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MicroRNAs AND HUMAN CANCER

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MicroRNAs (miRNAs) are a recently discovered family of short non-coding RNA molecules of about 19–24 nucleotides in length that are involved in regulation of gene expression. These small molecules have been found to regulate genes involved in diverse biological processes such as cell proliferation, development, differentiation, apoptosis and others. MiRNAs regulate gene expression at the post-transcriptional level either by inhibition of the target (mRNA) or by its destruction. Recent studies have shown that mRNA deregulation is a basic problem in studying pathogenesis of many malignant tumors. It has been recently shown that miRNAs are able to regulate thousands of target genes simultaneously. Thus, the key role of miRNA in carcinogenesis reveals a new layer in the molecular architecture of cancer. Patterns of altered miRNA expression in cancer may serve as molecular biomarkers for tumor diagnosis, prognosis of disease-specific outcomes, and prediction of therapeutic responses. Furthermore, miRNAs may serve as specific targets of new gene therapies.
Key Words: **microRNA, human cancer.**

INTRODUCTION

Currently, there is reliable evidence that DNA-sequences in the so-called "junk" genomic regions may play an important role in regulation of gene expression. These regions encode sequences known as microRNAs [1, 2], which belong to the class of small RNA molecules with a length of 19–24 nucleotides. Nearly all eukaryotic organisms have a large number of RNAs, which do not encode proteins, and are neither ribosomal nor transport RNAs.

Currently, hundreds of human miRNAs have been identified. It is estimated that there may be approximately 1000 miRNAs in the human genome. It is also known that a single miRNA can influence on the expression of several thousands of genes, thus controlling one third of the human genome [3]. MicroRNAs are able to regulate various biological processes such as cell growth (including stem cells), their differentiation and death [4], neuroprocesses and immune response [5, 6]. It becomes evident that these small molecules play a key role in biological processes of all systems and deregulation of their expression may have negative effect on the normal cell growth and differentiation.

More than 50% of genes of miRNA-encoding genomic loci reside in chromosomal regions unbalanced during tumorogenesis. Such miRNAs are called oncomirs (oncogenic microRNA) [7]. It is well established that deregulation of miRNA genes occurs in tumors of different localizations, and that miRNAs themselves can act as oncogenes (if their genes are amplified or hyperexpressed) or tumor suppressor genes (in case of deletions or repressions of miRNAs) [8].

There is increasing evidence that the altered miRNA expression plays an important role in carcinogenesis beginning from genomic localization, regulation at the level of transcription and processing and completing by post-transcriptional modification of molecules. This review presents the main historical events of discovery, biogenesis and disorders in miRNA genes related to malignization of human cells.

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Abbreviations used: LOH – loss of heterozygosity; miRNAs – microRNAs.

HISTORY OF microRNA DISCOVERY

The first miRNA, lin-4, was discovered in 1993 by Victor Ambros and his colleagues while studying heterochronic gene *lin-14* in worms. Lin-4 RNA controls the timing of *Caenorhabditis elegans* larval development [9]. It encodes a small RNA that is complementary to sequences in the 3'untranslated region (UTR) of lin-14 mRNA and acts as developmental repressor of the accumulation of lin-14 protein. This repression is essential for synchronization of numerous events of *C.elegans* larval development [10].

However, the most important functions of miRNA remained unknown until discovery of another *C. elegans* small RNA molecule, let-7. The *lin-4* and *let-7* genes are nonhomologous and act in a similar manner to trigger the transition to late-larval and adult stages [11]. Ruvkun et al. (2000) obtained the first evidences concerning ability of small RNAs to control gene expression. These investigators found that *let-7* RNA expression can be detected in a wide range of animal species including vertebrates, ascidian, mollusc, annelid and arthropods, further implying that small RNA-mediated translational regulation may be a widespread gene regulatory model present in many animal spices [12].

From the list of discovered and predicted miRNAs, several hundreds have been already cloned for human, mice, *C. elegans*, *Drosophila* and *Arabidopsis* (see http://microrna.sanger.ac.uk) [13]. The large number of miRNAs and homologous sequences of many miRNAs among organisms suggests that these RNAs might constitute an abundant component of the gene regulatory machinery with an ancient origin [14].

