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The arrangements of the locations of miR-619, miR-5095, miR-5096 and miR-5585 binding sites in the human mRNAs

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***Abstract*—The binding of 2,563 human miRNAs with the mRNAs of 12,175 human genes was studied. It was established that miR-619-5p, miR-5095, miR-5096 and miR-5585-3p bind with high affinity to the mRNAs of the 1215, 832, 725 and 655 genes, respectively. These unique miRNAs have binding sites in the 3'UTRs, CDSs and 5'UTRs. Groups of mRNAs in which the ordering of the miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites differed were established. The possible functional properties of these miRNAs are discussed.**

Keywords—cancer, human, miRNA, mRNA.

I. Introduction

MiRNAs, as a part of the RNA-induced silencing complex, bind to mRNAs and interfere with translation or promote mRNA destruction [2]. The study of the properties of miRNAs and their influences on the expression of the genes that participate in all key processes of cells was established in the last 20 years. The actions of miRNAs on the cell cycle [3], apoptosis [4], differentiation [5], growth and development in animals [6] have been shown. Connections among miRNA expression and the development of various diseases have been established. miRNA concentrations change in cancer [7]. Metabolic disturbances necessarily change miRNA concentrations in cells [8]. It is possible to normalize some processes using miRNAs [9]. The aforementioned roles do not encompass the full list of the biological processes in which miRNAs participate, which proves the importance of their biological functions.

Despite the appreciable successes in the study of miRNA properties, there are obstacles to establishing the target genes of miRNAs. There are miRNAs that bind to many mRNAs, and one mRNA can be the target of many miRNAs. These circumstances significantly complicate the study of the properties of miRNAs and their diagnostic and medical applications. There are more than 2,000 miRNAs in the human genome, and they are thought to act on 50% or more of genes. It will be difficult to draw unique conclusions about the participation of miRNAs in specific biological processes, and until those conclusions can be drawn, the connections between the majority of miRNAs and their target genes will remain unknown. Recently, we found a set of unique miRNAs that have hundreds of target genes and bind to mRNAs with high affinity. The binding sites unique to miRNAs are located in the 3'UTRs, CDSs and 5'UTRs of mRNAs. In present work, we studied some unique miRNAs that bind to the mRNAs of several hundred human genes.

II. Material and Methods

The human gene mRNAs were taken from GenBank (http://www.ncbi.nlm.nih.gov) using Lextractor002 script (http://sites.google.com/site/malaheenee/software). The nucleotide sequences of human miR-619-5p, miR-5095, miR-5096 and miR-5585-3p were taken from the miRBase site (http://mirbase.org).

The target genes for the tested miRNAs were revealed using the MirTarget program, which was developed in our laboratory. This program defines the following features of binding: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in the 5'-untranslated regions (5'UTRs), the coding domain sequences (CDSs) and the 3'-untranslated regions (3'UTRs) of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between the miRNAs and the mRNAs. The ratio $\Delta G/\Delta G_m$ (%) was determined for each site (ΔG_m equals the free energy of an miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on the mRNAs had $\Delta G/\Delta G_m$ ratios of 90% or more. We also noted the positions of the binding sites on the mRNA, beginning from the first nucleotide of the mRNA's 5'UTR. This program found hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The distances between A and C were equal to those between G and C, A and U, and G and U. The numbers of hydrogen bonds in the G-C,

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A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively. The free binding energies of these nucleotide pairs were taken as the same values (i.e., 3, 2, 1, and 1, respectively).

III. Results

A. Features of miR-619-5p, miR-5096, miR-5585-3p and miR-5095

The binding powers between the 2,563 tested hsa-miRNAs and the mRNAs of 12,175 human genes were calculated. Some of these miRNAs have greater numbers of target genes than others. For example, miR-619-5p, miR-5095, miR-5096 and miR-5585-3p are found to be capable of binding more 600 genes each. These miRNAs were termed unique miRNAs (umiRNAs). Additionally, the binding sites for these unique miRNAs are unusually located in the mRNAs. miR-619-5p, miR-5095, miR-5096 and miR-5585-3p have different miRNA binding site origins, lengths, quantities and miRNA binding site properties, among other features. Some characteristics of these unique miRNAs are outlined below.

