Regulatory Functions of MicroRNAs in Cancer Pathogenesis

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Abstract- MicroRNAs (miRNAs) are a large family of evolutionary conserved small non-coding RNA molecules that firstly discovered in 1993. They regulate gene expression of about 50% of protein-coding genes at the post-transcriptional level. MiRNAs can target numerous messenger RNAs and subsequent misexpression of them can affect many different signaling pathways. They are playing a pivotal role in cancer development by regulation of the genes expression which involved in the proliferation, survival, differentiation, apoptosis or metastasis of the cancer cells. Several treatment approaches such as inhibition of oncomiRs and restoration of tumor suppressor miRNAs have been established in certain types of cancers and some other miRNA-based strategies are in development for cancer prevention and treatment. Nowadays, cancer is the most important target of miRNA therapeutics and the specific mechanisms by which miRNA mediates cancer pathways needs more research and study.

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Introduction

MicroRNAs (miRNAs) are a large family of evolutionary conserved single-stranded small RNA molecules which occur as non-coding RNAs of 19-24 nucleotides in length (1,2). MiRNAs regulate gene expression of about 50% of any protein-coding gene at the posttranscriptional level (3). In the posttranscriptional level, miRNAs act through the degradation of their target mRNA or translational inhibition of the entitled mRNA (4). MiRNA coding genes are located either in intergenic regions or in the introns and are transcribed by RNA polymerase II into long primary transcripts called primary miRNAs (pri-miRNAs) (5). In the nucleus, these are processed by RNAase III endonuclease Drosha and double-stranded RNA-binding protein Pasha, into a structure called precursor-miRNA (pre-miRNA) (6). Pre-miRNAs are transported to the cytoplasm. There, they are cleaved, and a short RNA duplex molecule is generated (7). Later a helicase forms the mature miRNA. Mature miRNA is then assembled into the RNA induced silencing complex (RISC) (8,9). RISC regulates target mRNA's function by binding to it and silencing its expression (10-12). In addition, by acting on regulatory sequences of their target gene, miRNAs can promote the expression (13). MiRNAs mostly can target numerous mRNAs, thus in case of misexpression in a single miRNA, expression of several hundreds of proteins can be disrupted, and many different signaling pathways may be affected. This also can cause cancerous transformation (14). The processing of miRNAs is demonstrated in Figure 1.

MicroRNA discovery

MicroRNA was first discovered by Victor Ambros' laboratory in 1993 during research on Caenorhabditis elegans. Simultaneously, Gary Ravkun reported the first miRNA target gene, which resulted in the identification of a novel mechanism of posttranscriptional gene regulation (15). Later, Ravukon and Horvitz found let-7 in the same model nematode species. Also, a class of short (small) interfering RNA (siRNA) involved in the process of RNA interference was discovered. Following these findings, the various number of miRNAs have been discovered and reported in mammals, and more than 700 miRNAs, which were identified in humans, have been fully sequenced (16).

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MicroRNA and cancer

Proliferation, Differentiation, development, and metabolism are examples of multiple biological functions of this class RNAs. In addition, miRNAs are playing an important role in cancer, diabetes, autism, fragile X syndrome, Alzheimer's, and heart diseases (17-20). Cancer has been known as a common complex disease worldwide. Series of genetic and epigenetic factors alter certain balances, which cause uncontrolled cellular proliferation. Due to the complexity of cancer, a single therapeutic strategy will not be able to produce a lasting cure. MiRNAs, however, got the capacity to target several protein-coding genes at the same time. As a result, a small change in miRNA expression can lead to meaningful alterations in the expression profile of several protein-coding genes and, therefore, cause changes in cellular phenotype (21-23). MiRNAs can be classified as oncomiR and tumor suppressor miRNAs from which a large number of can be used as diagnostic and prognostic biomarkers of the cancers (24,25).

