

MICRORNAS AS REGULATORS OF APOPTOSIS MECHANISMS IN CANCER

VALENTINA PILECZKI^{1,2}, ROXANA COJOCNEANU-PETRIC^{1,3},
MAHAFARIN MARALANI⁴, IOANA BERINDAN NEAGOE¹,
ROBERT SANDULESCU²

¹The Research Center for Functional Genomics, Biomedicine and Translational Medicine, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

²Department of Analytical Chemistry, Faculty of Pharmacy, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

³Faculty of Biology, Babes-Bolyai University, Cluj-Napoca, Romania

⁴Molecular Medicine Department, Dokuz Eylul University, Izmir, Turkey

Abstract

MicroRNAs or miRNAs are small non-coding RNAs that regulate gene expression. Their discovery has brought new knowledge in biological processes of cancer. Involvement of miRNAs in cancer development includes several major pathways from cell transformation to tumor cell development, metastasis and resistance to treatment. The first part of this review discusses miRNAs function in the intrinsic and extrinsic pathways of apoptosis. Due to the fact that many miRNAs that regulate apoptosis have been shown to play a major role in tumor cell resistance to treatment, in the second part of the review we aim at discussing miRNAs potential in becoming curative molecules.

Keywords: microRNAs, cancer, apoptosis, cancer therapy

Introduction

Cancer in the modern era is becoming a burden on society, both in economically developed and undeveloped countries. Its incidence is continuously increasing because of the growth and aging of the population, along with the persistence of risk factors such as smoking, overweight, physical inactivity and others [1]. GLOBOCAN (Global Burden of Cancer Study) evaluations estimated approximately 14.1 million new cancer cases and 8.2 million deaths worldwide in 2012 [1]. Cancer is an illness characterized by the deregulation of multiple processes that lead to cell malignization, proliferation, and evasion of apoptosis [2].

Apoptosis is a naturally acquired process that normally plays an important role in the development and

life of multicellular organisms through the removal of damaged, aged, or autoimmune cells [3]. As a type I form of programmed cell death, it requires fine regulation, therefore the mechanisms of apoptosis are complex and involve many signaling pathways. Along these pathways, crucial changes may occur at any point, leading to tumorigenesis, metastasis and resistance to anticancer drugs [4,5]. These changes are determined by regulatory factors that switch on or off the genes controlling cell cycle and proliferation [6].

It is well known that most of the mammalian genomes are transcribed. Studies conducted in the last decade demonstrated that a large amount of DNA (deoxyribonucleic acid) is transcribed but not translated [7-9]. In these regard, human transcriptome broadly encodes for 10,000–32,000 long non-coding RNAs (lncRNAs), about 11,000 pseudogenes, around 9,000 small RNAs, and only around 21,000 protein-coding mRNAs (messenger RNAs) [10], thus indicating that most of the DNA transcripts

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Address for correspondence: valentinapilecki@gmail.com

do not encode for proteins [11-14]. miRNA are a class of small non-coding RNAs of 18-24 nucleotides in length that regulate gene expression at the post-translational level. In the last years, miRNAs have been a hot topic in cancer research. Their function and deregulation in cancer cell apoptosis evasion is not yet fully understood. The study of miRNA regulation in tumor cell apoptosis evasion might provide new information regarding the mechanisms of cancer development and tumor cell resistance to therapy. This review will discuss miRNA involvement in regulating apoptosis pathways focusing on their tumor suppressor and oncogenic function in cellular networks. And finally we will talk about the potentially therapeutic functions of miRNAs in cancer treatment.

