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MicroRNAs IN NORMAL AND CANCER CELLS: A NEW CLASS OF GENE EXPRESSION REGULATORS

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MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at posttranscriptional level. They are involved in cellular development, differentiation, proliferation and apoptosis and play a significant role in cancer. This review describes miRNA biogenesis, their functions in normal cells, and alterations of miRNA sets in cancer and roles of antitumorigenic and oncogenic miRNAs in cancer development.

Key Words: **microRNA, cancer, oncogene, tumor suppressor.**

MicroRNAs are a novel class of small, ~ 18–25 nucleotides long, non-coding RNAs that post-transcriptionally negatively regulate gene expression. The first miRNA, *lin-4*, was discovered in 1993 in *Caenorhabditis elegans* [62, 95]. The *lin-4* miRNA gene encodes a 22-nucleotide non-coding RNA that negatively regulates the translation of another gene, *lin-14*, by base-pairing to complementary sites within its 3′-untranslated region (3′-UTR) affecting the development timing. This type of regulation is an exception from the accepted concept of gene expression regulation. However, a significant number of recent studies have demonstrated that miRNA-mediated regulation of gene expression is a wide-spread phenomena in eukaryotic organisms that control the fundamental cellular processes such as development, proliferation, and apoptosis [6]. Moreover, altered miRNA profiles have been found in a variety of cancers indicating their significant role in cancer development [28, 35]. Hundreds of miRNAs have been identified in animals, plants and viruses, among them > 300 miRNA genes in the human genome [7]. Many miRNAs are highly conserved between variety of evolutionary distinguished species [6] supporting the hypothesis about important functions of these small molecules in organisms. In this review, we describe miRNA biogenesis, their functions in the cell, paying special attention to tumor cells.

miRNA BIOGENESIS AND MODES OF ACTION

miRNA genes are located mainly within introns of protein-coding and non-coding sequences, as well as in intergenic regions [81, 100]. In the first case, expression of corresponding miRNAs may be linked with transcriptional regulation of their host genes and, hence, reveals tissue specificity due to expression of different sets of genes [6, 66, 67]. In the second case, expression of miRNAs is regulated independently via their own regulatory elements [100]. In addition, a recent study has shown that a number of mammalian miRNAs are derived from DNA repetitive sequences, including LINE-2 transposable elements [86]. miRNAs are transcribed by RNA polymerase II producing long primary-miRNAs (pri-miRNAs) [53]. Within a pri-miRNA, the miRNA itself forms a stem-loop hairpin structure (Figure), which is excised in the nucleus by the RNase III endonuclease Drosha associated with double-stranded RNA-binding domain-containing protein DGCR8 (in mammals) or Pasha (in *Drosophila* and *C. elegans*) [26, 32]. Drosha asymmetrically cleaves both strands of the hairpin stem-loop at sites near the base of the primary stem-loop resulting in release 60–70-nucleotide pre-miRNA [31]. Pre-miRNA is exported to the cytoplasm by Ran-GTP-dependent Exportin-5 complex [98]. The cytoplasmic RNase III endonuclease Dicer1 with associated proteins TRBP and PACT in mammals excises a RNA-hairpin duplex from pre-miRNA. The fully mature miRNA incorporates in a single-stranded form into ribonucleoprotein complex termed as the RNA-induced silencing complex (RISC). In mammals, miRNAs negatively regulate their targets by either binding to imperfect complementary sites within the 3′-untranslated regions of their mRNA-targets [17], or by targeting specific cleavage of homologous mRNAs [33]. In the first case, miRNAs reduce protein levels of target genes by post-transcriptionally repressing target-gene expression without affecting mRNA levels of these genes, whereas in the second case, miRNAs induce the degradation of target mRNAs by the RISC. Interestingly, that miR-122 positively affects the replication of hepatitis C virus by binding to its 5′-noncoding region [49]. It is unclear whether this effect is unique or represents an unknown yet mechanism of miRNA action.