With a length of 22 nt, miR-619-5p is coded in an intron of the slingshot protein phosphatase 1 gene (*SSH1*). We found that miR-619-5p has 1811 binding sites on 1215 target mRNAs. Of those, 1772 miR-619-5p binding sites are located in 3'UTRs, 26 sites are located in 5'UTRs and 13 sites are located in CDSs. The mRNAs of 197 genes have completely complementary binding sites for miR-619-5p. The mRNAs of 27 genes have four binding sites. Seven genes have five binding sites, and the mRNAs of the *CATAD1, ICA1L, GK5, POLH,* and *PRR11* genes have six miR-619-5p binding sites. The mRNAs of the *OPA3* and *CYP20A1* genes have eight and ten binding sites, respectively. All of these sites are located in 3'UTRs.

With a length of 21 nt, miR-5096 is coded in an intron of the BMP2 inducible kinase gene (*BMP2K*). We found that miR-5096 has 997 binding sites on 832 target mRNAs. Of these, 984 miR-5096 binding sites are located in 3'UTRs, nine sites are located in 5'UTRs and four sites are located in CDSs. The mRNAs of 42 genes have completely complementary binding sites for miR-5096. The mRNAs of the *IP09* gene have four binding sites. The *PRR11* gene have five binding sites. The mRNAs of the *OPA3* and *CYP20A1* genes have six and 11 miR-5096 binding sites, respectively. All of these sites are located in 3'UTRs.

With a length of 22 nt, miR-5585-3p is coded in an intron of the transmembrane protein 39b gene (*TMEM39B*). We found that 725 target gene mRNAs have 844 binding sites for miR-5585-3p. Nine of these binding sites are located in 5'UTRs, two sites are located in CDSs and 833 sites are located in 3'UTRs. The mRNAs of the *CYP20A1* and *GPR155* genes each has four binding sites.

With a length of 21 nt, miR-5095 is coded in an intron of the sterol carrier protein 2 gene (*SCP2*). We found that 655 target gene mRNAs have 734 binding sites. 14 of these binding sites are located in 5'UTRs, eight sites are located in CDSs and 712 sites are located in 3'UTRs. The mRNAs of two genes have completely complementary binding sites for miR-5095. The mRNAs of the *OPA3,* and *SPN* genes each has four binding sites.

B. miRNA binding sites in 5'UTRs, CDSs and 3'UTRs

The miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites in the 5'UTRs, CDSs and 3'UTRs of several genes were predicted using the MirTarget program. Multiple miRNA binding sites are revealed to be in the 5'UTRs of several genes. For example, miR-619-5p has two binding sites in each of the 5'UTRs of the *ANAPC16, CYB5D2* and *PRR5* mRNAs and three binding sites in the *DNASE1* mRNA.

The mRNAs of some genes have binding sites for miR-619-5p, miR-5095, miR-5096 and miR-5585-3p within their 5'UTRs and 3'UTRs or CDSs and 3'UTRs. For example, the 5'UTRs and 3'UTRs of the *ATAD3C* and *CYB5RL* genes have miR-619-5p binding sites. The CDSs and 3'UTRs of the *C8orf44, ISY1* and *ZNF714* genes have miR-619-5p binding sites.

The 5'UTR and 3'UTR of the *ANAPC16* gene have miR-5095 miR-5096 and miR-5585-3p binding sites. The 5'UTR and 3'UTR of the *ATAD3C* gene have miR-5095 and miR-619-5p binding sites. The 5'UTRs and 3'UTRs of the *C14orf182* and *CYB5RL* genes have miR-5096 and miR-619-5p binding sites, respectively.

miR-5095 and miR-619-5p binding sites were found in the CDS and 3'UTR of the *ISY1* gene. The CDS and 3'UTR of the *ZNF714* gene have binding sites for miR-5096 and miR-619-5p, and the *C8orf44* mRNA has only a miR-619-5p binding site.