BIOGENESIS AND MECHANISMS OF ACTION OF MicroRNAs

Analysis of 186 miRNA genes provides information about the nonrandom distribution of miRNAs genes in the human genome. Ninety miRNA genes are located in 36 clusters, usually with 2 or 3 genes per cluster (median 2.5), and the part of them is located within introns of pre-mRNA genes [15].

Despite some differences in miRNA biogenesis between animals and plants, the most important events of this process remain similar. They can be represented as follows (by example of the miRNA biogenesis in animals):

- gene transcription by RNA-polymerase II and formation of primary miRNA gene transcript with stem- loop structure (pre-miRNA);
- formation of miRNA precursor molecules of about 70 nucleotides in length (pri-miRNA);
- final stage of maturation of effector 22-nucleotide double-stranded miRNA [16].

This space-division and coordinated process requires involvement of several cell complexes. The first two phases take place in the nucleus, and molecule maturation and gene regulation occur in the cytoplasm.

The primary miRNA molecule, pri-miRNA, have a length of about 1000 nucleotides with 7-methylguanosine at the 5' end and polyA at the 3' end. The pri-miRNAs are initially recognized by Drosha and its binding partner DGCR8, which is responsible for high-specific binding to immature miRNA molecule. The complex comprising these proteins was named the *microprocessor complex* [17, 18] which very conservative among all animals. Drosha as a component of microprocessor complex asymmetrically cleaves both strands of the hairpin stem at sites near the base of the primary stem loop, thus releasing a 60-to 70-nucleotide molecule known as pre-miRNA, that has a 5' phosphate and a 2-nucleotide 3'overhang [19].

The pre-miRNAs are transported to the cytoplasm by Exportin-5, in a Ran-GTP-dependent manner [20, 21]. The further cytoplasmic processing is that Dicer, a second RNase-III endonuclease, together with transactivation-responsive RNA-binding protein (TRBP) cleaves pre-miRNA molecule and forms two double-stranded nucleotides [22, 23, 24]. One strand of the miRNa duplex (plus-strand) binds to a large protein complex in an ATP-independent manner and forms *RNA-induced silencing complex* (RISC) [16]. The strand incorporated into RISC becomes the mature miRNA and guides the RISC complex to the target miRNA [25]. Binding to single-stranded RNA molecule is realized via PAZ-domain of Argonaute protein, and PIWI-domain is likely provides effector-nuclease function [26]. Ago protein is a compulsory component of RISC and it plays a key role in posttranscriptional gene silencing (PTGS). Four proteins of this family bound to miRNA through complementarity were found in humans [27].

More recently, it has become apparent that some miRNA can not use major biogenesis pathways. Mature of such molecules as *miR-320* and *miR-484* does not depend on Drosha and DGCR proteins [28], and *miR-451* matures by a Dicer-independent mechanism [29, 30]. Drosha-and DGCR-8-independent miRNAs represent a group of the so-called mirtrons and tailed mirtrons. Pre-miRNAs of these molecules mature by means of splicing and exonuclease cutting of strands [31, 32].

Interaction of miRNA with a target RNA in most cases results in gene repression. As opposed to other classes of small molecules of RNA microRNAs silence genes different from which their primary transcripts originate (hetero-silencing) [33].

Near perfect matching of the 5'end of miRNA (the seed region) to the target site within the 3'UTR leads to translational inhibition. The features within the 3'UTR of a particular mRNA that would accurately predict miRNA targeting have been expanded to include proximity of miRNA-binding sites, AU (adenosine, uracil) rich environments flanking the seed region, and preferential sites within the UTR at both ends [34]. Such regulation leads to decrease in protein level of the target gene with no decreasing in the number of mRNA molecules per cell.

An alternate pathway of miRNA is thought to lead not to translational inhibition but rather to mRNA destabilization through adenosine/uridine rich elements [35]. Ago2 protein bound to miRNA within the RISC complex is responsible for the target mRNA destruction [36]. These noncanonical types miRNA:mRNA interaction have not been extensively studied and thus cannot be bioinformatically predicted; however, they could be equally consequential to the better studied seed-matching mechanism leading to translational inhibition.

Recently obtained data have shown the new level of gene expression regulation. As shown in Fig. 1, the potential mechanisms of miRNA-mediated gene regulation are multifactorial and encompass interactions among different mechanisms.

