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Role of MicroRNA in the Regulation of Apoptosis

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Abstract

Daisy R Roy, Chandra SBC. Role of MicroRNA in the Regulation of Apoptosis. ARBS Annu Rev Biomed Sci 2008;10:63-74. MicroRNAs (miRNAs) are short, non-coding RNA molecules that suppress gene expression by selectively base-pairing to the complementary messenger RNA (mRNA). Post-transcriptional silencing of target genes by miRNA occurs either by targeting specific cleavage of homologous mRNAs or by targeting specific inhibition of protein synthesis. A number of studies with anti-sense miRNA have helped us in better understanding of miRNAs role in cellular pathways. Multi subunit protein complex containing DROSHA, an RNASE type III endonuclease, is one of the two main enzymes necessary for processing unprocessed miRNA. The influences of miRNA on plethora of biological processes like cell renewal and differentiation make miRNA quintessential in elucidating number of genetic processes. miRNA can be mediators and future targets for therapeutics in regulating cell growth and apoptotic pathways. The findings that miRNA down regulates BCL2 expressions in leukemia cell lines have reinforced the idea that their regulatory roles in apoptosis are very important. E2F1 of E2F family of transcription factors regulates apoptosis, an evolutionarily conserved programmed cell death. miRNA molecules modulate the translation of E2F1 mRNAs by binding to the sites in their 3'-untranslated region. Recent advances in miRNA are reviewed and the role of miRNA and its function in regulating apoptosis is discussed in this paper.

Keywords: miRNA, apoptosis, DROSHA, DICER

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1. Introduction

MicroRNAs (miRNAs) are small, endogenous, non-coding, (22-25 nucleotide) RNAs that bind to complementary sequences in the 3' untranslated region (3' UTR) of messenger RNA (mRNA). miRNAs get processed by a cellular nuclease, DROSHA, before being transported by an exportin-5 (XPO5)-dependent mechanism into the cytoplasm (He & Hannon, 2004; Kim, 2005; Lee *et al*., 2002; Lee *et al*., 2003). Once in the cytoplasm miRNAs are cleaved further by another ribonuclease type III enzyme DICER (Yi *et al*., 2003; Lund *et al*., 2004) and the resulting 17–24 nucleotide miRNAs associate with a cellular complex, the RNA-induced silencing complex that participates in RNA interference (Hutvagner *et al.*, 2004). The RNA-induced silencing complex-bound single-stranded miRNA guides this complex to mRNAs, complementary to the miRNA. Thus miRNAs, the cellular RNAs expressed in cells from plants to animals inhibit the ability of mRNAs to express protein products (Fig. 1).

MicroRNAs are fundamental, post-transcriptional regulators of target gene expression and they were first identified in the flat worm *Caenorhabditis elegans* in 1993 (Lee *et al*, 1993), but their significance was not appreciated until 2001 (Lee & Ambros, 2001). These non-coding RNAs have great importance in spectrum of biological processes like regulation of cell differentiation, cell cycle, apoptosis (Alvarez-Garcia & Miska, 2005), Embryo development and Brain development in Zebra fish (Weinholds *et al*., 2005; Schier *et al*., 2006). Also miRNAs play a role in oncogenesis and may serve as future targets for therapeutics. The role of miRNAs in cellular pathways is enhanced by the utilization of antisense molecules (Boutla *et al*., 2003; Hutvagner & Zamore, 2002; Meister *et al*., 2004). Antisense inhibition of human miRNA activity indicated the involvement of miRNA in apoptosis (Chan *et al*., 2005). The milestone in the development of research of miRNA is the evidence that its deregulation leads to many types of diseases like prostate cancer (Slack & Weidhass, 2006; Chandan & Sashwati, 2007).

MicroRNAs are key regulators of gene expression, and are differentially expressed among tissues and disease states. mRNAs containing multiple, non-overlapping miRNA binding sites are more responsive to miRNA-induced translational repression than those containing a single miRNA binding site (Doench *et al*., 2003; Zeng *et al*., 2002). Translation regulation of target mRNAs takes place by the strand separation of the double-stranded RNA (dsRNA) produced by DICER, and association of the single-stranded mature miRNA with the RISC (Hutvagner & Zamore, 2002). Selection of the active strand from the dsRNA appears to be based primarily on the stability of the termini of the two ends of the dsRNA (Khvorova *et al*., 2003; Schwarz & Zamore, 2002). The strand with lower stability base pairing of the 2–4 nucleotides at the 5' end of the duplex preferentially associates with RISC and thus becomes the active miRNA (Schwarz *et al*., 2003). A single miRNA can orchestrate multiple pathways involving the regulation of multiple genes.

