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REVIEW ARTICLE

MicroRNAs: Crucial Regulators of Stress

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Abstract: ***Background*:** Signaling pathways including gene silencing, cellular differentiation, homeostasis, development and apoptosis are regulated and controlled by a wide range of miRNAs.

***Objective*:** Due to their potential binding sites in human-protein coding genes, many studies have also linked their altered expressions in various cancer types making them tumor suppressors agents.

***Methods*:** Moreover, each miRNA is predicted to have many mRNA targets indicating their extensive regulatory role in cell survival and developmental processes. Nowadays, diagnosis of early cancer stage development is now dependent on variable miRNA expression levels as potential oncogenic biomarkers in validating and targeting microRNAs for cancer therapy.

***Results*:** As the majority of miRNA, transcripts are derived from RNA polymerase II-directed transcription, stress response could result on a general reduction in the abundance of these transcripts. Over expression of various microRNAs have lead to B cell malignancy, potentiated KrasG12D-induced lung tumorigenesis, chronic lymphocytic leukemia, lymphoproliferative disease and autoimmunity.

***Conclusion*:** Altered miRNA expressions could have a significant impact on the abundance of proteins, making them attractive candidates as biomarkers for cancer detection and important regulators of apoptosis.

Keyword: Apoptosis, cancer, miRNAs, regulators, stress, leukemia.

1. INTRODUCTION

1.1. Discovery of miRNAs and their Regulation of Gene Expression

MicroRNAs (miRNAs) have been shown to play a wide integral role in cellular processes by controlling mRNAs' stability and translation of their target genes [1]. Less than 2% of the human genome is transcribed into mRNA transcripts and is regulated by the non-coding RNAs [2-4]. Developmental studies in the 1990s revealed the first endogenous ~22 nucleotide (nt) regulatory ribonucleic acids (RNAs) in *C. elegans* and demonstrated that diverse 3' untranslated region (UTR) motifs mediated crucial modes of post-transcriptional repression of gene expression in *Drosophila melanogaster* [5, 6]. This posttranscriptional modification of gene expression is carried out by the action of a group of these single-stranded miRNAs. Many studies have adopted the essential tumor suppressing roles of miRNAs and their downregulated expression levels showed elevated progression and development in many cancer types including acute lymphoblastic leukemia and acute myeloid leukemia [7]. Aberrant miRNA expression and processing resulted in their deregulated expressions in a number of diseases. More than 60% of the human protein-coding genes have been encoded as potential binding sites for miRNAs indicating their massive regulatory roles in cell survival and developmental processes [8].

2. miRNA BIOGENESIS

miRNAs are defined as small, non-coding RNAs that repress the translation of target messenger RNAs (mRNAs) [9, 10]. Mature miRNAs function by the formation of hairpin transcripts that are processed into short RNAs that are associated with Argonaute (Ago) proteins and form the RNA-Induced Silencing Complex (RISC), a ribonucleoprotein complex mediating post-transcriptional gene silencing [9]. The RISC complex also guides complementary miRNAs to

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target mRNAs for degradation, destabilization, or translational inhibition by the Ago protein [9-11].

miRNA biogenesis starts with the transcription of miRNA genes by either RNA polymerase II/III into primary miRNA (pri-miRNA) transcripts [12, 13]. The long primary miRNA transcript is synthesized in the nucleus from non-coding DNA and processed by an RNAse enzyme named Drosha and the DGCR8 (DiGeorge Critical Region 8) protein, also known as Pasha, to a ~70 nt precursor miRNA (pre-miRNA). After nuclear processing, the pre-miRNA is exported to the cytoplasm by Exportin-5 (XPO5) in a complex with Ran-GTP [14]. It is in the cytoplasm where the miRNA is further processed by the RNAse enzyme Dicer to give rise to the mature ~22 nt miRNA. RISC, with its core Argonaute (Ago2) protein component, is the cytoplasmic effector of the miRNA pathway and binds to a single-stranded miRNA, guiding it to its target mRNAs [15]. A mature miRNA is able to regulate gene expression by either binding to the 3'UTR of its target mRNA (Fig. **1**) causing translational inhibition or even its targeted destruction. Yet, the consequences for mRNA targets are very different: mRNA degradation results in irreversible removal from the transcriptome, whereas the level of mRNA targets can remain constant or even increase upon inhibition of translation.

