (9). As a consequence, the controlled expression of miR-708 downstream of the ERUPR and its direct impact on ER calcium homeostasis could constitute a novel signaling circuit whose regulation could be involved in tumor growth and spreading (Fig. 3).

The folding and quality control machinery. Another miRNA-dependent regulation of BiP expression was shown to impact...
on tumor growth. Indeed, miR-30d, miR-181a, and miR-199–5p were found to target BiP expression and to be downregulated in prostate, colon, and bladder cancers (86). These three miRNAs cooperatively act to control BiP expression levels in untransformed cells, and their deregulation in cancers leads to the upregulation of BiP expression, thereby conferring to these cells a selective advantage. These three miRNAs are proposed to serve as therapeutic tools to suppress BiP expression and enhance chemosensitivity of cancer cells (86). Another example of a miRNA that targets a component of the ERAD machinery is provided by the mouse miR-183, which targets SEL1L, which may be involved in stemness maintenance of neural progenitor, and thereby induces mature neural derivatives differentiation (18) (Fig. 3).

The ERUPR. Three additional miRNAs were identified to directly target ERUPR transducers. This is the case for miR-214, which targets ATF4 downregulation, leading to bone formation inhibition (96). Moreover, Duan and colleagues (27) demonstrated that miR-214 targets XBP1 expression through a yet unclear mechanism. Furthermore, they showed that ER stress suppressed the expression of the miR-199a/miR-214 cluster in hepatocellular cancer cell lines through an NF-kB-dependent pathway (27). This indicated that the miR-199a/miR-214 cluster might represent another example of miRNAs being both regulators and effectors of the ERUPR. Moreover, miR-140 targets the transmembrane transcription factor OASIS (92), thereby leading to expression regulation of extracellular matrix encoding genes in pancreatic β-cells subjected to stress. Even more recently, miR-1291 was shown to directly target IRE1α within its 5’-UTR in hepatoma cells, thereby leading to the overexpression of the pro-oncogenic protein glypican-3 (67) (Fig. 3).

Collectively, these results demonstrate the existence of a highly connected network of miRNAs that target ER functions and homeostasis and add another layer to the regulation of ER stress signaling that might be highly relevant to physiology and pathophysiology.

MiRNAs Effectors of the ERUPR

The three arms of the ERUPR were shown to control the expression of select miRNAs as was nicely illustrated by the PERK-dependent induction of the miR-708 (9), miR-30-c-2* (15), or miR-211 (22) or repression of the miR-106b-25 cluster (36), by the ATF6-mediated repression of miR-455 (10) or the IRE1-dependent induction of miR-346 (8), and unexpectedly by the IRE1-mediated degradation of pre-miRs -17, -34a, -96, and -125b (56, 71) (Fig. 3). Although the above miRNAs are directly controlled by select arms of the ERUPR, Liu et al. (61) showed that miR-34b is transcriptionally silenced upon ER stress without characterizing the arm involved. This miRNA could function as a tumor suppressor by targeting SMAD3 (61).

PERK-dependent miRNAs circuits. MiR-708 expression is regulated by CHOP in brain and eyes and inhibits 1) neuronatin (NNAT), which controls intracellular calcium level and leads to metastasis in brain cancer (81), and 2) rhodopsin (RHO), most likely to prevent excessive production of the protein to enter the ER in mice (9). PERK signaling was also shown to regulate the expression of miRNAs that were themselves involved in the subsequent modulation of the ERUPR. For instance, PERK activation was found to promote the expression of miR-30-c-2*, a miRNA that in turn would repress the expression of XBP1 (15). Such a mechanism would provide the advantage of combining the transcriptional induction of XBP1 by ATF6 upon ER stress (101) followed by IRE1α-mediated splicing of XBP1 mRNA and then repression of XBP1 (unspliced and spliced) mRNA expression by miR-30-c-2* to turn off the XBP1-dependent stress response (15). It was recently demonstrated that PERK signaling induces miR-211, which in turn attenuates stress-dependent expression of the proapoptotic transcription factor CHOP. MiR-211 directly targets the proximal CHOP promoter, where it increases histone methylation and represses CHOP expression (22). Furthermore, upon ER stress mediated by hypericin in mouse embryonic fibroblasts, miR-106b-25 cluster expression is downregulated by ATF4 and NRF2, leading to an increase in BIM expression and a decrease in cell resistance to stress-induced apoptosis (36). These data show that miRNA-dependent signaling circuits are tightly regulated downstream of PERK to control PERK and IRE1 signaling pathways (Fig. 3).

ATF6-mediated repression of miR-455. ATF6 is activated upon ischemia and protects the heart from ischemic damage. Belmont et al. (10) have shown that miR-455, which is downregulated by ATF6, normally targets the expression of calreticulin (CALR). ATF6-mediated repression of miR-455 therefore leads to an increase in CALR expression in the pathologic heart, thereby decreasing hypertrophic growth (10).

