ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXI, N 2, 176 SECTIO D 2006

Department of Medical Genetics, Medical University of Lublin

KATARZYNA SKÓRZYŃSKA, AGATA FILIP, JACEK WOJCIEROWSKI

MicroRNAs, a novel class of small RNAs, and their role in oncogenesis

MicroRNAs (miRNAs) are an abundant class of 19–23 nucleotide noncoding RNAs, cleaved from hairpin precursors, which regulate gene expression at the post-transcriptional level in a sequence-specific manner.

MicroRNAs are processed in the nucleus by Drosha ribonuclease-III enzyme from primary miRNA (pri-miRNA) precursor transcripts into about 70-nucleotide pre-miRNAs, consisting of an imperfect stem-loop structure, which thereafter are exported to the cytoplasm by specific protein Exportin 5 and processed into mature microRNAs in a reaction requiring Dicer ribonuclease-III and Argonaute (AGO) family members (11). Mature microRNAs form miRNA:miRNA duplexes. An unwound single strand of such a duplex binds to Argonaute proteins finally forming siRigonaute ribonucleoproteins (known as miRNPs), which in association with other proteins is assembled into the RNA-induced silencing complex (RISC) subsequently acting on the target mRNA by its translational repression or mRNA cleavage (7). The preferential mechanism of action depends on complementarity between microRNA and its target messenger RNA. The complete accordance in nucleotide sequences results in mRNA degradation. The cleavage of complementary mRNA targets is known as post-transcriptional gene silencing (PTGS).

Although microRNA encoding loci are typically found in intergenic areas, they can also quite frequently be located in sense or antisense orientation within introns of host genes, including both protein-coding genes and non-coding ones, which implicate the possible mechanisms of microRNAs transcription regulation through their host-genes promoters (2). Additionally, certain microRNAs are clustered in polycistronic transcripts and therefore co-ordinately expressed.

Many messenger RNAs are regulated by microRNAs. Bioinformatic predictions of microRNAs targets, supported by a growing number of genome-wide experimental analyses, suggest that more than one-third of human mRNAs are influenced by microRNAs (7).

The first reported microRNA, *lin-4*, was originally identified in 1993 in *Caenorhabditis elegans* as a gene playing a crucial role in the timed regulation of development (11). Since this time, more than 300 microRNA genes have been identified in plants and animals. However, the computationally predicted number of microRNAs seems to be closer to 1,000 and the microRNAs registry is still very distant from being completed. The total number of microRNAs is estimated to represent about 1% of all genes.

MicroRNAs have been described as playing roles in a great variety of biological processes, including the regulation of gene expression, cell proliferation and death, development, stress resistance and fat metabolism. Transcription and chromosome structure, RNA processing and modifications, mRNA stability and translation, and protein stability and transport are influenced by microRNAs. MicroRNAs are expressed in a tissue-specific manner but very little is known about

how they are regulated to present the highly specific tissue patterns. Because of this specificity, the direct involvement of microRNAs in processes of oncogenesis could have been expected and, indeed, shortly after the discovery of tissue-specific expression pattern of microRNAs, abnormal microRNA expression during tumourigenesis was reported. Since this time a wide range of intricate connections between many neoplasms and different microRNAs has been described. Moreover, more than half of known microRNAs were reported to be mapped to chromosomal fragile sites and/or genomic regions involved in oncogenesis such as minimal loss of heterozygosity regions, minimal regions of amplification or common breakpoint regions (8). MicroRNAs can play the role of both a tumour suppression gene, inhibiting the development of tumour, and an oncogene, promoting neoplasm origin and/or its spreading. Just to give an example of possible mechanism of microRNAs action – the first event may occur in case when overexpressed microRNA could decrease expression of the oncogene target. The most common molecular mechanism leading to microRNAs overexpression is an amplification of the microRNAs encoding locus. In opposition, the molecular bases for microRNAs underexpression are deletion or methylation of their loci.

