Review

REGULATION OF THE UNFOLDED PROTEIN RESPONSE BY microRNAs

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Abstract: The unfolded protein response (UPR) is an adaptive response to the stress that is caused by an accumulation of misfolded proteins in the lumen of the endoplasmic reticulum (ER). It is an important component of cellular homeostasis. During ER stress, the UPR increases the protein-folding capacity of the endoplasmic reticulum to relieve the stress. Failure to recover leads to apoptosis. Specific cellular mechanisms are required for the cellular recovery phase after UPR activation. Using bioinformatics tools, we identified a number of microRNAs that are predicted to decrease the mRNA expression levels for a number of critical components of the UPR. In this review, we discuss the potential role of microRNAs as key regulators of this pathway and describe how microRNAs may play an essential role in turning off the UPR after the stress has subsided.

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Abbreviations used: 3'-UTR – 3'-untranslated region; 5'-UTR – 5'-untranslated region; AD – Alzheimer's disease; AP-1 – activator protein 1; ATF4 – activating transcription factor 4; ATF6 – activating transcription factor 6; BAK – Bcl-2-antagonist/killer; BAX – Bcl-2-associated X protein; Bcl-2 – B-cell lymphoma 2; BiP – binding immunogloblulin protein; C-2 – caspase-2; C-4 – caspase-4; C42B – human prostate cancer cells; Calu-3 – human lung adenocarcinoma; CDK4 – cyclin-dependent-kinase 4; CFTR – cystic fibrosis transmembrane conductance regulator; CHOP – C/EBP homologous protein; eIF-2α – eukaryotic initiation factor 2 alpha; ER – endoplasmic reticulum; ERAD – endoplasmic reticulum-associated degradation; ERGIC3 – endoplasmic reticulum-Golgi intermediate compartment protein 3;

INTRODUCTION

ER Stress and the UPR

The endoplasmic reticulum (ER) is the central organelle for the biogenesis and trafficking of membrane and secretory proteins and for Ca^{2+} and lipid homeostasis. Proteins that fail to achieve proper conformation are removed from the ER and undergo a process referred to as ER-associated degradation (ERAD) [1]. Accumulation of misfolded proteins, inhibition of protein degradation or other insults to the ER lead to ER stress, which activates the unfolded protein response (UPR) [2].

In mammals, the UPR is a multifunctional signaling pathway with distinct sensors and targets that regulate gene expression [2, 3]. It primarily serves as a cellular adaptive mechanism, activating cellular pathways that increase the protein-folding capacity and reduce protein influx into the ER by inhibiting gene expression at multiple levels [2]. It is evident that the UPR signaling pathways play important physiological roles in secretory cell development and function, as illustrated by plasma and pancreatic β cells, which respectively have expanded capacities to secrete large amounts of immunoglobulin and insulin [4].

When ER stress persists or cellular recovery mechanisms are defective, apoptosis occurs [2, 5-7]. UPR-associated cell death contributes to the pathomechanism of a number human diseases, including diabetes mellitus [2, 8], neurodegenerative disorders [9, 10], many types of cancer, chronic inflammation, and certain forms of conformational diseases that are characterized by a decreased ability of cells to respond to stress [2, 6].