Apoptosis is a conserved process by which organisms remove old, unnecessary, and unhealthy cells that are dangerous for the survival of the organism. Components regulating cell death have been identified and proteins are considered to be the primary effectors and regulators of cellular functions. However with research of past years it became clear that roughly 1% of predicted genes in animals and plants encode small noncoding RNAs known as miRNAs, which regulate gene function. Thirty percent of these miRNAs are highly evolutionarily conserved (Lai *et al*., 2004; Nakahara *et al*., 2005). The recent identification of potent miRNA cell death inhibitors in *Drosophila*, hints that such regulators are also likely to exist in mammals, and more generally the approaches and tools that are now available to probe roles for noncoding RNAs in the mechanism of cell death regulation (Brennecke *et al*., 2003; Xu *et al*., 2003) indicate the regulatory role of miRNAs.

Apoptosis plays critical role in development and tissue homeostasis (*Thompson, 1995; Savill et al.,* 2003*)*. Apoptosis in animals occurs by members of a family of proteases known as caspases (Cui *et al*., 2006). Caspases become activated in response to many different death signals and the active caspases cleave different cellular substrates, leading to cell death. Cells constitutively express caspase zymogens (inactive precursors) sufficient to bring about apoptosis. Thus, the key to cell death and survival signaling revolves around controlling the levels of active caspases in the cell. Following activation, caspase

activity is antagonized by the action of members of the IAP (inhibitor of apoptosis) family of cell death inhibitors, the only known cellular caspase inhibitors (Gupta *et al*., 2006).

The progress of apoptosis is regulated in an orderly way by caspase-cascade system and transforming growth factor beta (TGFB) signaling pathway (Fesik & Shi, 2001; Chen & Goeddel, 2002). Mostly, the activation of caspases results in initiation of apoptosis and this is the marker often used to analyze the induction of apoptosis (Fan *et al*., 2005). E2F1 is a transcription factor with an ability to activate genes required for synthetic phase of cell cycle. E2F1 acts as a specific signal for the induction of apoptosis by affecting the accumulation of P53 protein (Chiarugi *et al*., 1997; Mallikarjuna *et al*., 2006). Deregulation of E2F transcription factor expression leads to S-phase entry and P53 mediated apoptosis (Santosh *et al*., 2005).

Virus encoded miRNA protects some cells from entering apoptosis. miRNA encoded by the Latency-Associated Transcript gene (LAT) in the HSV-1 viral genome prevents normal cell death or apoptosis by RNA interference (Gupta *et al*., 2006). miRNAs encoded by cellular genes are known to be controlling gene expression, but this is one of the first miRNA found to be encoded by a viral genome to help HSV-1 maintain a latent infection for the lifetime of an infected individual (Perng *et al*., 2002). miRNA interferes with the translation of two cell proteins that are required for cell death: TGFB and SMAD family member 3 (SMAD3). The LAT miRNA binds to specific sequences of mRNA from these two genes and causes them to be degraded. Thus, the amount of TGFB and SMAD3 protein is reduced in the cell and apoptosis is prevented (Chiarugi *et al*., 1997; Yanna *et al*., 2007). Also as the latent virus is not producing any viral proteins, the immune system of the infected individual cannot detect the infected cell.

Latent HSV-1 infections occur in neuronal cells of the peripheral nervous system. When a latent infection is reactivated by different kinds of stress, HSV-1 proteins are synthesized and new infectious virus particles are formed (Thompson *et al*., 2001). These virus particles migrate along the neuronal axons to the epithelial cells of the skin. Viral growth in the skin, or other mucous membranes where nerves are found, causes cell damage and an immune reaction that result in a painful sore. miRNA may be a more general mechanism that latent viruses use to remain alive in the host cell (Ahmed *et al*.,2002). Finding a miRNA that interacts with the cellular TGFB pathway during latency offers the first target against the latent infection and offers a profoundly different approach to treatment. The recent findings on miRNAs function and its role in apoptosis are the subject of this review. The regulation of apoptosis by miRNAs could contribute to developing specific therapeutics for specific diseases, leading to the development of diagnostic tools.