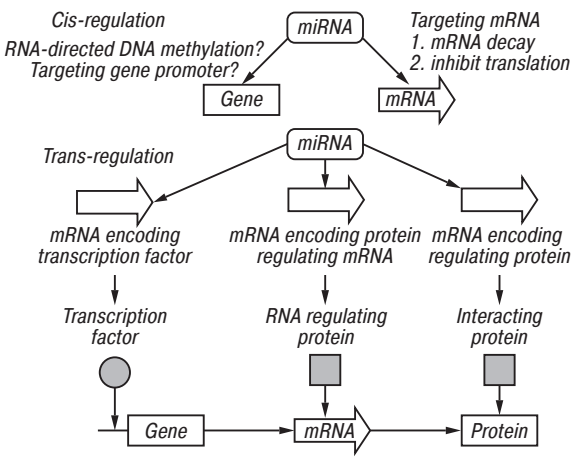

Fig. 1. Potential microRNA regulation mechanisms. *Cis-regulation*: microRNA direct targeting the mRNA and regulating the expression of the target gene at post-transcriptional levels (e.g., enhance mRNA degradation and inhibit translation). *Trans-regulation*: Following the expression changes of microRNA targeted specific genes (e.g., genes coded for transcription factors, genes coded for RNA regulating proteins, and genes coded for proteins that will interact with the target protein), subsequent effects may alter the transcription of other gene, levels of other mRNAs, or interactions among proteins, and thus microRNA may exert its functional effects through transregulatory mechanism(s) [37]

Cis-regulation including mRNA repression or mRNA decay is realized through the miRNA seed binding to the 3'UTR of a particular mRNA. However, it has been shown that after expression changes in miRNA-targeted specific genes (e.g., genes coded for transcription factors, genes coded for RNA regulating proteins, and genes coded for proteins that will interact with the target protein), subsequent effects may alter the transcription of other gene, levels of other mRNAs, or interactions among proteins, and thus miRNA may exert its functional effects through transregulatory mechanism [37].

MicroRNA AND CANCER

Relationship between cancer and miRNA was first revealed by Professor G. Calin and co-workers in 2002. They suggested that *miR-15a* and *mir16–1* could be the tumor suppressors involved in the pathogenesis of chronic lymphocytic leukemia because the expression of these genes is downregulated or suppressed in most cases with cell lymphocytic leukemia. Many different projects were done in this area since then. Many investigators have shown aberrant expression profile of some oncomirs between tumor and normal tissues [8, 38, 39].

Large-scale investigations on the role of some microRNA in carcinogenesis were done. Many projects are devoted to claster *mir-17–92* [15, 40–43], oncosupressor *let-7g* [11, 44, 45], oncogene *mir-21* [46–48] and some others.

The link between miRNAs and metastasis has been reported but these studies have been conducted using cell-lines and xenographic models [49]. Oncomirs (miR-10b, miR-9, miR-31 and miR-335), for which aberrant expression in breast cancer has been detected, can be taken as an example. The cell-line or model-based research cannot be extrapolated to humans because it is impossible to determine the role of microenvironment, link with disease prognosis and many other clinical parameters. However, there are literature data on the relationship between the level of miRNA expression and clinical-biological parameters such as survival, recurrence, predisposition to tumor progression and metastasis.

MiRNA GENETIC ALTERATIONS IN HUMAN CANCERS

Recent studies have shown that alterations in miRNA synthesis in human cancers are often related to tumor development, progression and metastasis. There is a hypothesis that deregulated synthesis of microRNAs, which in turn regulate protein synthesis, is one of the most important factors contributing to cancer development [50–52].

The finding that *miR-15a* and *miR-16–1* are located in a region that is frequently deleted in leukemia gave a hint that a connection might exist between the genomic position of microRNA and the location of cancer-associated genomic regions. Later on, deletions of *miR-15a* and *miR-16–1* were also detected in solid tumors, such as pituitary adenomas [53], ovarian and breast cancers [54]. Translocation of *miR-17–92* in acute T-cell leukemia was also shown [55]. The mir-142 gene, a marker of haematopoietic cells, was found at the breakpoint junction of a t(8;17) translocation, which causes an aggressive B cell leukemia due to strong up-regulation of a translocated MYC gene [56].