Abbreviations used (continued): ERp29 – ER stress protein 29; GADD153 – growth arrest and DNA-damage-inducible protein; GPC3 – glypican-3; GRP78 – glucose-regulated protein, 78 kDa; H9c2 – rat heart myoblasts; HCAEC – human coronary artery endothelial cells; HCC – human hepatocellular carcinoma; HEK293T – human embryonic kidney 293T cells; HeLa – human cervical cancer cells; HTM – human trabecular meshwork cells; HUVEC – human umbilical vein endothelial cells; IRE1 – inositol-requiring enzyme 1; JNK – c-JunNH2-terminal kinase; MCF-7 – human breast cancer cells; MEF – mouse embryonic fibroblasts; MHC – major histocompatibility complex; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; NIH 3T3 – mouse embryonic fibroblasts of the NIH 3T3 cell line; NRVMC – neonatal rat ventricular myocytes; PC12 – pheochromocytoma 12; PERK – protein kinase RNA-like ER kinase; PSMD10 – 26S proteasome non-ATPase regulatory subunit 10; PUMA – p53-upregulated modulator of apoptosis; TAP1 – transporter associated with antigen processing 1; TF – transcription factor; TXNIP – thioredoxin-interacting protein; UPR – unfolded protein response; VEGF – vascular endothelial growth factor; XBP1 – X-box binding protein 1; XBP1(s) – spliced XBP1
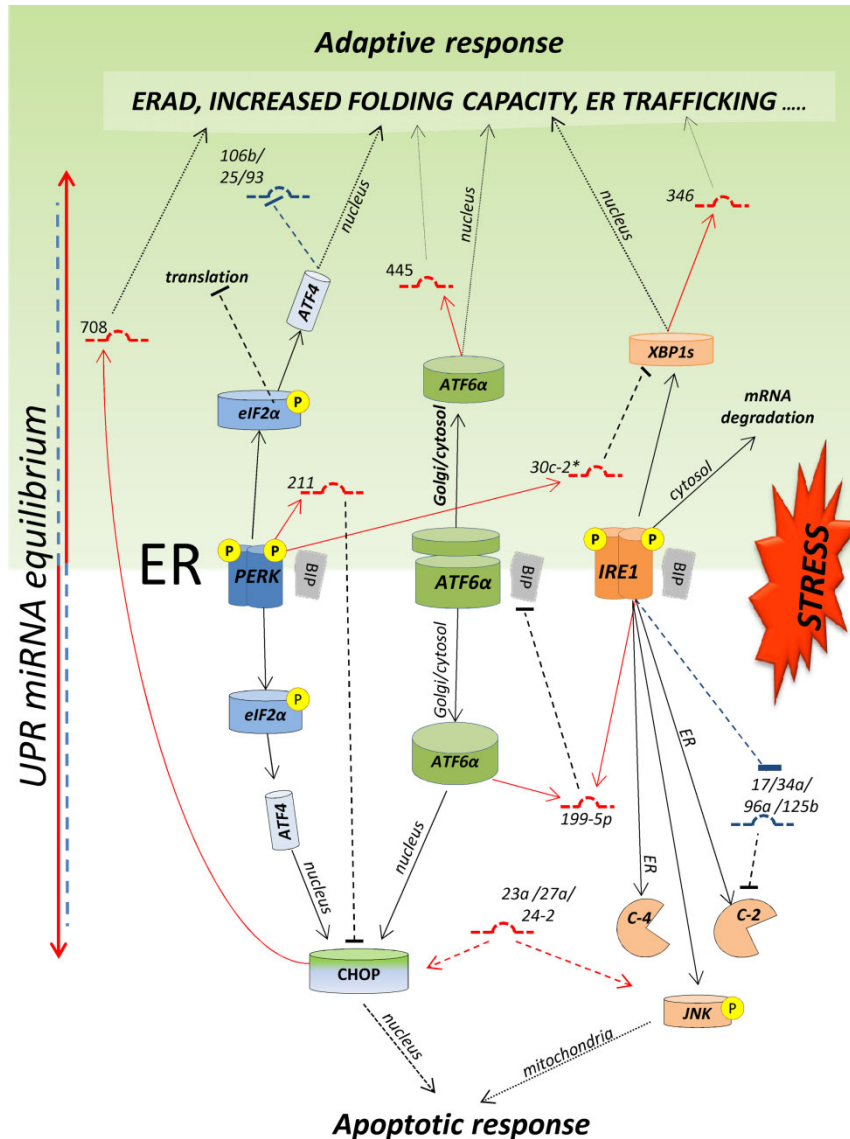
Fig. 1. The UPR includes both adaptive (green panel) and apoptotic (white panel) responses. Activation of PERK, ATF6 and IRE1 leads to inhibition of translation through eIF2α phosphorylation and degradation of ER-associated mRNAs by IRE1. IRE1, which is independent of XBP1, can mediate the rapid degradation of certain ER-localized mRNAs [16]. Activation of all three pathways also results in transcriptional activation through ATF4, ATF6α and XBP1, allowing long-term upregulation of genes that help cells adapt to persistent stress. The same sensors also initiate apoptotic signaling cascades, the best characterized of which are illustrated here. The miRNA component of UPR – induced miRs (red) and reduced miRs (blue) – contribute to both adaptive and apoptotic responses. Positive regulation is marked with →, while negative impact is denoted with ⊣. P (phosphate group), C-2 (caspase-2) and C-4 (caspase-4).