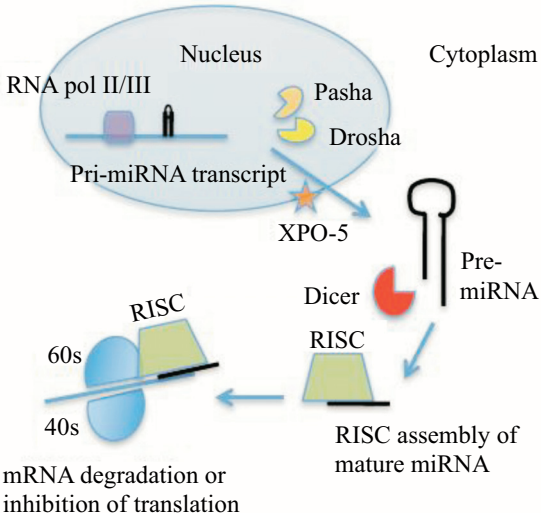

Fig. (1). miRNA regulation of gene expression. miRNAs are generated as primary (pri-miRNA) transcripts by RNA polymerase II/III in the nucleus. After being processed by the endonucleases Drosha and Pasha, the stem-loop structure of the precursor miRNA (pre-miRNA) is exported out of the nucleus by Exportin-5 (XPO-5). The endonuclease Dicer releases the mature miRNA, which is then assembled into the RNA-induced silencing complex (RISC). The RISC directs its bound miRNA to partially complementary mRNA 3' untranslated regions, resulting in inhibition of translation or targeted destruction of the mRNA [12-15].

3. REGULATION OF miRNA EXPRESSION

The questions related to what regulates miRNA is of major interest since these small RNA molecules are associated with many developmental and disease processes [16]. MiRNA expressions are encoded by genes, thereby, their expressions are regulated by various means including both the transcription regulation or processing [17, 18]. Another mode of regulation is linked to the nuclear export of pre-miRNA precursors or their localization to specific cytoplasmic compartments associated with the regulation of mRNAs' stability and RNA interference [14].

The location of miRNA genes within the genome is also a major feature in terms of transcriptional regulation. Several miRNA genes are located within the non-coding regions of other host genes leading to the cotranscription with the genes that contain them that are involved in the same biological pathways [19]. Other miRNAs are found to be located in the intergenic regions, having an independent transcription site. Although all primary miRNA transcripts are transcribed by RNA polymerase II and initiated by promoters, many transcription factors are not fully understood. Several transcription factors such as tumor suppressor p53 have been liked with the regulation of many miRNAs that are involved in apoptotic regulation and cell proliferation including miR-23a, miR-21, miR-34, miR-145, miR-192 and miR-215, miR-17-92 [20-22].

Posttranscriptional modification of miRNA is an important feature that needs to be taken into consideration as well [23]. Pre-mRNA processing including packaging and alternative splicing is under the control of a nuclear RNA-binding protein HnRNP A1. It plays an important in miRNA production by inducing conformational changes in the pri-miRNA transcripts that enhance its processing by Drosha and the DGCR8 protein [14]. Many miRNAs are shown to be regulated by post-transcriptional modifications such as let-7 family in embryonic cells, lin28 in *C. elegans* and miR-18 in HeLa cells [24, 25]. In humans, both lin28 and let-7 manipulated expressions are considered as important biomarkers for cancer progression, stem cell aging and pluripotency. Moreover, transgenic mice with different genetic variation lin28-let7 expressions have shown significant changes in body size and delayed onset of puberty [26].