To date, many details of miRNA-mediated gene expression regulation have been clarified. In contrast, regulation of miRNA expression is not fully understood. Epigenetic alterations play an important role in general regulation of gene expression [48], but little attention has been paid to miRNA genes. A recent study con-

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Abbreviation used: miRNAs – microRNAs.

ducted by Saito *et al*. [82] showed clearly the role of epigenetic mechanisms in expression of *mir-127* gene, which is located within a CpG island on chromosome 14q32.31. Additionally, the inhibition of histone deacetylase and the resulting rapid alterations in miRNA levels in breast cancer cells [83] further indicate the importance of epigenetic mechanisms in regulation of miRNA genes expression. On the other hand, miRNAs may be involved in regulation of chromatin structure (Figure). In support of this hypothesis, a recent prediction of miRNA target genes in humans contained various histone-modifying proteins, including histone methyltransferases, methyl CpG-binding proteins, and histone deacetylases [64]. Recent finding by Grosshans *et al*. [34] that chromatin-remodeling factor is one of let-7 predicted target genes in *C. elegans* provided extra evidence to this hypothesis. In addition, miRNAs can affect chromatin structure indirectly by regulating proteins that involved in the maintenance of chromatin organization. For instance, miR-106a involved in the histone H3 lysine 9 methylation and preservation of heterochromatin via regulation of retinoblastoma-1 protein [30]. In any case, the area of miRNAs/epigenetic changes relationships remains unexplored.

miRNAs AS REGULATORS OF DIFFERENT CELLULAR PROCESSES

miRNA genes represent only a small part (~0.5–3%) of the genome [7, 17], but they regulate approximately 20 to 30% of all human genes and there is an average 200 predicted targets per miRNA [54, 56, 63, 64]. Among these putative target-genes, there is a large group of genes involved in development, cell differentiation, apoptosis, transcriptional regulation, and other physiological processes [4–6, 16, 50, 51, 56, 70, 78, 80]. Possibly, not all predicted mRNAs are real targets of corresponding miRNAs. However, recent report about altered expression of hundreds of mRNAs in response to *in vivo* inhibition of miR-122 supports hypothesis of multiple targets for one miRNA [58].

To date, only a few miRNA targets have been identified and confirmed experimentally thus clarifying the mechanisms of their action. For example, the *let-7* family controls the timing of developmental processes in *C. elegans* [1, 17], and the involvement of miRNAs in developmental processes has also been shown in *Drosophila* [9]. Several studied miRNAs are involved in regulation of cell differentiation; thus, miR-31 in *Drosophila* [61] and miR-196a in mice [69] control axial patterning of the embryo. Brain-specific miR-124a and miR-9 affect neural differentiation in mouse embryonic stem cells [57]. A complex system of interacting miRNAs and transcription factors have been found to regulate cell fate determination in *C. elegans* [19, 20, 47, 99] and *Drosophila* [65]. In these models, the miRNAs and protein factors formed reciprocal negative feedback loops allowing the existence of only one of two stable states; the switch is gained by mutual re-