C. The arrangements of the locations of umi-RNA binding sites

The mRNAs that are targeted by miR-619-5p, miR-5096, miR-5095 and miR-5585-3p were established. The 5'UTRs of three target genes contained these miRNA-binding sites (Fig. 1). The degree of homology of the nucleotide sequences in these genes is high not only in the binding sites of the studied miRNAs but also across all mRNA 178 nt sequences. The distance between the miR-5095 and miR-5096 binding sites is 57-59 nt and that between the miR-5096 and miR-5585-3p binding sites is 46-47 nt. The miR-5095 and mir-619-5p binding sites partially overlapped. The greatest numbers of miR-619-5p, miR-5096, miR-5095 and miR-5585-3p binding sites are located in the 3'UTRs, and it is therefore possible that many target genes have umiRNAs binding sites. The data about the locations of the miR-619-5p, miR-5096, miR-5095 and miR-5585-3p binding sites and the degrees of homology of the corresponding nucleotide sequences in the mRNAs of 21 genes are presented in Fig. 2. The distances between the miR-5095 and miR-5096 binding sites are all 57-60 nt. The distances between the miR-5095 and miR-5096 binding sites in the mRNAs of 78 genes averaged 58.6±0.9 nt. Thus, the distances between miR-5095 and miR-5096 binding sites are highly conserved. The distances between the miR-5096 and miR-5585-3p binding sites are all 46-49 nt. The distances between the miR-5096 and miR-5585-3p binding sites in the mRNAs of 325 genes averaged 47.3±1.1.

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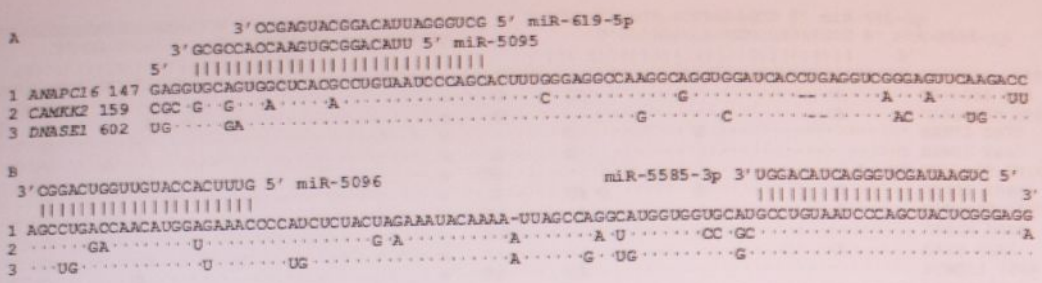

Fig. 1 the umiRNA binding sites located in 5'UTRs
Note: A. miR-619-5p and miR-5095 binding sites; B. miR-5096 and miR-5585-3p binding sites.