In mammals, the UPR is initiated through the activation of one or more of three ER transmembrane sensors (Fig. 1): inositol-requiring enzyme 1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6) [5]. These sensors are bound to the ER luminal chaperone BiP (binding immunoglobulin protein, also known as GRP78) in unstressed cells. The release of ATF6 transcription factor requires ATF6 translocation from the ER to the Golgi, where it is clipped by a serine protease, released into the cytoplasm, and translocated into the nucleus [11]. The two other sensors, IRE1 and PERK, are membrane proteins with ER stress-regulated oligomeric and dimerization domains, respectively. When ER stress occurs, IRE1 oligomerizes and becomes autophosphorylated [12], which activates it to function as an atypical endoribonuclease and splice X-box binding protein 1 (XBP1) mRNA to produce the potent transcription factor spliced Xbp1 or XBP1(s) [13]. To increase the protein-folding capacity of the ER, XBP1(s) and ATF6 promote the expression of chaperones, foldases and other membrane components that enhance ER function (Fig. 1) [14, 15].

PERK activation also requires dimerization and autophosphorylation. As a consequence, PERK phosphorylates eukaryotic initiation factor 2 alpha (eIF-2α), which inhibits translation to decrease the ER load [2]. Activating transcription factor 4 (ATF4) is preferentially translated upon the phosphorylation of eIF2α. It regulates the genes involved in redox homeostasis and amino acid metabolism (Fig. 1) [17-19]. Thus, the UPR acts at multiple levels to reduce ER protein load.

Transcriptional inhibition occurs through promoter hypermethylation and the formation of repressor complexes that contain ATF6 [20]. Protein synthesis is inhibited through the PERK-induced phosphorylation of eIF2α [21, 22]. Additional pathways are activated in order to minimize the ER protein load. These include post-transcriptional mRNA decay by IRE1 [16, 23] and ER-associated degradation. [13, 24]. Because ER stress activates both the ERAD and the UPR pathways, the biogenesis of numerous proteins with important cellular functions can be altered. A reduction in mRNA levels during ER stress can result from transcriptional repression, as previously described for CFTR [20, 25], or from reduced mRNA stability. The latter may result from the endonuclease activity of IRE1 [16, 23] or the activity of miRNAs [26, 27].

When ER stress is persistent and homeostasis is not restored, the UPR switches from a cytoprotective to an apoptotic function [28]. Several pathways have been directly implicated in ER stress-induced apoptosis (Fig. 1). The transcription factor CHOP, also known as growth arrest- and DNA damage-inducible protein (GADD153), is transcriptionally induced by ER stress. It sensitizes cells to ER stress [29, 30]. Under ER stress, IRE1 and PERK contribute to activation of the pro-apoptotic c-JunNH2-terminal kinase (JNK) [31, 32]. ER stress leads to the activation of human caspase-4 (C-4), localized to the cytoplasmic side of the ER membrane [33]. It recently became evident that there is cross-talk between the ER and the mitochondria, because persistent stress leads to the activation of caspase-2 (C-2) and subsequently the induction of BAX/BAK mitochondrial

apoptosis [34]. Furthermore, the p53 upregulated modulator of apoptosis (PUMA), p53 and Noxa proteins have been postulated to be novel components of the UPR apoptotic response [35, 36]. The precise regulation of the adaptive response of the UPR is crucial for cell death as well as survival.

MicroRNAs

MicroRNAs (miRNAs) are a class of short noncoding RNAs that introduce another level of regulation of gene expression. For details on miRNA biogenesis and their mechanisms of action please refer to the work of Treiber *et al.* [37] and the review by Nilsen [38]. They have the ability to selectively regulate expression at the post-transcriptional level, making them the perfect tool for selective modulation of various signaling pathways after mRNA synthesis [26], and they regulate protein expression via two distinct mechanisms: translational repression and mRNA degradation [27, 39, 40]. However, some more recent studies suggest that the latter is the more common mechanism for miRNA regulation [41]. They have been linked to a number of cellular processes, including apoptosis [42], inflammatory responses [43] and erythropoesis [44]. However, we are just beginning to understand their impact on cellular stress responses, such as hypoxia [45, 46], oxidative stress [47, 48], and various types of UPR activation, including insulin secretion [49] and B-cell differentiation [50]. During the UPR, mRNA and protein levels are decreased in order to reduce ER load, but surprisingly, the transcription of only a small percentage of genes is reduced [51, 52]. Therefore, mRNA degradation and the role of miRNAs during the UPR should be carefully considered.