2. The Impact of microRNA on Gene Expression

Central dogma, the backbone of an entire discipline of molecular biology states that the genetic information is transferred from nucleic acids to proteins through mRNA. Information is transferred from DNA to its perfect copy miRNA, by transcription and further into proteins, the final effectors of gene's action by translation. Identification of miRNA revealed a contrasting dimension in this process that expression of genetic information encoded in DNA, is regulated by the code in the miRNA itself (Siao, 2006). After the biosynthesis of miRNA (Fig. 1), the single stranded miRNA promotes posttranscriptional silencing such that the code in miRNA is complementary to the target mRNA. This miRNA acts to regulate gene expression by binding to target mRNAs, thereby blocking ribosome processing and hindering mRNA translation (Perkins *et al.*, 2005). More than 3000 miRNAs with diverse functions have been identified in animals, plants, and viruses and these small molecules regulate approximately 30% of genes in humans (Lewis *et al.*, 2005). miRNAs share limited sequence complementarities with their mRNA targets, so that each miRNA could possibly interact with scores of genes.

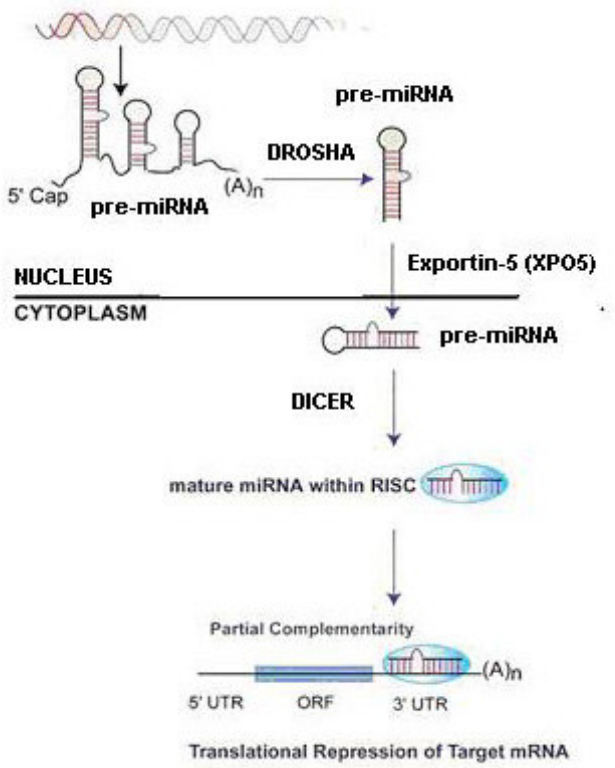

Figure 1. MicroRNA (miRNA) biogenesis pathway: The excision and activation of single-stranded miRNAs from precursor transcripts occurs through a multi-step process. Primary miRNAs (pri-RNA) transcribed in the nucleus are first processed by the RNASE III enzyme, DROSHA, and are then exported out of the nucleus. Once in the cytoplasm, they are subjected to second processing step by another RNASE III enzyme, DICER, resulting in mature miRNA. DROSHA: class 2 RNASE III enzyme responsible for initiating the processing of miRNA. DICER: a ribonuclease in the RNASE III family that cleaves double-stranded RNA (dsRNA) and precursor miRNA (pre-miRNA) into short dsRNA fragments. RISC (RNA-induced silencing complex): a multi-protein interfering RNA (siRNA) complex which cleaves (incoming viral) dsRNA and binds short antisense RNA strands which are then able to bind complementary strands.