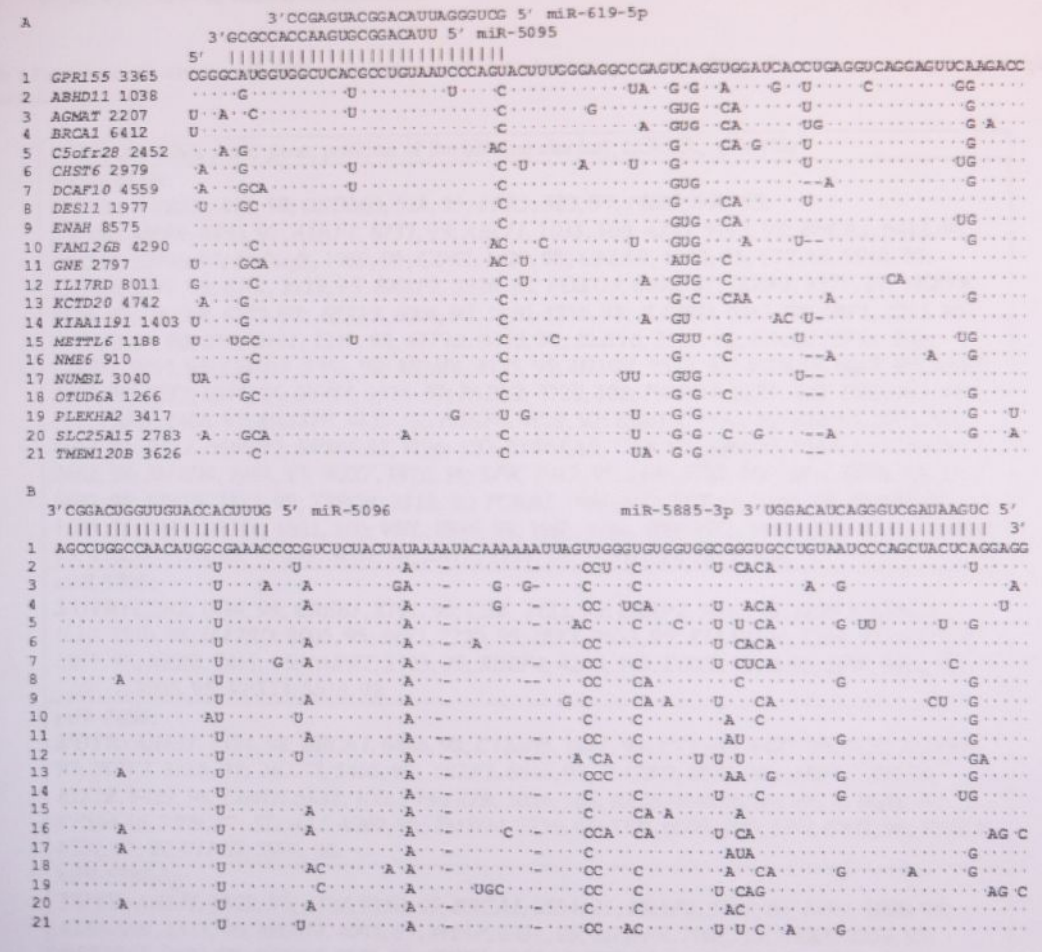

Fig. 2 the umiRNA binding sites located in the 3'UTRs
Note: A. miR-619-5p and miR-5095 binding sites; B. miR-5096 and miR-5585-3p binding sites.

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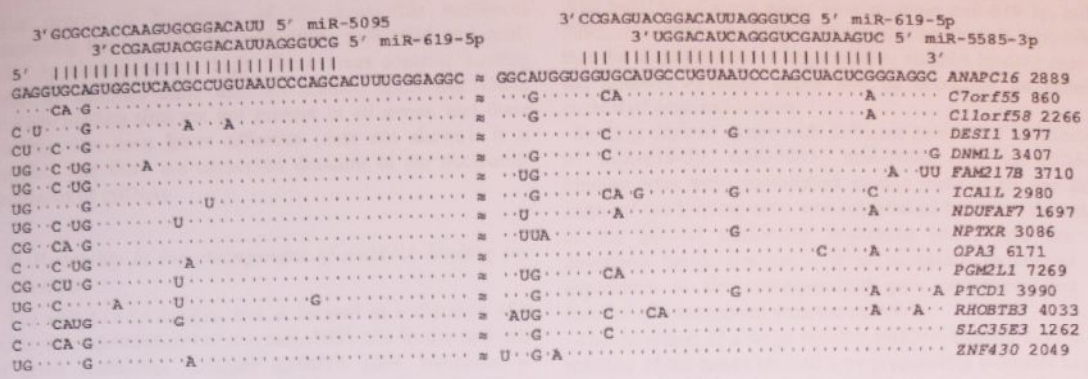

Fig. 3 the location of miR-5095, miR-5585-3p with two miR-619-5p binding sites in 3'UTRs
Note: ≈ indicates equal to 84 nt, which is not shown here.

Table 1 Features of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites in mRNA of tumor suppressor genes participating in breast and lung cancer