Apoptosis pathways

Apoptosis is triggered through two basic apoptotic signaling pathways, the intrinsic and extrinsic pathways, or the mitochondria apoptotic cascade and, respectively, the death receptor mediated pathway [15]. Both pathways eventually emerge and lead to the activation of a family of cysteine proteases called caspases, or the initiators and effectors of apoptosis that lead to specific morphological features, including chromatin condensation, DNA fragmentation, membrane blebbing, that finally cause complete cellular destruction [16]. The intrinsic apoptosis pathway is initiated by intracellular signals that affect the electron transport system of the mitochondria membrane, leading to the release of cytochrome c. Cytochrome c then recruits Apaf 1 (apoptotic peptidase activating factor 1) and pro-caspase 9 forming a complex known as the apoptosome. A series of Bcl-2 (B-cell CLL/lymphoma 2) family members like Bax (Bcl-2-associated X protein), Bak (Bcl-2-antagonist/killer), Bcl-X_L (BCL2-like 1 isoform 1), Bid (BH3 interacting domain death agonist), Mcl-1 (myeloid cell leukemia 1) and Bim (aliases BCL2L11 - BCL2-like 11 (apoptosis facilitator)) are important factors in apoptosis induction, and are involved in mitochondria membrane permeabilization and release of cytochrome c [17]. The extrinsic apoptosis pathway is initiated by the binding of death ligands to their corresponding cell surface specialized membrane receptors. These ligands are the Fas ligand (FasL), TNF- α (tumor necrosis factor), TNF related-inducing ligand (TRAIL), TNF-like weak inducer of apoptosis (TWEAK). Each of them is specifically recognized by a certain member of the TNF receptor family (e.g. Fas, TNFR, CD95, death receptor 4/5 and death receptor 3). Ligand - death receptor binding triggers the formation of the death-inducing signaling complex or DISC, which is formed by the association of Fas-associated death domain - containing protein (FADD) and the pro-caspases 8 and 10. As a result, this complex triggers the activation of effector caspase 3, 6 and 7 that execute the apoptosis process [15,18].

Also, numerous other factors that are not part of the apoptosis pathways play an important role often in multiple steps either in the intrinsic or extrinsic pathway. These factors are Smads, p53 (tumor protein p53), NF- κ B (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1), cellular inhibitor of apoptosis proteins (cIAPs) and death associated protein kinase (DAPK) [19].

MiRNAs involved in the regulation of the intrinsic pathway

In DLD1 colon cancer cell line, miR-491 is a direct target of BCL-X_L decreasing cell viability by inducing apoptosis, and determines tumor suppression in DLD1 derived tumors in nude mice [20]. MiR-133a up-regulation was observed to reduce cell proliferation, induce apoptosis and suppress tumorigenesis in osteosarcoma cell lines by targeting BCL-X_L and Mcl-1. miR-133a decrease in primary human osteosarcoma tissues was associated with tumor progression and patient prognosis [21]. BCL-X_L and EGFR (epidermal growth factor receptor) were observed to be regulated by miR-608, a tumor suppressor in human chordoma malignancy [22]. In pancreatic cancer, miR-365 was observed to be upregulated and promote tumor cell resistance to gemcitabine by directly targeting adaptor protein Src Homology 2 Domain Containing 1 (SHC1) and BAX [23]. miR-125b up-regulated expression in breast cancer determines the inhibition of Taxol-induced cytotoxicity and apoptosis. It was also observed to target the pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) [24]. Bcl-2 is negatively regulated by two miRNAs, miR-15 and miR-16, which are frequently deleted or down-regulated in the majority of chronic lymphocytic leukemias (CLL). Transfection of miR-15 and miR-16 as a cluster in MEG-01 cells that express high levels of Bcl-2 and do not express miR-15 and miR-16 reduced Bcl-2 expression and resulted in the activation of the intrinsic apoptosis pathway [25]. Ectopic expression of miR-204 and down-regulation of Bcl-2 determined colony formation and migration in gastric cancer cell lines, where down-regulation of the miR-204 and ectopic expression of Bcl-2 counteracted the miRNAs pro-apoptotic activity in response to 5-fluorouracil [26].

MiRNAs involved in the regulation of the extrinsic pathway

MiRNAs are regulating the extrinsic pathway as well. In tumor cells resistant to TRAIL induced cell death overexpression of miR-221 and miR-222 determined cell sensitivity to TRAIL and induction of apoptosis [27]. It was also observed that miR-221 and miR-222 modulate the expression of proto-oncogenes Kit (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) and p27^{kip1} that play an important role in cell cycle. Negative regulation of p27^{kip1} by miR-221 and miR-222 determined resistance

to tamoxifen in breast cancer cell lines [28]. In pancreatic cancer it was observed that FasL is a direct target of miR-21, that down-regulates the expression of the death receptor ligand determining resistance to gemcitabine-induced apoptosis [29]. MiR-590 is an oncomiR that regulates the expression of FasL in acute myeloid leukemia (AML) and promotes cell survival [30]. In osteosarcoma cells Fas expressions is inhibited by miR-20a, promoting tumor cell survival and enhance the metastatic capacity of the tumor cells [31]. In cholangiocarcinoma, where miR-25 expression is up-regulated it was observed that it protects tumor cells against TRAIL induced apoptosis [32]. Ovcharenko and colleagues indicated that several miRNAs target several proteins in the TRAIL pathway. They predicted that miR-182 and its homologue miR96 target FADD and caspase-3, while miR-145 and miR-216 target the receptors of TRAIL, DR4 and DR5 [32]. miR-K10a is a oncomiR that promotes tumor cell survival and down-regulates the expression of TWEAK receptor [33]. In myeloid leukemia was observed that FADD is targeted by miR-128a. Ectopic transfection of miR-128a determined Fas resistance in Jurkat cells by down-regulating FADD and antagonizing miR-128a induced Fas-mediated apoptosis by blocking FADD expression [34]. A scheme of both major pathways of apoptosis and there regulatory miRNAs is presented in Figure 1.