Our previous studies indicated that only a small number of miRNAs were induced to express under ER stress, while the majority were either unaffected or downregulated [51]. Recent studies continue to support the idea that UPR-adaptive transcription factors can be governed by miRNAs [53, 54], and transcription factors can regulate miRNA expression [51] or stability [55]. This clearly suggests that miRNAs have many important regulatory functions in various UPR pathways. They also govern the cellular response to ER stress by controlling both adaptive and apoptotic mechanisms. Behrman *et al.* [52] and our group [51] have shown that ER stress induced by unrelated mechanisms (blocking proteasomal degradation, blocking ER-dependent glycosylation or disturbing Ca^{2+} homeostasis) affects the miRNA expression patterns in different ways. Although most of the observed differences are presumably caused by basic mechanisms underlying the type of stress induction, some may be related to the different impact of those stressors on the dynamics of the UPR adaptive response. Therefore, a reasonable and testable hypothesis is that during the adaptive phase of the UPR, specific miRNA levels change dynamically to either help restore cellular homeostasis or target the cell for apoptosis. In this review, we discuss what is known about miRNA involvement in the UPR pathway, describe results obtained with *in silico* predictive methods to determine if key

UPR-related mRNAs could be targeted by miRNAs, and discuss how miRNAs could govern the adaptive phase of the UPR.

UPR miRNAs

Byrd and Brewer [56] and Maurel and Chevet [57] classified UPR miRNAs into proadaptive and proapoptotic groups. The proadaptive ones contribute to lowering ER load, increasing the protein-folding capacity, and improving the chances of cell survival. Thus, these miRNAs are closely related to the UPR-adaptive phase regulatory proteins XBP1(s), ATF6 and ATF4. Proapoptotic miRNAs contribute to the induction of UPR-related apoptosis. Since the UPR is a complex pathway that is in equilibrium between these two processes, all three crucial sensors (IRE1, PERK and ATF6) have both adaptive and apoptotic functions. Thus, UPR-associated miRNAs can act as adaptive or apoptotic, depending on their expression profile changes and specific targets, and the tissue type that is involved. Therefore, we will not use the proadaptive/proapoptotic classification here, but rather concentrate on the role of miRNAs in managing UPR equilibrium.

miR-708 was identified in the murine brain and retina by Behrman *et al.* as the first UPR-related microRNA [52]. They demonstrated that miR-708 expression is induced by C/EBP homologous protein (CHOP), and that the very abundant retinal protein, rhodopsin, is a direct target for this miRNA. Upregulation of miR-708 leads to a reduction in the ER protein load by lowering the amount of nascent rhodopsinin the ER. Although CHOP is normally considered an apoptotic UPR factor, it can also function adaptively [52].

XBP1(s) is a crucial enhancer of adaptive UPR activity [58, 59] and its regulation by miRNA is an excellent illustration of the complexity of the UPR. Byrd *et al.* reported an miRNA "link" between PERK and IRE/XBP1 [53]. They demonstrated that miR-30c-2*(miR-30c-2p) is upregulated during UPR in a PERK/NF-κB-dependent manner, and targets the 3' UTR of XBP1 mRNA [53]. Thus, by decreasing XBP1(s) mRNA, miR-30c-2* attenuates the activity of the UPR and is cytoprotective. In our studies, we found an opposite type of regulation in which XBP1(s) induced miR-346 expression during the initial stages of the UPR to decrease the protein load in the ER [51]. In this case, miR-346 targeted the 3' UTR of the ER antigen transporter TAP1 and decreased the expression of this ER peptide transporter. This inhibition of TAP1 expression inhibited MHC class I assembly in the ER, thereby lowering the ER protein load [51]. This established that miRNAs could either facilitate or attenuate the UPR and that the timing of their induction and their specific target selection was an essential feature of this regulatory process.

Similar effects on the regulation of miRNA expression were reported for another adaptive transcription factor, ATF6. In cardiomyocytes, activated ATF6 induced the expression of five miRNAs and reduced the levels of eight others, including miR-455 [60]. Expression of miR-455 inversely correlated with levels of the ER resident Ca^{2+}-binding protein calreticulin [60]. Elevating calreticulin levels

restored calcium homeostasis, which improved the ER folding capacities and was an important function of the adaptive response. Thus, in heart tissue, ATF6-related calreticulin upregulation has important cytoprotective effects.

Yang et al. [61] reported an alternative type of regulation of calreticulin by miRNA in hepatocellular carcinoma (HCC) cells. They demonstrated that inhibition of the most abundant miRNA in the liver, miR-122, resulted in accumulation of calreticulin, ER stress protein 29 (ERp29) and cyclin-dependent kinase 4 (CDK4), with each of these being direct targets of miR-122 [61]. This observed CDK4 induction contributed to the enhanced stability of its interacting protein, the 26S proteasome non-ATPase regulatory subunit 10 (PSMD10) [62]. Consequently, inhibition of miR-122 enhanced ERAD and cell survival [61]. As might be predicted, the reportedly high levels of miR-122 contribute to the UPR apoptotic response [63].