4. miRNA REGULATION OF THE STRESS RESPONSE

miRNAs are widely expressed and regulate essentially all signaling pathways, playing an important role in gene silencing and controlling a number of essential processes including cell proliferation, development, differentiation and apoptosis [7, 27, 28]. There are over 1000 human miRNAs currently identified in the miRNA database released October 10, 2010 (http://www.mirbase.org), even outnumbering kinases and phosphatases, indicating their extensive role in the regulation of cellular processes. Each miRNA is predicted to bind hundreds of target mRNAs, therefore a large portion of human genes are subject to miRNA regulation [29]. This binding morphology implies that the expression of the mRNA target is likely to be under the control of multiple miRNAs [30]. Consequently, nearly all developmental, physiological and disease-related processes appear to be regulated by miRNAs, at least to some degree [8]. Notably, miRNA processing defects may also enhance tumorigenesis [31]. Aberrant miRNA processing leads to altered expression of mature miRNAs,

often contributing to the disease. Over 120 miRNA transcripts demonstrate differential expression in response to hyperthermia [32] and interestingly HSP70 has also been shown to influence miRNA processing [33]. At present, this mechanism is, however, not well understood.

Many reports have shown deregulation of miRNAs in certain cancer types while others provided important pro-apoptotic functions. A group of miRNAs including miR-330, Let-7 family and miR-15a/16 have been downregulated in several cancer types including prostate, colon, breast and prostate. These preliminary analyses can be used as promising biomarkers for anticancer therapy [34-37]. Reduced levels of mature miRNAs were also reported in various tumors primarily due to epigenetic silencing or alterations in biogenesis pathways [38]. Nowadays, the diagnosis of early cancer stage development is now dependent on variable miRNA expression levels as potential oncogenic biomarkers in validating and targeting microRNAs for cancer therapy. Overexpression of various microRNAs including miR-155, miR-21, miR-29 and miR-17~92 has led to B cell malignancy, potentiated KrasG12D-induced lung tumorigenesis, chronic lymphocytic leukemia and Lymphoproliferative disease and autoimmunity, respectively [39, 40].

Cell survival is directly related to miRNA regulations and not surprisingly their expression is often altered in a number of diseases including cancer [41]. The majority of miRNAs are transcribed as a part of a cluster and their overexpression is implicated in many diseases such as cancer and cardiac hypertrophy [42]. Some pro-survival miRNAs act as suppressors and inhibit the expression of pro-apoptotic regulators APAF1, caspase-7 and NOXA [21, 43, 44]. Stress-induced factors such as hyperthermia have shown to decrease miR-23a levels resulting in the overexpression of the pro-apoptotic protein NOXA and leading to apoptosis [21]. Another study showed that the decrease in the abundance of miR-23a is due to CDK5 inhibition and this phenomenon could be reverted by the overexpression of the molecular chaperon HSP70 in stressed cells [45]. Hyperthermia has been shown to alter the expression profile of numerous miRNAs [21, 43, 45]. Screening of miRNA microarrays revealed that 123 different miRNA transcripts were affected by exposure of cells to hyperthermia with both increased and decreased expression of various miRNAs [21, 32, 43]. Whether altered miRNA expression contributes to heat-induced apoptosis is not known.

5. miRNA MODULATION BY P53

Recent reports show that misexpression of miRNAs, whether elevated or reduced, is seen in all types of cancer [7, 9]. As a protein that senses environmental stress and modulates the level of miRNAs, the tumor suppressor p53 regulates the expression of specific miRNAs at two different levels: transcription and processing. Upon DNA damage, p53 induces the transcription of some miRNAs that together promote tumorigenesis by blocking p53, thus preventing cell cycle arrest or apoptosis [46]. These miRNAs can be classified as oncogenic miRNAs. In addition, p53 enhances the processing of certain pri-miRNAs in cancer cells by associating with DDX5, a cofactor of Drosha [47]. Overexpression of these miRNAs can act as tumor suppressors, not necessarily by inhibiting cell growth, but by preventing the progression of cancer [9]. Interestingly, most p53 mutations found in cancers are located in a domain that is required for both the miRNA processing function and transcriptional activity [47]. Therefore, loss of p53 function in transcription and miRNA processing might contribute to tumor progression.