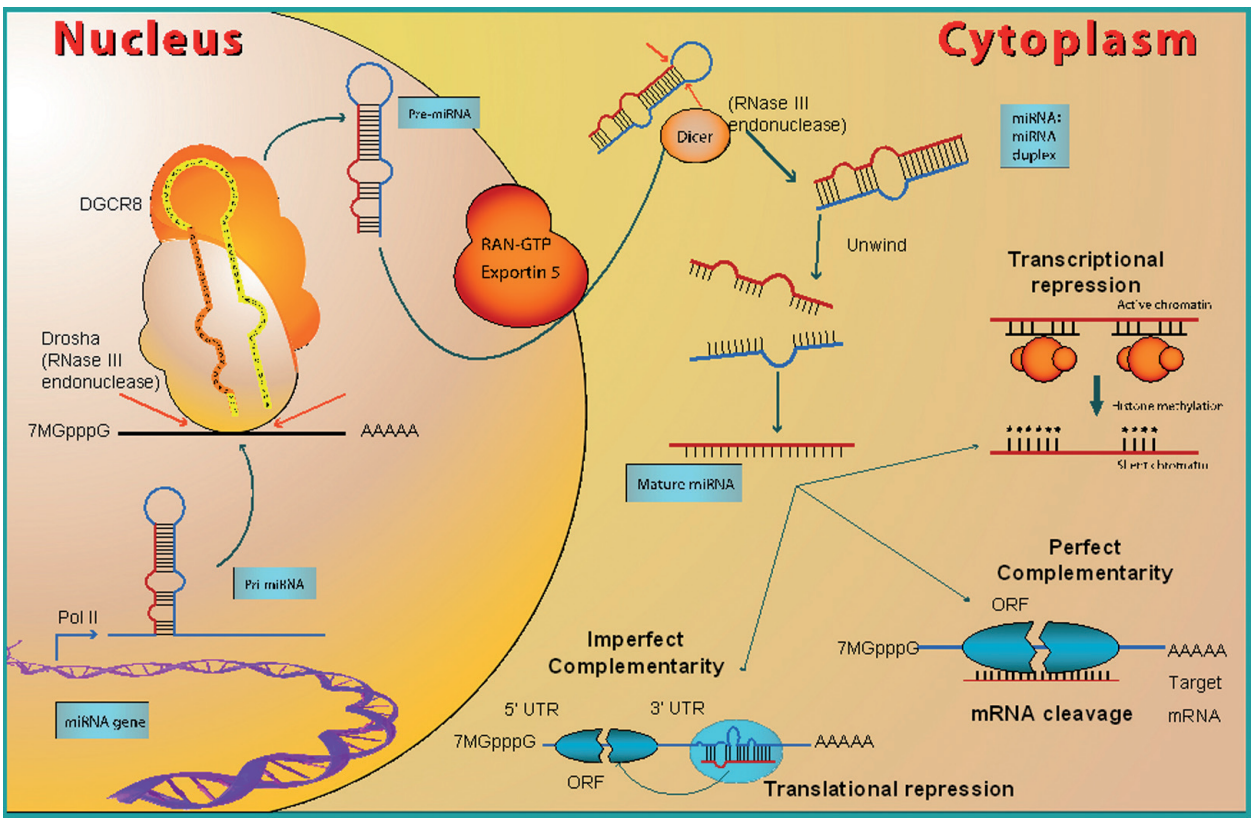

Figure. Biogenesis and cellular functions of miRNAs. Polymerase II transcribes miRNA gene forming long hairpin-bearing primary miRNA (pri-miRNA). The hairpin structure is excised by RNase III endonuclease Drosha, and resulting pre-miRNA is transported into cytoplasm by Exportin-5 in a RAN-GTP dependent way. The cytoplasmic RNase III endonuclease Dicer excises the top loop of the miRNA giving RNA-RNA duplex. After unwinding, one strand of the duplex is degraded, and another strand is mature miRNA. It can induce mRNA cleavage, if complementarity to 3′-untranslated regions of targets is perfect, translational repression, if complementarity is imperfect, and transcriptional repression due to interactions with chromatin

pression of miRNA expression through corresponding transcription factors.