Another miRNA group that enhances apoptotic signaling during the UPR is the miR-23a~27a~24-2 cluster [64, 65]. Induction of these miRNAs is sufficient to cause ER stress and induce genes associated with the PERK and IRE1 arms of the UPR to disturb ER calcium homeostasis, and to increase mitochondrial membrane permeability [63]. Conversely, the repression of this cluster correlates with induction of numerous pro-survival genes [63]. Thus, it has been suggested that miR-23a~27a~24-2 induction leads to the activation of PERK and IRE1 apoptotic signals [63].

Gupta et al. [66] also reported that the PERK/ATF4 arm of UPR induces the UPR apoptotic response in an miRNA-dependent manner. They reported that ATF4-driven repression of miR-106b~25 cluster members (miR-106b, miR-25 and miR-93) led to accumulation of their direct target, a Bcl-2 homology 3 family member, Bim [66]. Since Bim is a crucial inhibitor of the ER stress mitochondrial pathway [67, 68], UPR-dependent inhibition of endogenous miR-106b~25 members promotes cell survival. Importantly, they reported ER-stress downregulation of this cluster in several cell lines, suggesting that this is a general mechanism for regulating the UPR [66].

By contrast, the PERK-dependent miR-211 has pro-survival activity, as was recently demonstrated by Chitnis et al. [69]. PERK induces miR-211, which targets the 5' UTR of CHOP, causing transcriptional repression of this protein [69]. Therefore, miR-211 contributes to the UPR adaptive response.

So far, we have discussed the UPR miRNA expression profile changes that were mostly related to the adaptive phase of transcriptional activity. Interestingly, Upton et al. identified UPR-specific miRNA degradation as a novel mechanistic switch between apoptotic and adaptive responses [55]. Their work brings new insight into an alternative miRNA degradative mechanism. IRE1 is responsible for activation of both the adaptive and apoptotic responses of the UPR and has specific RNase activity. Prior to this, the function of IRE1 was mainly connected with XBP1 splicing and degradation of ER-associated mRNAs [70, 71]. Upton et al. demonstrated that IRE1 can also degrade miR-17, miR-34a, miR-96a and miR-125b [55], which are important for blocking BAX/BAK-dependent

apoptosis through specific binding to the 3' UTR of caspase-2 (C-2). Thus, IRE1 activation leads to a reduction in the levels of these mature miRNAs and to C-2 accumulation and subsequent apoptosis induction [72]. Interestingly, miR-17, miR-34a, miR-96a and miR-125b only inhibit the translation of C-2, leaving its mRNA levels unaffected and available upon IRE1 activation [55]. Furthermore, Lerner *et al.* reported that IRE1-driven reduction of miR-17 contributes to the accumulation of thioredoxin-interacting protein (TXNIP), which promotes sustained UPR-related apoptosis [73].

An alternative miRNA-IRE1 interaction was recently identified by Maurel *et al.* in hepatocellular carcinoma cells: IRE1 as a direct target of miR-1291 [74]. They postulated that in HuH7 cells, miR-1291-dependent attenuation of IRE1 leads to accumulation of a pro-oncogenic protein glypican-3 (GPC3) [74]. GPC3 levels are frequently elevated in HCCs [75] and contribute to the growth of HCC by stimulating canonical Wnt signaling [76]. Interestingly, stress-activated IRE1 did not increase GPC3 mRNA levels, and they demonstrated that miR-1291 binds to the 5' UTR of IRE1α [74]. However, in their latest study, Maurel *et al.* showed that miR-1291 targets the 3' UTR of GPC3 in HCCs [77]. The potential links between the UPR, miR-1291, IRE1 and GPC3 require further clarification.

BiP (GRP78), the major ER chaperone and signaling regulator, was shown to be a direct target of miR-30a, miR-181a and miR-199a-5p [78]. These three miRNAs are downregulated in prostate, colon and bladder tumors, and in a number of human cancer cell lines. Induction of these miRNAs in C42B prostate cancer cells attenuated BiP expression and induced apoptosis [78], meaning these miRNAs may be a part of the apoptotic UPR response. Another study by Dai *et al.* [54] demonstrated that miR-199-5p is induced in hepatocytes during thapsigargin-induced ER stress, and targets BiP mRNA along with ATF6 and IRE1α. Inhibition of this miRNA resulted in uncontrolled, sustained ER stress and apoptosis activation. Their work demonstrated that the transcription factor AP-1, activated by IRE1 and ATF6, is responsible for inducing miR-199-5p and thereby creating a negative feedback loop [54, 79]. Thus, during the UPR in normal hepatocytes, this miRNA promotes cell survival through IRE1 mRNA degradation.