3. Biogenesis of microRNA

MicroRNAs are transcribed into primary transcripts (pri-miRNA) by RNA polymerase II and then processed by two processing events. The RNASE-III family of enzymes DROSHA and DICER sequentially cleave the primary transcript (He & Hannon, 2004; Lee *et al*., 2002; Lee *et al*., 2003; Kim, 2005). Pri-miRNAs, which are several kilo bases long, are poly-adenylated and capped (Cai *et al*., 2004; Cullen, 2004; Lee *et al*., 2003). The pri-miRNAs is processed by the complex composed of DROSHA and its associated binding partner PASHA (or its human homolog, DGCR8), a dsRNA binding protein. Excision of a 65–75 nucleotides takes place and it results in stem-loop miRNA precursor called a pre-miRNA (Lee *et al*., 2003; Meister *et al*., 2004).

The pre-miRNAs processed are then recognized and transported from the nucleus to the cytoplasm via the Ran-GTP dependent nuclear transmembrane protein, exportin5 (XPO5), where they are then subjected to a second cleavage step by DICER (Yi *et al*., 2003; Lund *et al*., 2004). Processing by DICER results in the production of a small double stranded mi-RNA duplex containing 2-nucleotide-

long 3' overhangs (Bernstein *et al*., 2001). These double-stranded molecules are unwound by a yet unidentified helicase and a single mature strand can be asymmetrically incorporated into the RNA-induced silencing complex (RISC) (Khvorova *et al*., 2003; Schwarz *et al*., 2003). There they act by translational repression (by a cleavage-incompetent RISC) or by mRNA degradation (by a cleavage-competent, Slicer-containing RISC) (Schwarz *et al*., 2003; Cullen, 2004; Fan *et al*., 2005). The mechanism by which activated RISC complex locates the mRNA targets in the cell is not known completely but it has been shown that the process is not coupled to ongoing protein translation from the mRNA (Sen *et al*., 2005). Functional analysis of mammalian miRNA is understood by altering the endogenous levels of miRNAs with miRNA specific reagents like antisense oligonucleotides that reduce miRNA activity (Hutvagner *et al*., 2004; Meister *et al*, 2004).

The pre-miRNAs are predicted to form irregular hairpin structures containing various mismatches, internal loops and bulges (Jones, 2004). The structure of pre-miRNAs is among the factors that may influence the rate at which specific miRNAs emerge in cells. The pre-miRNAs harboring miRNAs at either the 5'or 3'-side revealed the conservation of two high stability regions (positions 4–8 and 15, 16). This may suggest that these regions are involved in determining either the binding or cleavage specificity of the processing enzymes. When DICER cleaves the pre-miRNA stem-loop, two complementary short RNA molecules are formed, but only one is integrated into the RISC complex. This strand is known as the *guide strand* and is selected by the argonaute protein, the catalytically active RNASE in the RISC complex, on the basis of the stability of the 5' end (Preall *et al*., 2006). The remaining strand, known as the *anti-guide* or passenger strand is degraded as a RISC complex substrate (Gregory *et al*., 2005). miRNAs base pair with their complementary mRNA molecules and induce mRNA degradation by argonaute proteins, the catalytically active members of the RISC complex. It is as yet unclear how the activated RISC complex locates the mRNA targets in the cell, though it has been shown that the process is not coupled to ongoing protein translation from the mRNA (Sen *et al*., 2005).

RNA-induced silencing complex (RISC) (Bernstein *et al*., 2001) is responsible for the gene silencing observed due to miRNA expression and RNA interference. The pathway in plants varies slightly due to their lack of DROSHA homologs; instead, DICER homologs alone affect several processing steps (Kurihara & Watanabe, 2004). Efficient processing of pre-miRNA by DROSHA requires the presence of extended single-stranded RNA (ssRNA) on both 3' and 5' ends of hairpin molecule. The mechanism of plant miRNA biogenesis requires at least three cleavage steps. Experiments demonstrated that these motifs could be of different composition while their length is of high importance if processing is to take place at all (Zeng *et al*., 2003).

4. Mechanism of microRNA Action in the Cell

Hundreds of miRNAs have been identified in plants and animals most of which are conserved between species (Gregory *et al*., 2002; Wittbrodt *et al*., 2006). This suggests that miRNAs have diverse and important regulatory roles in organisms. Two mechanisms for regulation of gene expression by miRNAs have been reported. Firstly, target RNAs having perfect complementary sequences to the miRNA are cleaved by ribonucleases in the RISC complex (Llave, 2002) as shown in Fig. 2. Secondly, target RNAs containing sequences imperfectly complementary to the miRNA are subjected to translational control.