IRE1-mediated direct regulation of miRNA expression. Following ER stress, Bartoszewski et al. (8) have shown that XBP1s induces miR-346 expression, which inhibits TAP1 expression and reduces major histocompatibility complex class I-associated antigen presentation observed upon ER stress. Recently, IRE1α has been shown to cleave pre-miRNA [(56, 89); pre-miRs -17, -34a, -96, and -125b] at sites distinct from those cleaved by Dicer (Fig. 2C) and presenting some degree of divergence with the IRE1 cleavage consensus sites previously defined by Oikawa and colleagues (71). These miRNAs normally repress translation of caspase-2 mRNA, and their cleavage leads to elevation of this initiator protease protein levels followed by activation of the mitochondrial apoptotic pathway (89). Moreover, IRE1α-mediated destabilization of miR-17 was shown to increase TXNIP mRNA stability. In turn, elevated TXNIP protein leads to the activation of the NLRP3 inflammasome, causing procaspase-1 cleavage and interleukin 1β (IL-1β) secretion, thereby increasing the systemic or local inflammatory response (56) (Fig. 3).

CONCLUSION AND PERSPECTIVES

The information presented in this review clearly points towards the existence of a miRNA network that constitutes an additional layer in the regulation of the ER stress-induced unfolded protein response. As such, miRNAs, either regulators or effectors of the ERUPR, were demonstrated to have differential impact on stress cellular outcomes (Fig. 4). Indeed, depending on the nature of each miRNA and on its up- or downregulation, the impact on cell fate can be dramatically modulated, leading to either prosurvival or prodeath effects. As shown in Fig. 4, at the present time, miRNAs associated with the IRE1 arm of the ERUPR display prosurvival properties, whereas those associated with either the PERK or the ATF6 arm show more balanced effects. It is very interesting to note
that several miRNAs are identified as both modulators and effectors of the ER\textsuperscript{UPR} (miR-708 and miR-214). This indicates that feedback regulatory loops might exist that could either amplify or repress ER stress signals. In the case of miR-708 and miR-214, both were found to play prodeath roles (Fig. 4). Interestingly, in addition, miRNAs are often described to regulate multiple mRNA targets that encode proteins belonging to the same signaling pathway (88). Using the miRWalk database, we identified the miR-515 family as potentially targeting the three ER stress sensors and consequently, this family of miRNAs could represent a master regulator of the unfolded protein response that should be experimentally tested in physiologically relevant models. A more extensive analysis of the predicted miRNAs targeting ER\textsuperscript{UPR} or ER homeostasis component could therefore represent an approach to identify novel ER\textsuperscript{UPR} regulatory pathways in physiology and disease.

As for other canonical genes, it is well established that miRNAs display cell/tissue specificity. Moreover, ER\textsuperscript{UPR} signaling has also been demonstrated to present various degrees of sensitivity and activation in specific cells of the organism. As a consequence, one could predict that physiological or pathophysiological modulation of specific miRNAs expression in tissues might control ER\textsuperscript{UPR} activation threshold and duration, thereby affecting cell fate upon stress (or not). A combination of miRNA specificity towards select arms of the ER\textsuperscript{UPR} with tissue specificity might also represent an interesting avenue to uncover novel ER\textsuperscript{UPR} regulatory pathways.

As illustrated in many instances in this review, numerous miRNAs associated with ER stress were identified in a cancer context and to play either prooncogenic or tumor suppressor roles. Moreover, several general studies have shown that miRNA expression is globally decreased in tumor tissues compared with their normal counterparts. This could be a consequence of the tumor cell’s poor differentiation (33, 72). Three main mechanisms were designated as responsible for miRNA deregulation in cancer (62). First, the alteration of genetic and epigenetic mechanisms was suspected as miRNAs loci are frequently localized in fragile chromosomal sites and are often altered (methylation, deletion, amplification, translocation). Second, the change in transcription factor activities that control miRNA expression was also identified as a possible mechanism as MYC and P53, which are key transcription factors in tumorigenesis, control numerous miRNA expression changes. Third and finally, miRNA biogenesis could be affected by the decreased expression of Dicer, Drosha, XPO5 or the mutations present in these genes.

The observation that in the past 10 years ER stress has become an unambiguous actor in cancer development again argues in favor of a pathophysiological relevance of the ER stress response-miRNA connection in cancer that will most likely be extendable to other diseases. The ER\textsuperscript{UPR} is affected in various cancers, and mutations in key genes of the ER\textsuperscript{UPR} have been identified. Overall, studies presented in this review allow us to hypothesize that the ER\textsuperscript{UPR} might be involved in second and third miRNA expression regulation mechanisms. Indeed, the transcription factors ATF4, CHOP, ATF6c, and XBP1s could also contribute to the altered expression of miRNAs. In addition, IRE1α could affect the maturation of pre-miRNAs.

In conclusion, this manuscript extensively reviews the relationships existing between ER stress signaling and miRNA regulation. It depicts an intricate regulatory network that presents physiologically and pathophysiological relevant properties and that provides a novel layer of regulation in the control of ER homeostasis.

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M.M. and E.C. prepared the figures; M.M. and E.C. drafted the manuscript; M.M. and E.C. edited and revised the manuscript; M.M. and E.C. approved the final version of the manuscript.

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