MicroRNAs in cancer therapy

Due to their role in cancer biology and main function in gene regulation, miRNAs have been in the last years major sources of interest for developing new treatment strategies in different pathologies. The aberrant expression of miRNAs has been linked to various human disease, including cancer. Furthermore, these small molecules' deregulation in malignant pathologies is connected to apoptosis evasion that leads to tumorigenesis and drug resistance [35]. In tumor initiation and progression miRNAs can act as either oncogenes or tumor suppressors which, as described above, are strongly related to the apoptosis phenomenon. Therefore, restoring their function can reduce or even abrogate diseases including tumors in animal models [36]. miRNA anti-tumor based therapies can be used alone or synergistically with the generally approved cancer therapies [37,38]. Targeting specific miRNAs should help minimize off-target toxicity [39] and may also have a potential use in reducing tumor cell drug resistance [40]. In small cell lung cancer, miR-100 presents chemo-resistance properties [41] and also, epigenetic inhibition of miR-199b-5p reduced drug resistance in chemoresistant ovarian cancer [42]. For this, investigators are aiming to design new molecular therapy that target and block oncogenic miRNAs expression and others that stimulate over-expression of tumor suppressor miRNAs.

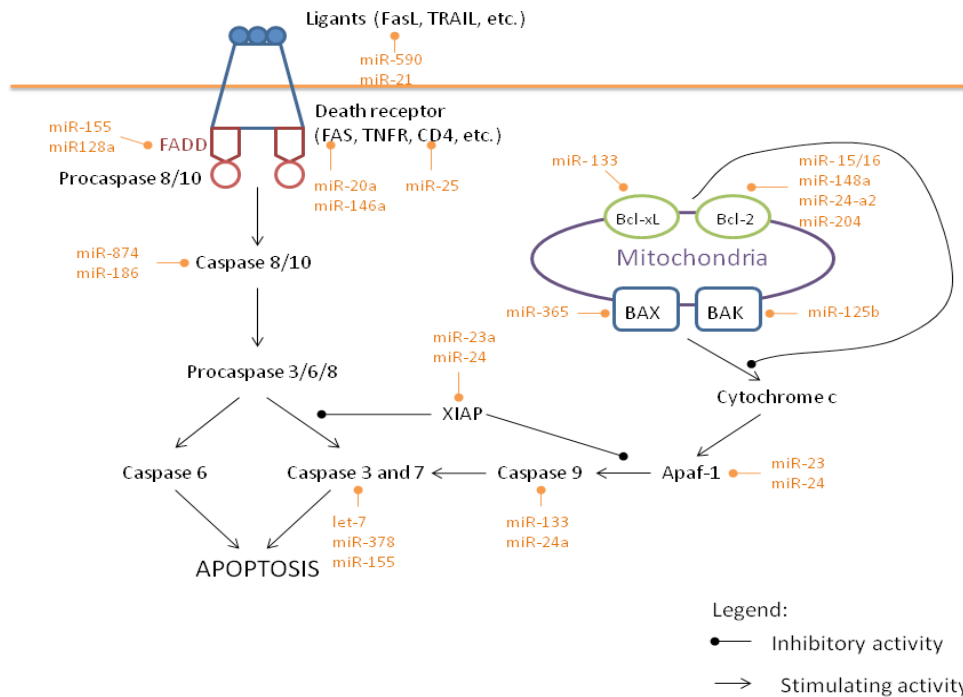


Figure 1. Genes and miRNAs involved in the regulation of the intrinsic and extrinsic apoptosis pathway. MiRNAs that regulate apoptosis are shown in the diagram in light orange.