Tumor formation is the result of alterations in miRNA expression by decreasing the expression of necessary genes that are needed in the proliferation or survival of the cells. However, in another study, it has been indicated that cancer progression or tumorigenesis does not contribute directly to miRNA (26). It is not completely found out that the changes in miRNA expression are either because of the pathological state of cancer or the cancer is the direct reason for it. Nevertheless, miRNA expression is affected directly or indirectly by several alterations that happen in cancer cells. Some changes such as gene mutations, changes in epigenetic regulation of miRNA, abnormalities in miRNA genes, or proteins that are involved in their construction and genomic rearrangements are some of the examples of alterations that might affect miRNA expression.

One of the main factors of changing miRNA expression in tumor cells in the presence of miRNAs in tumor-related genomic regions or fragile genomic areas. This specifically causes influences miRNA and mRNA connectivity features, which can be named the direct effect of the mutations. Incomplete translational processes are the result of altered miRNA interactions (27). MiRNAs are capable of regulating a broad set of genes efficiently and silence target genes simultaneously. Since cancer is a heterogenic disease, miRNA's characteristic features are beneficial for treatment. MiRNAs target cancer cells in spite of targeting endothelial cells and fibroblasts. This helps the inhibiting of angiogenesis and tumor fibrosis. Therefore, the required process during metastasis and tumor formation is blocked (28). Moreover, dysregulated miRNAs are implicated in the pathogenesis of cancer due to having an effect on oncogenes and/or tumor suppressor genes (29,30).

MicroRNAs function

The role of miRNA in cancer has already been intensively evaluated, and either clinical studies or in vitro and in vivo experiments demonstrated their importance on this occasion (23). MiRNA's role was first studied in association with chronic lymphocytic leukemia (CLL) (31). Later multiple miRNAs were reported in accordance with plenty of other cancers (32-40). The dysregulation of miRNAs is also linked to cancer in various studies (41-44). It has been proved that they play important roles in metastasis, initiation, and progression, as well as therapeutic resistance (44). There also exists researches describing miRNAs playing two separate acts in carcinogenesis (to be both as "oncomirs" and as "tumor suppressors") (45-48). In support of this thought, scientists demonstrated the fact that miRNA expression can be up- or down-regulated in cancer cells in comparison with normal cells. They also seem to be deregulated in hematological malignancies, as well as many solid tumors (42,49,50). When it comes to location, however, about 50% of miRNA genes are embedded in genomic instability regions (51). This strengthens the evidence of cancer being related to miRNAs. Besides, miRNAs regulate 20-30% of all protein-coding genes (52,53), which supports the probability of miRNA's signature, providing efficient information about tumors (49).

Notably, expression patterns of miRNAs are tumor- and tissue-specific (32,54,55). For example, miR-155 is a multifunctional miRNA and is involved in inflammation, immune response, and cancer development (56), over-expressed in leukemia and lymphoma (57-60), and down-regulated in melanoma, gastric cancer, ovarian cancer, and endocrine tumors (61-65). These capabilities make miRNAs valuable agents for diagnosis and therapy of certain kinds of cancer. Another example of cancer-specific miRNAs is miR-21, which has been investigated by several groups. Subsequently, scientists found out that miR-21 is over-expressed in malignancies like a breast (50), colon (66), lung (32), liver (67), thyroid (68), and leukemia (32). These findings suggested that this oncomir is a good example of a cancer-specific miRNA (69).

Dysfunction or misexpression of miRNA can affect a broad range of processes involved in tumor progressions

such as metastasis, apoptosis, angiogenesis, and cell cycle regulation (70-75). There are reports of five families of miRNA who target cell cycle regulators. These miRNAs are the let-7, the miR-15a/16 cluster, miR-34 families, the miR-17/20 cluster, and the miR-221/222 cluster. The entitled miRNAs are capable of controlling cell cycle checkpoints. Malfunction of these miRNAs may cause a rise in cell proliferation, which is necessary for tumor growth (76). However, some studies demonstrated miRNAs as anti-apoptotic regulators of key pathways in cancer. These miRNAs are highlighted to maintain cancer cell survival and drug resistance contribution (77). Besides, pro-apoptotic miRNAs serving as anti-cancer agents (78). The miRNAs also play a key role in the metabolism of cancer cells (79). They regulate nutrient uptake, targeting transporters, and metabolic enzymes and modulating cancer cell metabolism. They increase the accumulation of materials to control metabolic flux and support proliferation (80,81). We summarized some important onco-miRNAs and their functions in Table 1.