By using bioinformatics and public databases of genomic regions that are prone to alteration in cancer, such as minimal regions of LOH, minimal regions of amplification, common breakpoint regions and fragile sites, Calin et al. (2004) analyzed the location of, known that time, microRNAs in the human genome and found they frequently reside (> 50%) in such genomic regions. Later on, these results were confirmed by different methods and at different cancers [39, 54, 57]. Hua Dong et al. (2011) [58] indentified 409 trans-expression quantitative trait locus and 27 trans- miRNA eQTL for loss of heterozygosity (LOH) mutation in glioblastoma.

Amplification of certain genes also plays a role in carcinogenesis, for example amplification of the *mir-17–92* cluster in lymphoma [59]. By analizing *miR-17–19* cluster at the 13q31 locus, He et al. (2005) found that expression of 5 microRNAs is significantly correlated with the gene dosage of *C13orf25* across the all analyzing cell lines. Zhang et al. (2006), using an array-based comparative genomic hybridization (aCGH), showed correlation between gene copy number of microRNA and cancer. Starczynowski et al. (2010) [60] showed that seventy-seven percent (542 of 706) of miRNAs mapped to leukemia-associated copy-number alterations in the cell lines, but only 18% (99 of 542) of these miRNAs are expressed above background levels. Differential PCR revealed deletions in *miR-186* (15%), miR-135a-1 (33%), miR-548d-1 (42%), miR-548d-2 (21%) and miR-512–2 (33%) genes, whereas deletion or amplification was detected in *miR-135b* and *miR-135a-2* in 23% and in 15% of medulloblastomas, respectively [61].

MicroRNA biogenesis malfunctions can result in serious consequences for a cell and ultimately for the whole organism. Deletions, disorders in the processing pathway or abuse of regulation of microenvironment play a certain role in cell transformations [62]. Deletions or malfunction of microRNA biogenesis (Dicer or DGCR8 proteins) can lead to changes in proliferation and differentiation of stem cells [63, 64]. Malfunction of Dicer due to deletion of its locus most frequently leads to malignization. It is notably that Dicer is haploinsufficient oncosupressor that is even deletion of a part of this gene could lead to malignization [65]. Kumar et al. (2009) [66] showed that 27% of various tumors have a hemizygous deletion of the gene that encodes DICER1. TARBP2 is mutated in some colon and gastric cancers with microsatellite instability, and TARBP2 frameshift mutations correlate with DICER1 destabilization [67].

Of particular interest is the fact that *DGCR8* was originally identified as a gene that maps to the chro-

mosomal region 22q11.2, a region whose monoallelic deletion accounts for >90% of patients with DiGeorge syndrome, the most common human genetic deletion syndrome [68].

Genetic changes in the 3′ UTR of target genes can both create or destroy a miRNA binding site. For example, Jiang et al. (2010) [69] identified a mutation in the *miR-155* binding site of the SOCS1 3′ UTR in a breast tumor that reduced *miR-155* repression. Chromosomal translocations can remove miRNA binding sites from their regulated oncogenes [70]. Alternative polyadenylation can relax miRNA-mediated regulation of known oncogenes [71]. Cooperativity or competition of miRNAs for mRNA target site binding with other RBPs, such as ELAVL1 (HuR), DND1 and PUM1, can also de-repress target expression [72–75].

EPHIGENETIC ALTERATIONS OF miRNA IN HUMAN CANCERS

Recently, accumulating studies have shown that a subgroup of miRNAs is regulated epigenetically. The finding that CpG islands within introns can act as promoters suggests that perhaps intronic miRNAs that have CpG islands upstream within the same intron could be transcribed from their own promoters that are regulated by DNA methylation [76, 77].

Scott et al. (2006) [78] showed that in breast cancer cells, inhibition of the histone deacetylase (HDAC) rapidly altered the expression levels of nearly 30 microRNAs, indluding let-7a. Moreover, Saito et al. (2006) [79] found that the combined treatment of human bladder cancer cells with a DNA-demethylating agent and an inhibitor of HDAC caused the significant upregulation (>3-fold) of 17 out of 313 microRNAs. Another example of important role of epigenetic alterations is role of *miR-9–1* in breast cancer [80]. MicroRNA regulate proteins that play role in epigenetic repression of genes *DNMT, HDAC, HMGA2* and *PcG* [81–83]. Thereby, there is overlapping of functions and crossregulation of regulatory epigenetic agents and microRNAs.