The studies discussed above are clear examples of how miRNAs contribute to UPR regulation. Other miRNAs that have recently been reported on may also be related to the UPR. Using HCC cells, Dai *et al.* identified miR-221/222 as direct regulators of the p27kip protein, which is responsible for G1 arrest [80, 81]. ER stress-related repression of miR-221/222 allows for the accumulation of p27kip, thereby promoting G1 arrest. Thus, in HCC cells, miR-221/222 downregulation has a cytoprotective effect [80]. Since miR-221/222 levels are usually lower in normal liver tissues, this pro-survival function may only be valid in cancer cells [80].

Another miRNA that is correlated with ER-stress is miR-204 in human trabecular meshwork (HTM) cells [82]. Induction of this miRNA sensitizes cells to ER stress-related apoptosis by lowering the levels of BiP and CHOP [83].

However, none of the affected adaptive response factors were confirmed as direct targets of miR-204 and its role in the UPR needs further clarification.

Hart *et al.* recently reported the possible role of miR-490-3p in ER trafficking machinery regulation [84]. Furthermore, a direct interaction between miR-490-3p and ER-Golgi intermediate compartment protein 3 (ERGIC3) has been demonstrated in HCC cells [85]. Since the function of ERGIC3 is unclear, further studies are needed to evaluate its role in ER trafficking, as well as the role of miR-490-3p in this pathway.

Afonyushkin *et al.* described a correlation between miR-663 and the ATF4 arm of the UPR [86]. In their study, inhibition of miR-663 during the UPR in aortic and venous endothelial cells led to reduced ATF4 protein levels and inhibition of ATF4 transcriptional target expression [86]. Although induction of miR-663 during the UPR had ATF4-related pro-adaptive effects, further studies are necessary to understand this mechanism.

Wang *et al.* studied osteogenic differentiation and bone formation and identified ATF4 as a direct target of miR-214, which is a member of the miR-199a~214 cluster [87]. Duan *et al.* reported that miR-214 level reduction during ER stress contributes to XBP1(s) induction in HCCs [88]. Further studies are required to validate role of this miRNA during the UPR (Table 1).

Table 1. Summary of microRNA effects on UPR signaling. Indirect regulation or targets are marked with "*"; unidentified effects or unknown regulation are marked with "?".

miR	Cell type	ER stress impact on miR expression	Regulation (indirect*)	Target(s)	UPR effects	Ref.
miR-708	MEFs	Induction	CHOP	Rhodopsin	Balances the ER protein-folding capacity	[52]
miR-30c-2*	HeLa, NIH-3T3, MEFs	Induction	NF-kB	XBP1	Controls XBP1 levels as the UPR proceeds	[53]
miR-346	HeLa Calu-3	Induction	XBP1(s)	TAP1	Balances the ER protein-folding capacity	[51]
miR-455	Murine cardiomyocytes, NRVMCs	Repression	ATF6	Calreticulin*	Improves ER folding (restores calcium homeostasis)	[60]
miR-122	HCCs	Induction	?	Calreticulin* ERp29*	Induces apoptotic response	[61]
miR-23a~27a~24-2	HEK293Ts	Induction	?	Pro-survival ER stress proteins*	Induces apoptotic response	[63]

miR	Cell type	ER stress impact on miR expression	Regulation (indirect*)	Target(s)	UPR effects	Ref.
miR-106b~25 cluster	MCF-7, H9c2, PC12	Repression	ATF4*	BIM	Prevents apoptotic response	[66]
miR-211	NIH-3T3s MEFs	Induced	PERK	CHOP	Prevents apoptotic response	[69]
miRs -17, -34a, -96a, -125b	MEFs	Degraded	IRE1-mediated degradation	C-2	Regulates adaptive to apoptotic response switch	[55]
miR-17	HEK293s MEFs	Degraded	IRE1-mediated degradation	TXNIP	Promotes UPR related apoptosis	[73]
miR-1291	HCC-derived HuH7 cells	?	?	IRE1	?	[74]
miRs-30a, -181a, -199a-5p	C42B	Repressed	?	BIP	Prevents apoptotic response	[78]
miR-199-5p	HCCs	Induced	AP-1 (downstream of IRE1 and ATF6)	BIP, ATF6 and IRE1	Negative feedback loop: induces apoptotic response up on sustained ER stress	[54]
miR-221/222	HCCs	Repressed	CHOP*	p27^{kip}	Induces apoptotic response	[80]
miR-204	HTMs	Repressed	?	BIP*, CHOP*	?	[82, 83]
miR-490-3p	HCCs	?	?	ERGIC3	? Related to ER trafficking	[84]
miR-663	HUVECs, HCAECs	Induced	?	Unknown suppressor of ATF4*	Prevents apoptotic response	[86]
miR-214	HCCs	Repressed	NF-kB*	ATF4 XBP1(s)*	Prevents apoptotic response	[88]