MicroRNA could have either a positive or negative regulatory impact on gene expression, depending on the base pairing of miRNA, which influences the structure and composition of the mRNA ribonucleoprotein (RNP). miRNAs could bind to protein factors and thereby recruit them to specific mRNAs, or they could alter mRNA secondary structure, and thereby indirectly control the binding of other regulatory factors (Ambros, 2001).The function of miRNAs in gene regulation is due to its complementarity to a part of one or more mRNAs. Animal miRNAs are usually complementary to a site in the 3' UTR whereas plant miRNAs are usually complementary to coding regions of mRNAs. In animals, the primary effects of most miRNAs are translational repression or destabilization of mRNAs by deadenylation. In contrast, plant miRNAs appear to act mainly by guiding cleavage of specific target mRNAs, with the mechanism of cleavage being similar to cleavage guided by short interfering RNAs

(siRNAs). The primary mode of action of animal miRNAs is by annealing of the miRNA to the mRNA which inhibits protein translation and also sometimes cleaves the mRNA (Doench & Sharp, 2004). In plants, miRNAs have perfect or near perfect complementarity to their mRNA targets (Rhoades *et al*., 2002). Upon binding to their mRNA targets, the miRNA-containing RISCs function as endonucleases, cleaving the mRNA (Llave *et al*., 2002). In plants this occurs by the formation of dsRNA through the binding of the miRNA which triggers the degradation of the mRNA transcript through a process similar to RNA interference (RNAi) (Hutvagner *et al*., 2004). In contrast to plant miRNAs, the complementarity between animal miRNAs and their targets is usually restricted to the 5' region (nucleotides 2-8 or 2-7) of the miRNA, i.e. to the 3' region of the target site (Lai *et al*., 2004). In the absence of extensive complementarity between the miRNA and the target, binding of the RISC blocks translation of the target mRNA into protein, rather than catalyzing its cleavage into two pieces (Olsen & Ambros, 1999). miRNAs function mostly in association with a complement of proteins collectively termed the miRNP. Unlike miRNA pathway, RNAi is a different technique in which exogenous, dsRNAs complementary to known mRNAs are introduced into a cell to specifically destroy that particular mRNA, thereby diminishing or abolishing gene expression.