A very intriguing problem is miRNA patterns in stem cells and their changes during differentiation. Generally, stem cells possess a specific set of miRNAs which is replaced during development [42, 88]. A key characteristic of stem cells is their capacity to divide for long periods. In this respect, stem cells are similar to cancer cells, which are also capable of escaping cell cycle arrest. Therefore, there is growing interest to elucidate the mechanisms responsible for indicated properties. Results of recent experiments suggest miRNA involvement in stem cell self-renewal [22, 84]. *Drosophila* with a null mutation of Dicer-1, which is required for miRNA processing, reduced by 80% germline stem cell division [37]. It is known that the transition from G1 to S phases of the cell cycle is negatively regulated by Dacapo (Dap), an inhibitor of cyclin-dependent kinase. In the mutant *Drosophila*, Dap was over-expressed, possibly due to absence of Dap-down-regulating miRNAs. Therefore, miRNAs are required for germline stem cells to transit the G1/S checkpoint by repressing the G1/S inhibitor Dap. These results allow speculating that miRNAs could have a similar role in cancer cells [37].

In addition to important roles of miRNAs in regulation of various cellular physiological pathways mentioned above, a recent observation of targeting repetitive sequences, such as *Alu* elements, by miRNAs indicates a crucial role of miRNAs in defense of the mammalian genome via silencing of foreign DNA sequences preventing and maintaining stability of the genome [87].

miRNAs AND CANCER

Taking into account an important role of miRNAs in regulation of the key processes of cell life and death, the involvement of microRNAome deregulation in disease development, including cancer, can be predicted. Indeed, recent studies showed a link between altered miRNA patterns and cancer [10,12, 25, 28, 31, 44]. Altered miRNA patterns in tumor *versus* non-tumor cells have been found in chronic lymphocytic leukemia [13], B-cell lymphomas [27, 39, 55,], Burkitt's lymphoma [73], breast cancer [45], lung cancer [46, 90, 97], colorectal cancer [74], glioblastoma [18], follicular thyroid carcinoma [94], cholangiocarcinoma [72], and hepatocellular carcinoma [75]. Additionally, detailed studies reveal that more than half of miRNA genes are located at sites in the human genome associated with amplification, deletion or translocation in cancer, suggesting direct relationship between miRNA abnormalities and cancer pathogenesis [11, 14, 31, 60, 101].

Generally, there are two approaches linking miRNAome deregulation to cancer in the context of diagnosis and prognosis: (i) comparison of global miRNA profiles in cancer and non-cancer tissues; and, (ii) search for individual miRNAs that may have diagnostic and prognostic significance in certain types of cancer. For example, chronic lymphocytic leukemia is accompanied by loss of miR-15a and miR-16-1 located in frequently deleted chromosomal region [13]; in lung cancer, the let-7 miRNA is down-regulated, and its reduced expression correlates with poor survival of patients [90]; miR-155 is over-expressed in B-cell lymphomas [27, 55].

miRNAs involved in cancer can have either pro- or anti-tumorigenic action [52]. Anti-tumorigenic, or tumor-suppressing miRNAs act as inhibitors of cell proliferation and stimulators of apoptosis. Contrarily, group of miRNAs acting in the opposite direction by stimulating cell proliferation and inhibiting of cell death is termed "oncogenic miRNAs" [35].

Table summarizes available information regarding cancer-related miRNAs and their targets. Tumor-suppressor miRNAs are frequently down-regulated or deleted in cancer and, respectively, their targets are over-expressed. These include transcription factors and other regulatory proteins stimulating cell growth and proliferation. Oncogene *RAS* is negatively regulated by let-7 miRNA, which is down-regulated in human lung cancer [46]. Two members of BCL family, BCL2 and BCL6, are targets of miR-15a/miR16-1 and miR-127, respectively. Both miRNAs are often deleted or down-regulated in leukemia and lymphomas [13, 27] and increased level of 2 and BCL6 proteins suppresses apoptosis and promotes cell proliferation [24]. miR-143 regulates extracellular signal-regulated kinase 5 (ERK5), a MAP kinase that is activated by growth factors and involved in regulation of cell proliferation [76].