Cell types: human breast cancer cells (MCF-7); human cervical cancer cells (HeLa); human coronary artery endothelial cells (HCAEC); human embryonic kidney 293 cells (HEK293); human embryonic kidney 293T cells (HEK293T); human hepatocellular carcinoma (HCC); well differentiated hepatocyte derived cellular carcinoma cell line (HuH7); human lung adenocarcinoma cells (Calu-3); human prostate cancer cells (C42B); human trabecular meshwork cells (HTM); human umbilical vein endothelial cells (HUVEC); mouse embryonic fibroblasts (MEF and NIH-3T3); neonatal rat ventricular myocytes (NRVMC); pheochromocytoma 12 (PC12); rat heart myoblasts (H9c2).

The UPR is especially important in Alzheimer's disease (AD), where its initial activation may have a neuroprotective function, but sustained activation may lead to neurodegeneration [89]. Schonrock *et al.* have shown that amyloid-β (a known factor contributing to AD) can cause neuronal miRNA deregulation [90]. Changes in miRNA expression profiles that correlate with AD were also identified and confirmed by Wang *et al.* [91]. The ASAP (amyloids as sensors and protectors) hypothesis proposed by Murray *et al.* postulates that amyloid proteins serve as stress sensors and in response trigger protective cellular mechanisms including the UPR [92]. Thus, the link between amyloid proteins and UPR-related miRNA should be very carefully examined.

THE POSSIBILITIES

Although the number of identified UPR-adaptive response miRNAs is growing, we are only beginning to understand their role in this pathway. The majority of studies are limited to a specific cellular model, such as HCC, while the field lacks information on more general cell line-independent miRNA mediators of the UPR. During the past five years, we have witnessed great strides in the development of molecular biology bioinformatics tools, including miRNA target identification software. In parallel, the popularization of nucleic acid expression array technology and the increased availability of miRNA and mRNA expression profiling have dramatically expanded the possibilities for understanding mRNA regulation. We applied *in silico* analysis to predict the other microRNA mediators of the UPR adaptive response. We limited the scope of our analysis to the main UPR adaptive response factors: XBP1 (NM_001079539), ATF6 (NM_007348), ATF4 (NM_001675), IRE1 (NM_001433), PERK (NM_004836) and BiP (NM_005347). We selected miRNAs that were predicted by the majority of these software programs. Our approach allowed us to confirm 8 out of 14 previously validated miRNAs that had target sequences in the 3' UTR. Less strict analysis (including non-conserved miRs) confirmed 2 more direct interactions. However, for many of the discussed miRNAs, the mRNA targets are either unknown or the miRNA target sequences are located in the 5' UTR. Although this approach is limited to 3' UTR sequences (due to software design), and requires further experimental validation, this information will be useful in future investigations.

Bioinformatics tools

Initially, we used miRecords, a web server that allows for parallel prediction analysis with multiple independent webserver miRNA prediction software [93]. The "Predicted Targets" component of miRecords integrates the predicted targets of the following miRNA target prediction tools: DIANA-microT_[94, 95], MicroInspector_[96, 97], miRanda_[98, 99], MirTarget2_[100], miTarget_[101], PicTar__[102, 103], PITA__[104], RNA22__[105], RNAhybrid__[106], and TargetScan/TargetScanS_[107]. We sorted the predicted miRNAs based on the

number of independent software binding prediction "hits". Next, for the preselected miRNAs, the predicted binding to the 3' UTRs was independently confirmed via a detailed analysis using TargetScan 6.2, miRanda, DIANA-microT, PicTar and miRSearch 2.0 [108]. The final results are limited to miRNAs that are conserved among mammals and predicted to have a high score by at least three of the five web servers.