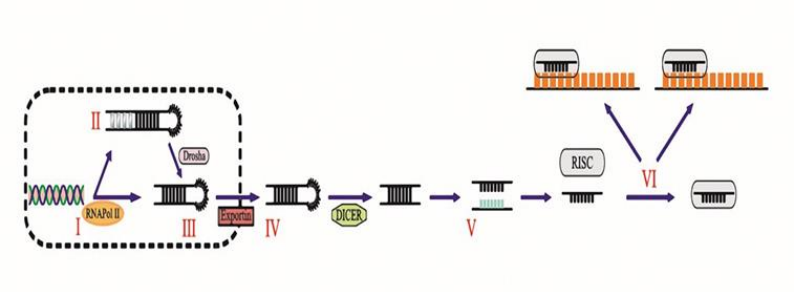

Figure 1. MicroRNA processing. RNA polymerase II and proper transcription factors excite the transcription of the microRNA gene (I) in the production of a pri-miRNA. The primary transcript (II) is then processed by an RNAase III enzyme called Drosha to produce a ~ 65 nucleotide (nt) pre-miRNA. The pre-miRNA, which has a short stem of 2–3 nt 3′ overhangs (III), is then exported by exportin 5 (EXP5) to the cytoplasm for additional processing. In the cytoplasm, the precursor microRNA subsequently processed into a mature 19–24 nucleotide duplex (IV) by an enzyme called Dicer. Afterward, the duplex is separated into a primary and secondary strand (V); then, the primary strand is embedded into the RISC (RNA-induced silencing complex). In the next step, the microRNA with RISC targets complementary mRNA transcripts (VI) at the seed region to induce either block translation (right) or mRNA degradation (left)

Table 1. Some important onco-miRNA and their function

MicroRNAS	Target gene(S) or Protein	Function
miR 15 and miR 16	NA	B-cell lymphocytic, chronic Leukemia
miR-17 ~ 92 cluster	NA	Lung and other malignancies
	Myc	Tumorigenesis and angiogenesis
miR-21	Pdcd4, BMPRII & LRRFIP1	Promote apoptosis through activation of caspases
miR-155	TP53INP1	Overexpression in pancreatic cancer and breast cancer progression
miR-371 ~ 3	LATS2	Cell proliferation and tumor development

NA: Not applicable

Metastasis-mediating miRNAs have also been discovered. They regulate distinct steps of the metastasis, affecting both signaling pathways in the cancer cell and interactions of cells with one another and with tumor stroma. According to studies, they can activate or suppress metastasis (82,83). Some miRNAs are implicated in suppressing apoptosis and stimulating tumorigenesis (84). Other miRNA families contribute to both tumor growth and metastasis (38). They are able to silence multiple oncogenes and are down-regulated in several tumors (85,86). Pro-metastatic miRNAs, however, are another example of metastasis-promoting miRNAs (87,88). Subsequently, due to the important role of miRNAs in cancer, there exists a wide range of strategies based on miRNA in oncology. They can be used for cancer classification (49,89) or tissue origin identification of cancers with the unknown primary origin (90,91). Their expression can serve as a useful prognostic or diagnostic marker (92-95). Interestingly, miRNA signatures have been established as predictive factors of response to therapy (96-100) and drugs (101,102).