Hypermethylation of *miR-9* loci is observed in various malignant tissues, including breast, lung, colon, head and neck cancers, melanoma and acute lymphoblastic leukemia [80, 84–86]. Wong et al. (2011) [87] showed that in primary lymphoma samples, *miR-124–1* preferentially hypermethylated in B- or NK/T-cell lymphomas and associated with reduced *miR-124* expression. Also the methylation levels of the CpG islands of *miR-34b/c* [85, 88], *miR-137* [89] are correlated with mature expression levels in various cancers.

miR-203 is located in fragile genomic region, and it targets *ABL1* and *BCRABL1*, an oncogenic fusion gene generated by the Philadelphia translocation [90]. Epigenetic silencing of *miR-203* enhances activation of the *BCR-ABL1* fusion gene, resulting in an elevation of tumor cell growth rate. Also it is frequently observed in other types of malignancies, including oral cancer, hepatocellular carcinoma, etc. [89, 91].

Many transcriptional factors regulate microRNA expression through type-specific manner and many microRNAs are under regulation of oncosupressors such as *TP53, MYC* and *RAS* [92], for example *miR-21* which are regulated by *RAS* gene [93]. MicroRNA molecules correlate with their transcriptional regulators through the feedback, so it is clear that disorder at one stage can lead to malfunction of the whole system.

From these observations it may be concluded that miRNA genes undergo different genetic alterations during tumorigenesis. Biogenesis disorders, deletions, translocations, increase in gene copy number and epigenetic alterations have been found to lead to changes in microRNA expression and tissue-/site-specific pattern of microRNA, thus influencing on pathogenesis and molecular-genetic characteristics of cancer.

CONCLUSION

Recent researches concerning the role of miRNA in human cancer pathogenesis have provoked a great interest of both the research scientists and clinical oncologists. The high specificity of miRNAs compared with mRNAs indicates that these small molecules can serve as highly informative cancer biomarkers [94, 95]. Furthermore, miRNA can be extracted from a wide range of tissue and body fluid samples, that also make them useful potential markers for the molecular analysis [96].

The perspective of using miRNA in clinical practice is not restricted by only early detection of the disease. It is also possible to use them in cancer therapy. In this case it is necessary to rely on dualistic functions of miRNA. If miRNA acts as tumor suppressor, it is necessary to increase the level of its expression by either increasing copying or deleting repression of the encoded gene or guiding the mature miRNA into the tumor cell. If miRNA acts as oncogene, it is necessary to inhibit its expression. One of the approaches to inhibit miRNA expression is the use of “sponges” which represent mRNA and have multiple complementary sites for particular miRNAs. High expression of these mRNA-sponges leads to specific inhibition of the whole family of miRNA with similar binding sites [97].

It is also necessary to take into consideration side effects of miRNA -based therapy. miRNAs and interfering RNAs (siRNAs) share many common features. However, interfering RNA is able to repress the target mRNA both in perfect matching of seed region within the 3’UTR of a particular mRNA and in nearly perfect complementarity. Although siRNAs can be easily designed to target one specific target with perfect complementarity, it can be difficult to computationally predict repression of other target genes through imperfect sequence marching, via a miRNA-like mechanism [98]. These unpredictable interactions can lead to negative side effects of miRNA therapy [99, 100]. In this respect, miRNAs are preferential because they are able to recognize and repress a large number of target mRNAs. However, this is suggested not to result in side effects because miRNA therapy will be directed to the recov-

ery of physiological level of miRNA expression, which is detected in the normal tissue.

Before clinical use of miRNAs, some problems should be solved. They include both the role of miRNA in cellular pathways and mechanisms of regulation and expression and the search for the critical miRNAs involved in cancer development. The influence of antitumor therapy on the levels of miRNA expression has not been extensively studied. Few data are reported in the literature about the effect of chemotherapeutical agents on the miRNA expression levels in tumor cells that in turn can result in drug resistance [101, 102].

Both the basic and clinical researches in the miRNA field are currently very important. The results of these studies will allow the researches to determine the miRNA status in normal and transformed cells as well as to assess the possibility of application of miRNA molecules for modulation of cell growth, proliferation and metabolism. Understanding of the role of miRNAs is allowing new insights on the molecular basis of cancers, and new biomarkers for cancer diagnosis, prognosis and therapy.

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