Table. Selected tumor suppressor and oncogene miRNAs

miRNAs	Targets	Type of cancer	References
Tumor suppressor miRNAs (downregulated or deleted in cancer)			
miR-15a	BCL2	B-cell chronic lymphocytic	8, 11, 13, 15, 24, 27
miR-16-1	BCL2	Adenomas, leukemia, lymphomas, pituitary	
let-7	RAS	lung cancer	2, 46, 90, 97
miR-143	ERK5	breast, colon and lung cancer	3, 45, 74, 97
miR-145	?		
miR-127	BCL6	Bladder, colon and prostate cancer	82
Oncogene miRNAs (upregulated in cancer)			
miR-155	?	B-cell lymphomas, Burkitt's lymphoma, breast, colon, lung, thyroid cancer	27, 45, 55, 73, 91, 92, 97
The miR-17-92 cluster	E2F1 PTEN TGFBR2	lymphomas, breast, colon, lung, pancreas and prostate cancer	38, 39, 92
miR-21	PTEN	breast, colon, glioblastoma, liver, lung, pancreas, prostate, stomach cancer	18, 5, 59, 72, 92, 97
miR-372	LATS2	testicular germ cell cancer	93
miR-373	LATS2		
miR-106a	RB1	colon, liver, lung, pancreas, prostate cancer	59, 92, 97
miR-9	CDH1	breast cancer	45
Both tumor suppressor and oncogene miRNAs			
The miR-17-92 cluster:			
miR-17-5p	E2F1		77
miR-20a	E2F1		77
miR-17-5p	AIB1	breast cancer	41
miR-130a	MAFB		29

Over-expression of oncogenic miRNAs negatively regulates tumor-suppressor genes including retinoblastoma 1 (RB1; a regulator of the cell cycle), large tumor suppressor homolog 2 (LATS2; an inhibitor of cyclin-dependent kinase 2), E-cadherin (CDH1; involved in cell-cell adhesion), transforming growth factor-β receptor II (TGFBR2). PTEN (phosphatase and tensin homolog), a target of two miRNAs, miR-21 and the miR-17-92 cluster, encodes a phosphatase

that inhibits PI-3 kinase pathway; the last promotes cell survival/growth [72].

Several target genes have been found for the miR-17-92 cluster that includes seven miRNAs: miRs-17-5p, -17-3p, -18, -19a, -19b, -20, and -92. The miR-17-92 cluster is located on human chromosome 13q31, a region that is easily amplified in several types of cancer including lymphomas [89]. A remarkable feature of this cluster is its capacity to function as both oncogene and tumor suppressor with the result depending on the real situation in the cell. Using a mouse model of c-Myc-induced B-cell lymphoma, He *et al.* [39] found that enforced expression of the miR-17-92 cluster dramatically accelerates disease development with a simultaneous decrease in apoptosis, indicating that these miRNAs act primarily by suppressing cell death.

O'Donnell *et al*. [77] showed that *c-Myc* transcriptionally regulated the miR-17-92 expression. In addition, two miRNAs in this cluster, miR-17-5p and miR-20, regulated the transcription factor E2F1, functioning both as oncogene and tumor suppressor, at posttranscriptional level. E2F1 and c-Myc are known to induce each other's expression. In the absence of other controls, this can set up a positive feedback loop leading to over-expression of both genes with destructive consequences for normal cell-cycle regulation. At high level of expression, E2F1 favors apoptosis induction through the ARF-p53 pathway [35]. Therefore, dampening of translation efficiency by the miR-17-92 cluster might shift the E2F1 action to enhanced proliferation. Generally, the loop c-Myc/miR-17-5p-miR-20a/E2F1 ensures precise control by *c-Myc* of target gene expression with simultaneous activation of their transcription and restriction of their translation. Therefore, this cluster reveals, on one hand, oncogenic action stimulating cell proliferation and, on the other hand, suppressor activity via negative regulatory feed-back loop c-Myc/miR-17-5p-miR-20a/E2F1 [50]. Recently one more tumor suppressor action of this cluster has been found: in breast cancer, miR-17-5p repressed translation of the oncogene AIB1 ("amplified in breast cancer 1") [41].