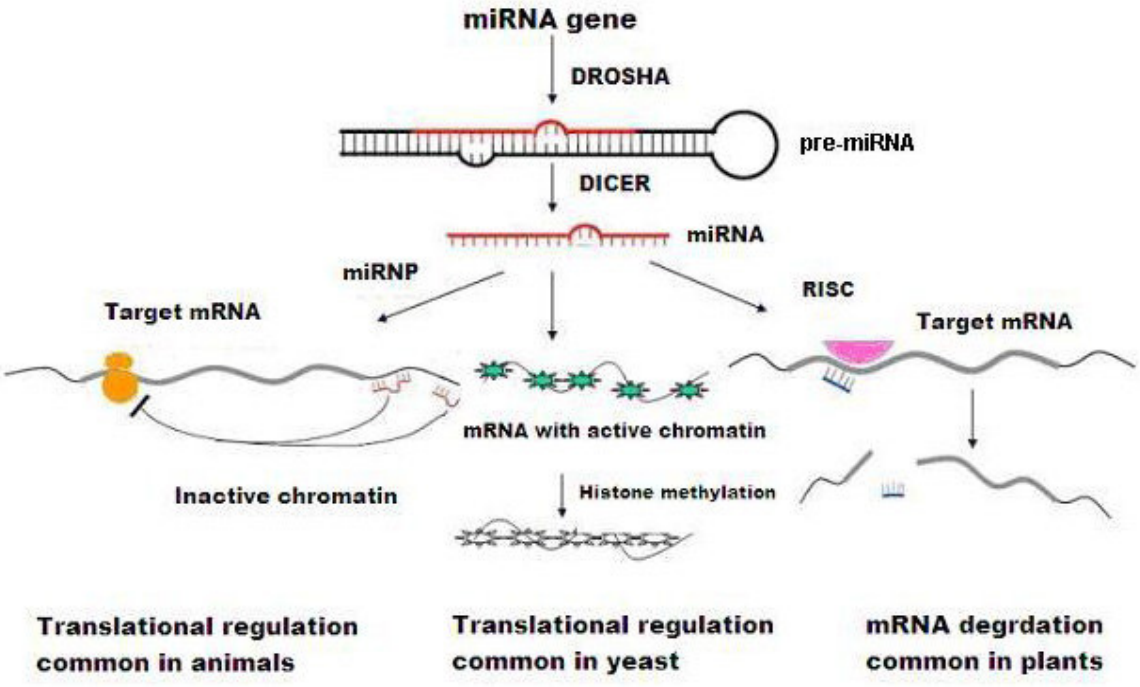

Figure 2. Action of microRNA (miRNA) in cells: binding of the miRNA to a target messenger RNA (mRNA) alters the RNA secondary structure, displacing proteins, and/or directly recruiting other proteins, and/or exposing binding sites for other proteins. miRNAs also target methylation of genomic sites corresponding to targeted mRNAs. Pre-miRNA: precursor miRNA. miRNP: novel class of ribonucleoproteins containing numerous miRNAs. RISC (RNA-induced silencing complex): multi-protein interfering RNA (siRNA) complex which cleaves (incoming viral) dsRNA and binds short antisense RNA strands which are then able to bind complementary strands.

5. MicroRNA and Apoptosis

MicroRNAs have emerged as important regulators of development and control processes such as cell fate determination and cell death (Lee *et al*., 1993; Wightman *et al*., 1993; Moss *et al*., 1997; Abrahante *et al.*,2003; Brennecke *et al*., 2003; Johnston & Hobert, 2003; Lin *et al*., 2003; Chang *et al.*, 2004; Chen *et al.*, 2004). Changes in the level of miRNAs altered the control of growth or apoptosis in

some cancers (McManus, 2003; Xu *et al*., 2004). Reductions in the levels of MIRN15A and MIRN16, MIRNLET7A1 or MIRN143 plus MIRN145 have been reported in chronic lymphocytic leukemia (CLL) (Calin *et al*., 2002), lung cancer (Takamizawa *et al*., 2004), and colon carcinoma (Michael *et al*., 2003) respectively. miRNAs regulate differentiation, proliferation, and apoptosis, the important processes in neoplastic transformation (Miska, 2005). Many miRNAs map to fragile sites or cancer-associated regions of chromosomes (Calin *et al*., 2004). In some contexts, miRNAs resemble tumor suppressors, and in other situations, they appear to have oncogenic potential. In the highly malignant human brain tumor glioblastoma, MIRN21 was strongly over expressed. When MIRN21 was knocked down in glioblastoma-cultured cells, caspases were activated, causing an increase in cell apoptosis. This suggests a role for a MIRN21 as a suppressor of apoptosis in this malignant tumor (Chan *et al*., 2005).

MicroRNAs regulate apoptotis both as pro- and anti-apoptotic molecules. Apoptosis is induced in various cell types by different pathways. Among them one of the pathways is TGFB-induced apoptosis (Schuster & Krieglstein, 2002) which is further mediated in two ways: the SMAD pathway or the death associated protein 6 (DAXX) pathway. In the SMAD signaling pathway (Feng & Derynck, 2005), TGFB dimers binds to a type II receptor which phosphorylates a type I receptor (Fig. 3A). TGF type I and II receptors can only be distinguished from each other by peptide mapping and the receptor here is a serine/threonine receptor kinase, The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD). SMAD3, an R-SMAD, then binds to the common SMAD (coSMAD), SMAD4 and forms a heteromeric complex. This complex then enters the cell nucleus where it activates the mitogen activated protein kinase 8 (MAPK8) pathways which then triggers apoptosis.