Expression studies of various tumours have revealed some specific alterations in miRNAs profiles. For example, *miR-26a* and *miR-99a* are demonstrated to be underexpressed in lung cancer cells lines. In 2004 Takamizawa et al. reported that *let-7* overexpression in A549 lung adenocarcinoma cell line results in inhibition of cells growth and negatively regulates RAS expression (12). Also *in vivo let-7* has been reported to inversely correlate with RAS expression. The major study of 143 cases of non-small lung carcinoma revealed that patients classified according to *let-7* expression pattern had significantly shorter postoperative survival, directly associated with reduced *let-7* expression. One year later Karube et al. reported that also RNA-specific endonuclease Dicer, converting precursor forms of microRNA into their mature product, demonstrates reduced expression levels in a fraction of lung cancers with a significant prognostic impact on the shorter survival of surgically treated cases (9).

Another evidence for microRNAs involvement in solid tumours pathogenesis has been reported by Michael et al. who have found reduced levels of *miR-143* and *miR145* in precancerous and neoplastic colorectal tissue (adenocarcinoma Dukes' stage B). The decreased abundance of these both miRNAs probably can reflect early changes in the cellular composition of tumours, compared with normal mucosae, and might be directly involved in the processes that lead to neoplasia (10).

In comparison to still very few attempts of microRNAs expression studies in solid tumours, the most impressive progress has been recently made in investigations of microRNAs association with haematological malignancies. It should be emphasized that the first evidence for direct involvement of microRNAs in oncogenesis was reported in 2002 by Calin et al. who demonstrated that *miR-15* and *miR-16* located at chromosome 13q14, a genomic locus deleted in more than 65% of cases of chronic lymphocytic leukaemia (CLL), were deleted or down-regulated in most cases (about 68%) of this malignancy (4). The 13q14 loss in CLL is the most frequent chromosomal abnormality found in this disease, very often described as the sole genetic alteration. This data suggests that tumour suppressor genes at 13q14 must be involved in CLL pathogenesis and *miR-15/miR-16* might represent the targets of inactivation by hemizygous and/or homozygous loss in this disease. Deletions at 13q14 can be also observed in other malignancies as mantle cell lymphoma (50% of cases), multiple myeloma (16–40%), prostate cancers (60%) but microRNAs expression pattern in these diseases still waits to be explored. However, in 2005 Bottoni et al. shortly reported that *miR-15a* and *miR-16-1* are expressed at lower levels in pituitary tumours, in which 13q14 region has been recently shown as frequently deleted, as compared to normal pituitary tissue (1).

In 2002, Calin et al. demonstrated in CLL an inverse correlation between *miR-15a* and *miR-16-1* expression and the expression levels of arginyl-tRNA synthetase (RARS), an enzyme associated with the

cofactor p43 in the aminoacyl-tRNA synthetase complex. The p43 protein regulates local inflammatory response and macrophage chemotaxis, and seems to have anti-neoplastic properties (4).

In 2004 Calin et al. published the results of the genomewide expression profiling of miRNAs isolated from 38 individual CLL cell samples (5). The pattern of microRNAs expression in CLL was compared to normal CD5+ B cells. The most striking differences between CLL cells and control were shown in the pattern of expression following microRNAs, mostly located inside different fragile sites and being down-regulated in the majority of analysed CLL samples: *miR-183*, *miR-190*, *miR-24-1* and *miR-213*. Calin and co-workers also determined the specific signature profile associated with the expression of mutated IgV_H, a favourable prognostic marker in CLL. To five differentially expressed genes, which could be used as distinguishing markers for samples with mutated and unmutated IgV_H, belonged *miR-186*, *miR-132* and *miR-16-1* positively associated with unmutated $IgV_{H \text{ and } miR\text{-}102}$ and *miR-29c* positively associated with mutated IgV_H. A unique microRNA signature was reported to be associated with prognostic factors such as mutations in the IgV_H or high expression of the 70-kd zeta-associated protein (ZAP-70+) and disease progression in CLL (5).

Two years later, in 2006, Cimmino and Calin et al. reported that *miR-15a* and *miR-16-1* expression is inversely correlated to *BCL2* expression in CLL and both microRNAs are negative regulators of *BCL2* at a posttranscriptional level (6). Since 1998 *BCL2* gene has been known to be strongly overexpressed in CLL, but mechanisms of *BCL2* up-regulation have remained completely unexplained. Therefore, the data presented in the following Calin's study have a great significance, especially since *miR-15a* and *miR-16-1*, natural antisense *BCL2* interactors, can be used as a powerful tool in a novel therapeutic strategy for CLL.