In order to achieve inhibition of the oncogenic miRNAs, several blocking methods have been designed, such as: antisense oligonucleotides, antagomirs, locked nucleic acid (LNA) constructs and sponges, all known as antimiRs [43]. Silencing of dysregulated miRNAs requires that the antimiRs oligonucleotides are chemically modified in order to ameliorate their biostability and binding affinity [44]. antimiRs work by inhibiting miRNA expression in cancer, which will further lead to the up-regulation of tumor suppressor proteins and inducing apoptosis and thus blocking tumor formation *in vitro* and *in vivo*. Antisense oligonucleotides are usually modified in order to increase nuclear resistance by adding 2'-O-methyl and 2'-O-methoxyethyl groups at the 5' end of the strand [45]. By using these types of molecules, Cimmino and collaborators demonstrated that miR-15 and miR-16 expression is inversely correlated to Bcl2 in MEG-01 human megakaryocytic cell line [46]. *In vivo* experiments were also conducted by using an O-methyl modified cholesterol-conjugated antagomirs that target the liver specific miR-122. Silencing effects were observed up to 23 days [47]. A new and more effective way to silence miRNAs families in the last years has emerged, the miRNA sponges. They are ectopically expressed RNA fragments that present multiple miRNA target sites and fulfill their function by sequestering miRNAs and stopping them from achieving their natural goal [48]. Up to date, most of the oncomiRs seem to play a role in apoptosis, therefore serving as potential target in cancer therapy.

In other situations, the restoration of a certain tumor suppressor miRNA into diseased tissue is needed in an attempt to reestablish various cellular functions to normal. This is a technique that involves delivering synthetic miRNAs or miRNA mimics into target cells, elevating their expression levels, and leading to the suppression of a harmful gene. MiRNA mimics are small, chemically modified double-stranded RNA molecules that mimic the endogenous mature miRNA [49]. An effective method to deliver these therapeutic molecules is either the use of engineered viruses, or the use of nanoparticles [50, 51]. Viral vectors have had significant success in laboratory studies and limited efficiency in animal models due to immunogenicity, chromosomal incorporations and unspecific target of the tumors [52]. With all of these, a successful study was published by Kota and colleagues were an intravenous injection of adeno-associated virus 8 (AAV8)-expressing miR-26 induced tumor apoptosis in Myc-induced liver tumors [53]. With the discovery of the nanoparticles, most of the viral delivery issues were resolved. In a study conducted by Huang et al a new anionic lipopolyplex nanoparticle that could carry miR-29b was designed and showed great results *in vitro* and *in vivo* by specifically targeting and binding to the AML cells [54].

In the development of cancer a series of miRNAs were observed to be misregulated and thus influence tumor

cell survival and sustain apoptosis inhibition. As previously described down-regulated miRNAs like miR-15, miR-16, and let-7 are correlated with the regulation of anti-apoptotic genes thus leading to the activation of apoptotic signaling pathways in cancer cells [26]. In a study on breast cancer done by Singh and Saini, it was demonstrated that Bcl is a direct target of three miRNAs (miR-195, miR-24-2 and miR-365). They individually led to a significant inhibition of the Bcl protein and induced apoptosis in a hormone dependent breast cancer cell line [55]. All of these put together, the new discoveries regarding miRNA regulatory activity and importance in different pathways, challenges presented by targeted delivery of these molecules led to their clinical implementation. Nowadays most clinical trials use miRNAs as biomarkers in patient stratification, prognosis and drug efficacy. And only one uses these molecules as therapeutical agents in cancer [56]. MRX34 is a chemically designed mimic of miR-34a delivered with the help of liposomal nanoparticles. miR-34a is a tumor suppressor that targets p53 gene and it was observed to present the capacity of blocking tumor cell migration and resistance to chemotherapy in liver cancer [57].

Conclusion

Since their discovery, our understanding of microRNAs biology, mechanisms and functions have grown exponentially. Their dysregulation have been linked to many diseases, especially cancer. Gene expression profiling of miRNAs has led to the understanding of their role in cancer biology and tumor related pathways. An important pathway in all stages of tumor development and metastasis is the apoptotic pathway. Dysregulation in apoptosis mechanisms lead to genesis and development of malignant tumor. Drug resistance has been also associated with apoptosis pathways inhibition. Studies in this field have highlighted miRNAs as key regulators of cell death. In an attempt to re-establish their function, various molecules have been designed to either block the expression of oncomiRs or to boost the expression of tumor suppressors. Therefore future research will identify and help integrate new relevant clinico-pathological data regarding miRNAs utility in cancer treatment and help establish miRNA-based therapeutics.

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