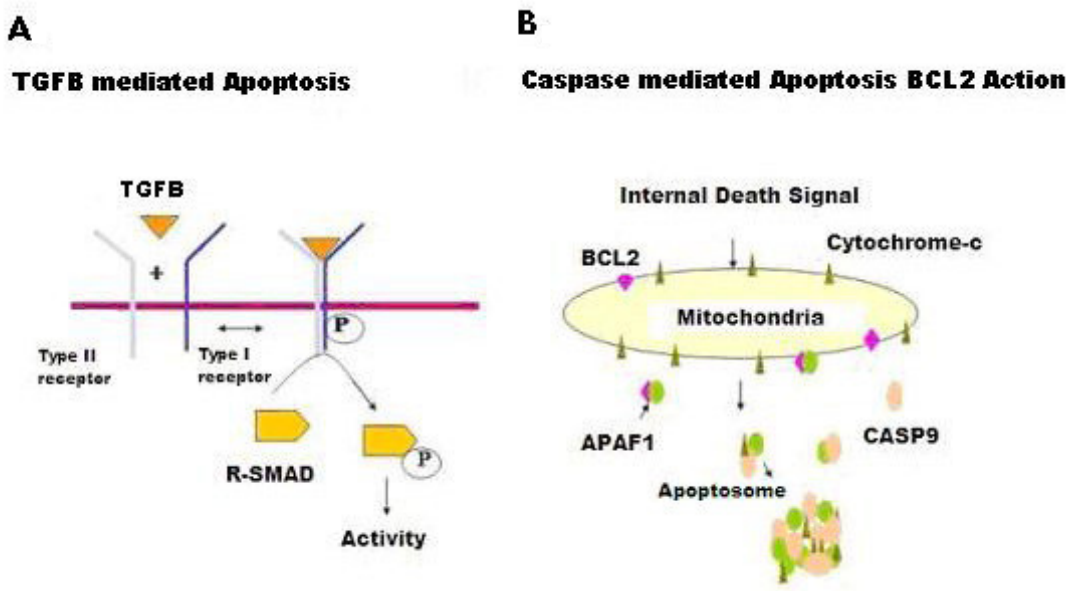

Figure 3. A: Cytokines of the transforming growth factor beta (TGFB) super family peptides regulate apoptosis through SMAD proteins by binding to type I and II serine/ threonine kinase receptors. Phosphorylated SMADs, form complex with mediator SMADs and move out of the nucleus to act as components of transcription factor complexes. B: BCL2 is an integral membrane protein located mainly on the outer membrane of mitochondria. Over expression of BCL2 prevents cells from undergoing apoptosis by preventing the efflux of cytochrome c. Cytosolic cytochrome c is necessary for the initiation of the apoptosis. TGFB is a protein that comes in three isoforms called TGFB1, TGFB2 and TGFB3. TGFB controls proliferation, cellular differentiation, and other functions in most cell types. R-SMAD: R-SMADs or receptor regulated SMADs are a class of intracellular proteins involved in cell signaling. Type I and II receptors: serine/threonine receptors distinguished from one another by peptide mapping. APAF1 (apoptotic protease activating factor 1): a cytosolic protein involved in cell death or apoptosis. CASP9: Caspase-9 is an initiator caspase. The aspartic acid specific protease Caspase-9 has been linked to the mitochondrial death pathway. It is activated during programmed cell death.

The B cell lymphoma-2 (BCL2) proteins are a family of anti-apoptotic proteins (Lim *et al*., 2008). The pro-apoptotic proteins found in the cytosol act as sensors of cellular damage or stress and relocate to the surface of the mitochondria where the anti-apoptotic proteins are located. This interaction between pro- and anti-apoptotic proteins disrupts the normal function of the anti-apoptotic BCL2 proteins and can lead to the formation of pores in the mitochondria and the release of cytochrome C and other pro-apoptotic molecules from the intermembrane space (Harris & Thompson, 2000). This in turn leads to the formation of the apoptosome and the activation of the caspase cascade which leads to the death of the cell by apoptosis (Fig. 3B). miRNA encoded by the HSV-1 *LAT* gene confers resistance to apoptosis and its anti-apoptotic effect is due to the down regulation of the SMAD signaling pathway (Gupta *et al*., 2006). The latency-associated transcript (*LAT*) of herpes simplex virus-1 (HSV-1) is the only viral gene expressed during latent infection in neurons (Jones, 2003). *LAT* inhibits apoptosis and maintains latency by promoting the survival of infected neurons (Perng, *et al*., 2000). MiRNA to-LAT exerts its anti-apoptotic effect by downregulation of TGFB1 and SMAD3 expression (Abrahante *et al.*, 2003; Brennecke *et al,*. 2003; Chang *et al.,* 2004; Chen *et al.,* 2004; Cui *et al*., 2006), both of which are functionally linked in the TGFB pathway of apoptosis.