In response to the environmental stress, p53 modulates a number of specific miRNAs at their transcription and processing level. Many cancers contain p53 mutations located in a domain that is required for miRNA processing and its transcriptional activity [47]. Therefore, loss of p53 function could contribute to tumor progression by preventing stress-induced miRNA transcription and processing. Due to their overexpression in several human cancers, these miRNAs have been implicated as oncomirs, acting as tumor suppressors by preventing cancer progression [9].

6. miRNA REGULATION OF APOPTOTIC PROTEINS

Numerous studies have uncovered highly specific miRNA profiles during development, tumorigenesis and apoptosis [41, 48]. It is currently undisputed that miRNAs function as important regulators of apoptosis. Recent studies show that miRNAs can regulate the expression of key apoptotic regulators including the Bcl-2 family proteins. Zhang and colleagues found that miR-221 and miR-222 directly target PUMA mRNA translation in various types of cancer cells [17]. Many regulators such as miR-15, miR-16, and miR-34a were also documented to downregulate Bcl-2 in Chronic Lymphocytic Leukemia (CLL) [27, 49, 50]. Additionally, RNA interference (RNAi) mechanisms have been shown to regulate apoptosis directly by targeting these miRNAs and ultimately altering the expression of PUMA in glioblastoma, A549 lung and MCF-7 breast cancer cell lines (human breast cancer cell line with estrogen, progesterone and glucocorticoid receptors) [51]. A knockout of miR-221/miR-222 results in restoration of the pro-apoptotic PUMA protein and its activity inducing cancer cell apoptosis and inhibition of tumor growth in these cells (Fig. **2**). miR-221 and miR-222 are only a few of the miRNAs shown to target and alter the expression of PUMA, and may, therefore, be potential therapeutic targets in the treatment of certain types of cancers [51]. Alternatively, Ouyang and colleagues found miR-29a to target PUMA mRNA when studying forebrain ischemia [52]. Another very recent study on the liver carcinoma cell line, HepG2, has raised the possibility that miR-483-3p modulates PUMA and that its induced expression can protect cells from apoptosis [53]. miR-BART5, an Epstein-Barr Virus (EBV) encoded miRNA is another miRNA that has been shown to have anti-apoptotic activity targeting PUMA expression in Nasopharyngeal Carcinoma (NPC) and in EBV-associated gastric carcinoma latently infected with EBV to protect these cells from apoptosis. Interestingly, this was the first evidence that some viruses are able to modulate cellular apoptosis through miRNAs [54]. Moreover, overexpression of miR-24-2 impairs the pro-survival of Bcl-2 by binding to