Another miRNA, miR-130a, also exhibits both tumor suppressor and oncogene action. This miRNA targets the transcription factor MAFB that plays a dual role in carcinogenesis acting as both oncogene and tumor suppressor [79].

Despite the fact of the established link between miRNAs deregulation and cancer, very little is known regarding miRNA changes during early stages of carcinogenesis. He *et al*. [40] showed that in non-tumor tissues adjacent to papillary thyroid carcinoma, miR-221, highly expressed in tumor cells, was also up-regulated — probably reflecting an early event in pathogenesis. In hepatocellular carcinomas, miR-23 and miR-21 expression was enhanced in preneoplastic nodules compared to normal liver, and further increased in tumors [59]. First signs of miRNA alterations during carcinogenesis require extensive studies to determine the key miRNAs that could reflect early events in cancer development.

CONCLUSIONS AND PERSPECTIVES

Discovered recently, miRNAs have been unexpectedly recognized as new global regulators of gene expression that control the key processes in the cell — growth, development, apoptosis. miRNAs are able to simultaneously regulate many mRNAs forming regulatory network that can act in a flexible manner for precise and quick effects on gene expression.

A prominent role of oncogene and tumor-suppressor miRNAs in cancer renders them as a useful tool for diagnostic and prognostic purposes [68, 97]. miRNA profiles are very informative, reflecting the developmental progress and differentiation state of tumors; moreover, they better than mRNA profiles distinguish cancer and non-cancer tissues [25, 68] and in some cases are changed already at early stages of cancer development prior clinical signatures of disease [11, 75]. Altered expression of specific miRNAs has been found in a diversity of cancers giving a promising perspective to use such miRNAs as targets for anticancer therapy. One approach may be treatment with precursors of tumor suppressor miRNAs that are often down-regulated in cancer. For example, the let-7 miRNA may be useful in treatment of lung cancer [85]; as demonstrated on human cancer cells, transfect ion with its precursor suppressed proliferation and simultaneously decreased RAS and c-MYC proteins [2]. In case of oncogene miRNAs, an effective approach might be using antisense olidonucleotides to inhibit respective miRNAs due to competition with mRNAs for binding miRNAs [23, 36]. Antisense therapy has been successfully tested *in vitro* [43, 71], and chemically modified anti-miRNAs termed 'antagomirs' could inhibit specific miRNAs and subsequently upregulated their targets *in vivo* [58]. However, before wide practical use, a number of questions should be clarified. They include miRNA roles in cellular pathways and mechanisms of regulation of their expression in general and search and confirmation critical miRNAs involved in the development of given type of cancer in particular. Finally, a fully unexplored area is effects of anticancer therapy on the miRNA expression. Some data indicate that such treatment can alter miRNA profiles in cancer cells and result in resistance to anticancer drugs [21, 72]. Therefore, both fundamental and clinic-related studies are needed to better understand roles of miRNAs in normal and cancer cells and modulate cellular growth, proliferation and metabolism using miRNAs.

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МикроРНК В НОРМАЛЬНЫХ И ОПУХОЛЕВЫХ КЛЕТКАХ: НОВЫЙ КЛАСС РЕГУЛЯТОРОВ ЭКСПРЕССИИ ГЕНОВ

Микро РНК (miRNAs) — это малые некодирующие РНК, негативно регулирующие экспрессию генов на посттранскрипционном уровне и принимающие участие в развитии, дифференцировке, пролиферации и апоптозе клеток, а также выполняющие важную роль в опухолевом процессе. В обзоре обсужден биогенез miRNA, функции этих молекул в нормальных клетках, изменения набора miRNA в опухолевых клетках и роль противоопухолевых и онкогенных miRNAs в опухолевой прогрессии.
Ключевые слова: **микроРНК, рак, онкоген, опухолевый супрессор.**