Contrastingly, miRNAs negatively regulate BCL2 at the posttranscriptional level causing induction of apoptosis. BCL2 repression by these miRNAs induces apoptosis in the leukemic cell line model chronic lymphocytic leukemia (CLL). CLL is the most common human leukemia and is characterized by predominant non-dividing malignant B cells over expressing the anti-apoptotic BCL 2 protein. miRNAs are of considerable therapeutic significance because they are natural antisense BCL2 interactors that could be used for therapy in tumors over expressing BCL2. The mechanism of BCL2 gene expression regulation explains the inverse correlation between the expression of BCL2 protein and miRNA in CLL cells (Cimmino *et al.*, 2005). In majority of CLL cells, frequent deletions and downregulation of the MIRN15 and MIRN16 genes at the chromosome locus 13q14 are observed (Calin *et al*., 2002). This finding suggests a role for MIRN15A and MIRN16A as repressors of BCL2 expression and possible inducers of apoptosis (Cimmino *et al*., 2005).

MicroRNA regulation has a major impact on the proper regulation of a cell, and thus of the organism. Knockdown studies with parts of the miRNA processing machinery indicate that an organism can not survive in its absence (Hutvagner *et al*., 2004). The impact of individual miRNAs on their target genes is not known much because of complication in target prediction. However, it is likely that miRNAs function similar to transcription factors. Their impact on target regulation can vary from minor to significant depending on a variety of factors.

6. Concluding Remarks

MicroRNAs are non-coding RNAs that have been found to play significant roles in a great variety of processes, including transcription and chromosome structure, RNA processing and modifications, mRNA stability and translation, and protein stability and transport (Ambros, 2001; Schwarz & Zamore, 2002; Storz, 2002). Investigation of miRNA expression in *Caenorhabditis elegans, Drosophila melanogaster, Arabidopsis thaliana* and humans has identified the biological functions of several miRNAs. In *Drosophila melanogaster, mir*-14 has a role in apoptosis and fat metabolism (Xu *et al*., 2003) and the *bantam* miRNA targets the gene hid involved in apoptosis and growth control (Brennecke *et al*., 2003). Increasing *mir*-14 levels resulted in a converse set of phenotypes – decreased triacylglycerol (TAG) and diacylglycerol (DAG) levels. The expression of *mir*-14 specifically in the *Drosophila* fat body (the equivalent of our adipose tissue) can drive total adult TAG levels down to roughly a fifth of their normal levels (Xu *et al*., 2004). Thus, there is a relationship between the effects of *mir*-14 on cell death and TAG and DAG levels.

MicroRNAs regulate apoptotis both as pro- and anti-apoptotic molecules. The latency-associated transcript (*LAT*) of herpes simplex virus-1 inhibits apoptosis and maintains latency by promoting the survival of infected neurons (Perng *et al*., 2000). miRNA to LAT exerts its anti-apoptotic effect by downregulation TGFB1 and SMAD3 expression, both of which are functionally linked in the TGFB pathway of apoptosis. Also miRNAs negatively regulate BCL2 at the post transcriptional level causing

induction of apoptosis. BCL2 repression by these miRNAs induces apoptosis in a leukemic cell line model (chronic lymphocytic leukemia, CLL) the most common human leukemia (Cimmino *et al.*, 2005).

Understanding the role of miRNA in regulating apoptosis could contribute to developing specific therapeutics for specific diseases. Future research should be able to answer and comprehend the standard questions like where, when and how are miRNAs expressed? What genes do miRNAs control? And what cellular processes are regulated by miRNAs? If miRNAs are important in everyday working of cells as the research suggests, then exploiting them could play a role in every aspect of cellular defects, from screening and diagnosis, to prediction of treatment outcomes and for treatment itself. Also if miRNAs only regulate the translation of, but not the stability of target mRNAs, this may partially explain the reason behind non-correlation of gene expression profiles based on mRNA analysis with protein expression data (Kern *et al*., 2003).

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