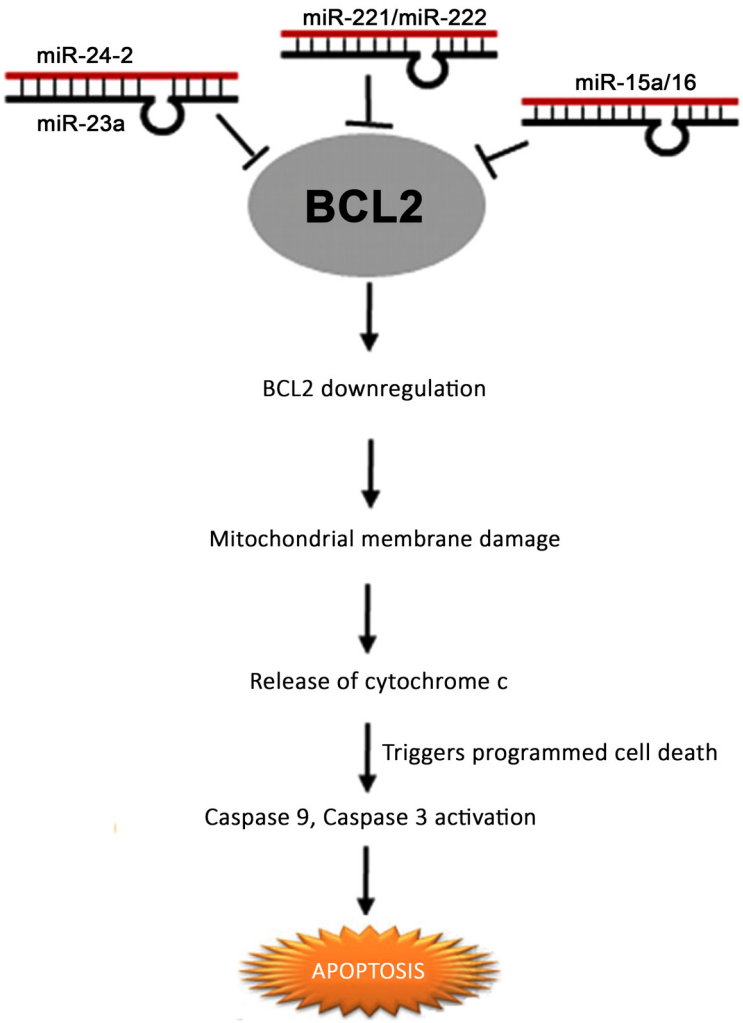

Fig. (2). Diagram showing a mechanism of cellular apoptosis induction by miRNAs. Downregulation of the anti-apoptotic protein BCL2, miR-23a, miR-24-2, miR-15a/16 and miR-221/miR-222 reduce mitochondrial membrane potential and cause release of apoptotic molecule cytochrome c into the cytosol. Cytosolic cytochrome c leads to activation of a caspase cascade and ultimately results in apoptosis [21, 22, 34, 35, 51].

its 3'UTR and activated apoptotic pathways in the Human Embryonic Kidney (HEK 293T) and breast cancer (MCF7) cells [55]. Transcribed as part of the miR-23a~27a~24-2 cluster, miR-23a is deregulated in several diseases including cancer where expression is elevated [42, 56]. This cluster has been found to be amplified in a mouse model of colon cancer and the elevated miR-23a expression was shown to promote the transition of indolent adenomas to invasive colorectal cancers [57]. Elevated expression of miR-23a was also documented in human colorectal cancers and colon cancer cell lines. miR-23a has been shown to target APAF1 (Chen *et al*., 2014), Caspase-7 (CASP7) [44], and NOXA [21] and together with our finding that it also targets NOXA, it reveals that this miRNA coordinately antagonizes the expression of multiple apoptotic regulators and therefore its downregulation during proteotoxic stress ensures effective apoptotic pathway activation (Table **1**). Because a large number of miRNAs contribute to the development of cancer and other diseases, the discovery of miRNA pathways has opened up a novel therapeutic approach for the treatment and these findings highlight the importance of miRNAs as promising therapeutic molecules.

7. THERAPEUTIC ROLES OF miRNAs

Evasion of apoptosis is both a hallmark of cancer and is involved in tumorigenesis and drug resistance. Overcoming chemotherapeutic drug resistance remains a key challenge in the fight against cancer. This drug resistance may be in large part due to resistance to apoptosis. Where apoptosis can be effectively induced, dramatic clinical responses are often seen [58]. This reinforces the basic concept that achieving efficient apoptosis is essential for optimizing therapeutic responses and therefore the clinical outcome.

Recently miRNAs have been shown to be important in regulating apoptotic proteins and therefore cell death. The field of miRNAs based pharmacogenomics is still relatively new but given their involvement in the regulation of apoptosis and the understanding that most chemotherapeutic drugs kill cells by this mechanism, core apoptosis pathway-

Table 1. Validated miRNAs targeting pro- or anti-apoptotic proteins.