miRNAs predicted to target XBP1 mRNA

miR-449a and miR-449b. Downregulation of miR-449 has been demonstrated in gastric cancers [109]. Induction of these miRNAs correlates with induction of apoptosis and p53 upregulation [109], suggesting that they may be incompatible with cancer cell survival in certain types of cancer.

miR-34a has been already assigned to IRE1-related apoptosis in the UPR [55]. However, if this prediction is validated, miR-34a could create an additional link between IRE1 and XBP1, allowing IRE1 to regulate the accumulation of XBP1 during the UPR.

miR-34c-5p has been identified in cervical carcinoma cells as an effector of p53 [110].

miRNAs predicted to target ATF6 mRNA

miR-203 has a previously demonstrated tumor suppressive role [111-117]. It mediates subversion of stem cell properties during mammary epithelial cell differentiation and promotes the mesenchymal-to-epithelial transition [118].

miR-424 has been implicated in the regulation of angiogenesis [119]. Recent studies have shown that its downregulation plays an important role in the epithelial defense against microbes [120].

miRNAs predicted to target IRE1 mRNA

miR-506 has been shown to function as an anti-oncogenic miRNA in malignantly transformed cells [121] and breast cancer cells [122].

miR-124 is very abundant in the brain, where it governs neurogenesis [123-125]. Furthermore, it has been shown to play a suppressive role in cancer progression [126, 127].

miRNAs predicted to target PERK mRNA

miR-320 is a negative regulator of vascular endothelial growth factor (VEGF) during high glucose stress in human vascular endothelial cells (HUVEC) [128]. Furthermore, it acts in stromal fibroblasts to curtail tumor progression [129].

miR-454. There is currently no literature data available regarding the role of this miRNA.

miRNAs predicted to target BiP mRNA

miR-495 has been reported to induce cancer progression and hypoxia resistance in breast cancer cells when present at high levels [130]. Its tumor suppressor action has also been reported in other types of cancer cell lines [131, 132].

miRNAs predicted to target ATF4
Due to relatively short 3' UTR sequence of ATF4 (below 100 bases), none of the identified miRNAs meet the selection criteria.

MiRNAs mentioned above, along with their predicted direct targets are summarized in Table 2.

Table 2. MicroRNAs that are predicted to decrease mRNA expression levels for a number of key components of the UPR. The web servers that confirmed the mRNA targets are listed.

miRNA	Target	Web Server	Ref.
miRs:-449a ,-449b	XBP1	miRanda, TargetScan, DIANA-microT	[109]
miR-34a	XBP1	miRanda, TargetScan, DIANA-microT	[55]
miR-34c-5p	XBP1	miRanda, TargetScan, DIANA-microT	[110]
miR-203	ATF6	miRanda, TargetScan, DIANA-microT, PITA, RNAhybrid	[111-118]
miR-424	ATF6	miRanda, TargetScan, miRSearch	[119, 120]
miR-506	IRE1	miRanda, TargetScan, DIANA-microT	[121, 122]
miR-124	IRE1	miRanda, TargetScan, DIANA-microT	[123-127]
miR-320	PERK	miRanda, TargetScan, DIANA-microT, PicTar	[128, 129]
miR-454	PERK	miRanda, TargetScan, DIANA-microT	-
miR-495	BIP	miRanda, TargetScan, DIANA-microT, MirTarget2, PITA, RNAhybrid	[130-132]

CONCLUSION

It has become evident that cellular stress responses play an important role in gene expression regulation under both physiological and pathological conditions. The extent and specificity of these processes are not clear. The results discussed in this review clearly indicate that miRNAs are important mediators of cellular UPR stress responses. However, we are just beginning to understand the complexity of how miRNAs impact the UPR. While the mechanisms underlying the adaptive/apoptotic "UPR switch" still require further clarification, miRNAs are clearly an important part of this puzzle. Most of the information available is from steady-state analysis, and what is clearly needed is a thorough dynamic/temporal analysis of the UPR-induced miRNA profiles. As discussed in this review, studies clearly indicate that UPR-related alterations in miRNA levels can affect both adaptive and apoptotic responses. Furthermore, we need to emphasize that many miRNAs are expressed in tissue- and age-specific patterns, suggesting that miRNAs have cell type-specific functions [133, 134]. Many of

the studies discussed here are limited to specific cancer cell lines, so more general miRNA-mRNA expression profiling studies in other cell lines are required. These data combined with the results of further *in silico* studies will provide critical clues for defining the role of miRNAs in the UPR.

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