MicroRNA in cancer therapy

Since miRNAs discovery, a debate has risen that miRNAs could be regarded as a promising biomarker to

improve response to cancer treatment (26). The advent of miRNA-based therapy, however, has opened new avenues to use targeted therapy for clinical applications, since there are some limitations for current cancer therapies (103). All of the applications in the previous section are possible when dealing with primary tumors. The fact that miRNAs are more stable than mRNAs is the key point since this stability enables them to be detected in the circulation and serve as biomarkers. Circulating miRNAs can be measured with regard to a wide variety of cancers (104). Therefore, studies are currently highlighting their employment in cancer therapeutics (105). Based on several studies in recent years, miRNAs can be used as highly potential molecules in CRC therapy (30). Several therapeutic strategies associated with miRNAs can prevent cancer progression (Figure 2).

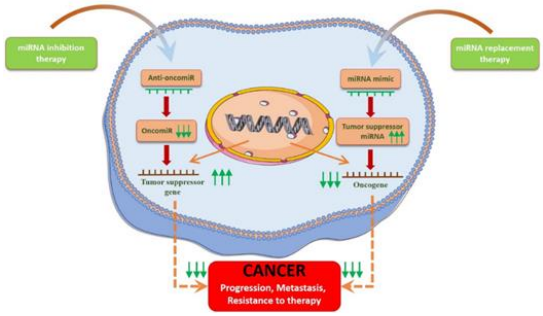

Figure 2. Different miRNA-based therapeutic strategies against cancer progression, metastasis, and resistance to therapy

Some examples include cutting oncogenic miRNAs by artificial miRNAs, which are capable of pairing with mRNAs, inhibition of the entitled oncogenic miRNAs, inducing the tumor suppressor miRNAs, or decreasing miRNA expression using various epigenetic factors like promoter methylation. To reduce the miRNA expression, antisense oligonucleotides can also be used. They are particularly paired with miRNA (26). One type that is artificially made is Antagomir (106). In comparison with other cancer treatment methods, these molecules are less toxic and create stable inhibition (27).

MiRNAs can also be agents or targets of cancer therapy according to their function, stage of cancer, and type of cancer (107). In order to use combined therapies targeting multiple miRNAs, tumor-secreted miRNA, who are messengers and/or effectors, must be characterized as the first step. Due to the correlation between their levels and metastasis, circulating or exosomal miRNAs can be quantified to select patients with high risks of metastasis in a certain type of cancer. As a result, these patients will benefit from a preventive strategy that targets the miRNA effectors (108). There also have been studies indicating that treatment interventions such as inhibition of oncomiRs and restoration of tumor suppressor miRNAs might be beneficial for certain types of cancers (25).

In addition to the fact that cell-free miRNAs are functionally effective in metastatic progression, they are also nominated for potential novel therapeutic targets (108,109). An interesting advantage of cell-free miRNAs is that their expression levels can be monitored when treatment is started (110). In this regard circulating miRNAs are discovered to be potential diagnostic and therapeutic agents in association with cancers (111). Another aspect of targeting miRNAs is that they are observed to be beneficial for improved response to drugs. Hence, circulating miRNA's expression level in blood is useful for prognosis determination (102,112). Moreover, compared to other gene-therapy methods and drug molecules, miRNA showed low toxicity (101). Accordingly, in case of safe delivery to cancer cells, miRNA-based therapeutics seems to be promising anti-cancer guardians.

Once miRNAs discovered, significant progress in the identification of these novel family has confirmed that these small and non-coding RNAs are a numerous class of regulatory RNAs. Also, the skeleton of a biochemical mechanism for their functions in gene regulation has specified. The most attractive part of miRNA therapeutics is their capability to target any genes, which is not possible or difficult by protein-based drugs or small molecules. Nowadays, cancer is the most important target of miRNA therapeutics among the numerous diseases being studied. We briefly clarified the particular roles and the importance of miRNAs in the regulation of gene expression. Additionally, the specific mechanisms by which miRNA mediated repression needs more research and study.

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