The latest findings of Calin's group present the view that CLL is a genetic disease in which the main alterations occur at the level of transcriptional/post-transcriptional regulation of the malignant cells genome (3). These alterations are the result of mutations in miRNA transcripts, reported as frequent events which can be also found in the germ-line. This finding suggests that a special predisposition to CLL and many associated malignancies may exist.

In 2005, He et al. reported that the *mir-17-92* polycistron is located in a region of DNA, amplified in different human B-cell lymphomas such as diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma and primary cutaneous B-cell lymphoma. B-cell lymphoma samples and cell lines were compared to normal tissues (8). The levels of the primary or mature microRNAs derived from the *mir-17-92* locus were often substantially increased in these neoplasms. In a mouse B-cell lymphoma model increased expression of the *mir-17-92* cluster acted with c-myc expression which resulted in an acceleration of tumour development.

In conclusion, as very important regulators of gene expression, miRNAs may play a crucial role in genetic predisposition to disease. They can also be analysed in patients as prognostic factors because of their specific signature profile associated with particular diseases. In future, miRNAs can be also used as a novel therapeutic tool, but this approach, although very promising, should wait for the better understanding of a complex network of associations between microRNAs and their target genes.

REFERENCES

1. Bottoni A. et al.: *miR-15a* and *miR-16-1* down-regulation in pituitary adenomas. J. Cell Physiol., 204, 280, 2005.
2. Caldas C., Brenton J. D.: Sizing up miRNAs as cancer genes. Nat. Med., 11, 712, 2005.
3. Calin G. A, Croce C. M.: Genomics of chronic lymphocytic leukemia microRNAs as new players with clinical significance. Semin. Oncol., 33, 167, 2006.

4. Calin G. A. et al.: Frequent deletions and down-regulation of micro- RNA genes *miR15* and *miR16* at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA, 99, 5524, 2002.
5. Calin G. A. et al.: MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc. Natl. Acad. Sci. USA, 101, 11755, 2004.
6. Cimmino A. et al.: *miR-15* and *miR-16* induce apoptosis by targeting BCL2. Proc. Natl. Acad. Sci. USA, 103, 2464, 2006.
7. He L., Hannon G J.: MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet., 7, 522, 2004.
8. He L. et al.: A microRNA polycistron as a potential human oncogene. Nature, 435, 828, 2005.
9. Karube Y. et al.: Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer Sci., 96, 111, 2005.
10. Michael Z. M. et al.: Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol. Cancer Res., 12, 882, 2003.
11. Nelson P. et al.: The microRNA world: small is mighty. Trends Biochem. Sci. 28, 534, 2003.
12. Takamizawa J. et al.: Reduced expression of the *let-7* microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res., 64, 3753, 2004.

SUMMARY

MicroRNAs are an abundant class of 19–23 nucleotide noncoding RNAs which regulate gene expression at the post-transcriptional level. MicroRNAs play roles in a great variety of biological processes, including development and cell proliferation, differentiation and apoptosis. Transcription and chromosome structure, RNA modifications, translation, protein stability and transport are influenced by microRNAs. MicroRNAs are expressed in a tissue-specific manner and exhibit a unique signature profile associated with particular diseases. In this review, mechanism of microRNAs action and their involvement in pathogenesis of particular malignancies such as chronic lymphocytic leukaemia, malignant lymphomas, lung cancer and colorectal neoplasia are elucidated.

MikroRNA, nowa klasa krótkich RNA, i ich rola w onkogenezie

MikroRNA są bogato reprezentowaną grupą krótkich 19–23 nukleotydowych niekodujących RNA, które regulują ekspresję genu na poziomie posttranskrypcyjnym. MikroRNA odgrywają rolę w ogromnej ilości procesów biologicznych, takich jak rozwój osobniczy oraz proliferacja, różnicowanie i apoptoza komórek. Transkrypcja i struktura chromosomów, modyfikacje RNA, stabilność białek i ich transport są kontrolowane przez mikroRNA. MikroRNA ulegają swoistej tkankowo ekspresji. Unikalny profil ekspresji mikroRNA jest specyficzny dla poszczególnych nowotworów. Powyższa praca poglądowa podsumowuje wyniki dotychczasowych doniesień na temat mechanizmów działania i roli mikroRNA w chorobach nowotworowych, szczególnie w przewlekłej białaczce limfocytowej, chłoniakach złośliwych, raku płuc oraz jelita grubego.