Anti-Apoptotic	Pro-Apoptotic			
NOXA and PUMA	**Mcl-1**	**Bcl-2**	**BCL-xL**	**Bcl-w**
miR-149-5p	miR-29	miR-15/16	Let-7c/g	Let-7a-3p
miR-23a	miR-30	miR-24	miR-133a	miR-107
miR-23b	miR-101	miR-34a [98]	miR-326	miR-122
miR-192	miR-125b	miR-125b	miR-377	miR-125b
miR-125b	miR-133a	miR-153	miR-491	miR-133b
miR-155	miR-133b	miR-155	miR-608	miR-203
miR-194	miR-153	miR-195 miR-204		miR-335
miR-215	miR-181	miR-206		
miR-34a,b,c	miR-193a	miR-365		
	miR-302b	miR-497		
	miR-320	miR-1290		
	miR-512	miR-15a/16		
		miR-221		
		miR-222		
		miR-23a		
		miR-24-2		

targeting miRNAs have good potential as predictors of anticancer drug efficacy [41]. This, along with the abundance and wide range of activity of miRNAs, makes them attractive candidates as biomarkers for cancer. In addition, the modulation of miRNA expression with miRNA mimetics or antagomirs may be a possible therapeutic avenue [28]. One advantage of using miRNAs as therapeutic targets is that they often regulate multiple mRNA targets that belong to the same signaling pathway or protein complexes at the same time [59, 60]. The key is to identify which miRNAs and which targets are involved in each particular disease. There are ongoing efforts to deliver miRNA mimics or perfectly complementary antagomir inhibitors to increase or decrease, respectively, the levels of specific miRNAs *in vivo*. In cases where a specific miRNA-mRNA target interaction should be modulated, short oligonucleotides termed as "target protectors" have been successfully applied in mammalian cells [61]. The idea of a target protector is that a single-strand oligonucleotide could specifically interfere with a single target while leaving the regulation of the other targets of the same miRNA unaffected. Ultimately, this avenue of research will lead to better understanding and treatment of diseases in which miRNAs mediate the modulation of mRNA expression. Once better understood, the expression of the miRNAs can be altered effectively to restore the expression of the target mRNA and protein.

The importance of miRNA regulatory activities in multiple apoptotic pathways leads us to overcome many stress challenges by delivering these molecules in clinical implementations. Misregulation of miRNAs was observed in multiple cancer development situations accompanied by the inhibition of cellular survival and the activation of apoptotic pathways. Recent clinical trials used miRNAs that are potential biomarkers for patient diagnosis, stratification and drug treatments for therapeutical agents in cancer [62]. Anti-apoptotic Bcl-2 genes were downregulated upon the overexpression of miR-15, miR-16, and let-7, leading to the activation of cellular apoptotic pathways in cancer cells [27]. Another study demonstrated that Bcl protein was a downstream target for a group of microRNAs including miR-24-2, miR-195 and miR-365. Overexpression of these microRNAs led to induced apoptosis by directly inhibiting Bcl protein in a clinical breast cancer treatment [63]. Another clinical treatment used miR-34a mimic as a potential therapeutic strategy for blocking tumor cell migration in liver cancer patients [64].

CONCLUSION

The critical role that miRNAs play in the regulation of stress responses has only recently gained significant attention [65]. Their roles in apoptosis and tumorigenesis are well-documented and not surprisingly many apoptotic regulators including Bcl-2 family members NOXA, PUMA and caspases are regulated by miRNAs that in many cases have altered patterns of expression in several types of cancer such as lung, colon, breast and prostate cancer, and are therefore considered to be promising targets for anticancer therapy. There are only a few reports on miRNA expression in heat-stressed cells [66]. The recently discovered role of miRNAs as regulators of gene expression opened up several new areas of research and suggested that these can be used as important biomarkers. In general, the expression of some miRNAs was found to be enhanced while others' expressions reduced in studies using both cell cultures and whole animals [67-69].

Studies indicate that directly targeting the apoptotic machinery through the manipulation of miRNA expression may offer a new hope for improved therapy for cancer and other autoimmune and infectious diseases. Cell lines that stably express the miRNAs under study here will allow the examination of their role in the heat-induced regulation of apoptotic protein expression and also other regulators of apoptosis that are implicated in the process of controlling. More importantly, stably transfected shRNA knockdown cells can be created to abolish miRNA expression with the hope of restoring apoptotic protein expression under conditions of cellular stress.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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