miRNA: mRNA part: gene, position of the binding site (nt), $\Delta G/\Delta G_m$ ratio (%)
miR-619-5p: **5'UTR:** *AURKA*, 426, 98; *GSDMD*, 524, 95; *PRR5*, 523, 97; *PRR5*, 660, 95. **3'UTR:** *AHRR*, 4450, 97; *APAF1*, 6737, 95; *ARLI1*, 1033, 100; *ATM*, 9793, 98; *BRCA1*, 6412, 98; *BRCA2*, 10746, 97; *C12orf5*, 1769, 95; *CD82*, 1420, 98; *CFLAR*, 1932, 95; *CFLAR*, 5910, 95; *CREB1*, 2797, 98; *CRK*, 2129, 95; *ERAP2*, 3626, 98; *FOXO3*, 6098, 97; *IKZF3*, 3377, 97; *IKZF3*, 5526, 97; *IKZF3*, 6772, 97; *IKZF3*, 6906, 97; *IL10*, 1216, 98; *IL17RD*, 8011, 98; *IRF1*, 2235, 95; *IRF1*, 2659, 98; *KIAA0101*, 1210, 98; *KIF1B*, 9415, 98; *KLK10*, 2139, 95; *LIMD1*, 5735, 100; *LIMD1*, 5897, 95; *LIMD1*, 5763, 98; *MDM4*, 3975, 95; *MDM4*, 7553, 95; *MTHFR*, 6861, 95; *NEK8*, 2417, 98; *NIT1*, 1375, 95; *NOX4*, 3325, 97; *PARK2*, 3729, 100; *PDCD4*, 3221, 100; *PECAM1*, 871, 98; *PPARA*, 2406, 97; *RASSF6*, 4152, 98; *RBBP4*, 4019, 97; *RBBP4*, 4236, 95; *RBBP5*, 3971, 95; 4019, 97; *RBL1*, 3669,97; *RPS6KA6*, 7136, 100; *RPS6KA6*, 7268, 97; *SMAD5*, 3147, 95; *SMYD4*, 2662, 98; *SMYD4*, 2961, 97; *SOX7*, 1976, 98; *SPN*, 3917, 95; *SPN*, 5287, 100; *SPN*, 6018, 95; *SPN*, 6633, 95; *STAT3*, 3131, 98; *TBRG1*, 3312, 98; *TCEB1*, 1964, 100; *TCEB1*, 2100, 95; *TNFSF10*, 1583, 95; *TNFRSF10A*, 1621, 100; *VHL*, 2989, 98; *VHL*, 3764, 100; *VHL*, 3898, 100; *VPS53*, 3967, 95; *VPS53*, 5126, 95; *VPS53*, 5684, 98; *XAF1*, 2751, 97; *ZC3H12D*, 2812, 100.
miR-5095: **3'UTR:** *CD82*, 1414, 98; *CREB1*, 2791, 95; *CRK*, 2123, 95; *ERAP2*, 3620, 98; *IKZF3*, 6766, 95; *IL10*, 1210, 98; *IL17RD*, 8005, 95; *IRF1*, 2229, 95; *IRF1*, 2653, 95; *KIAA0101*, 1204, 95; *MTHFR*, 6855, 95; *NEK8*, 2411, 95; *PARK2*, 3723, 95; *RBBP4*, 4230, 100; *SOX7*, 1970, 95; *SPN*, 3911, 95; *TBRG1*, 3306, 95; *VPS53*, 5678, 98.
miR-5096: **3'UTR:** *ARLI1*, 1534, 98; *BRCA1*, 6486, 98; *C12orf5*, 1841, 98; *C12orf5*, 6427, 98; *FOXO3*, 6038, 97; *IKZF3*, 3315, 97; *IKZF3*, 5465, 97; *IKZF3*, 6846, 97; *IL17RD*, 8085, 100; *IRF1*, 2597,98; *KIF1B*, 9489, 98; *LIMD1*,5837, 100; *PPP2R1B*, 3054, 100; *RASSF6*,4226, 98; *RBL1*, 3609, 97; *RPS6KA6*, 7209, 97; *SLC4A1*, 4269, 98; *SMYD4*, 2736, 98; *SPN*, 6093, 100; *SPN*, 6702, 98; *VPS53*, 6331, 97; *ZC3H12D*, 2886, 98.
miR-5585-3p: **3'UTR:** *ARLI1*, 1598, 95; *ATM*, 9950, 95; *BRCA1*, 6554, 95; *ERAP2*, 3767, 95; *IRF1*, 2800, 95; *KIAA0101*, 1351, 95; *MDM4*, 4041, 97; *MTAP*, 2431, 98; *MTHFR*, 7003, 95; *NEK8*, 2559, 95; *PPP2R1B*, 3124, 98; *RBBP4*, 4376, 95; *STAT3*, 3268,95; *TBRG1*, 3443, 95; *VHL*, 4041, 97; *ZC3H12D*, 2955, 98.

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The degree of homology of the nucleotide sequences containing the miR-619-5p, miR-5096, miR-5095 and miR-5585-3p binding sites is high. These areas containe binding sites for miRNAs other than the studied umiRNAs. Other miRNA binding sites are not present in all genes, and these binding sites have lower affinities (data not shown). It is possible that there are conserved domains in the nucleotide sequences of mRNAs.

D. Variability in the arrangement of umiRNA binding site locations

The miR-619-5p binding site is located at a distance of 6 nucleotides downstream of the miR-5095 site in the majority of genes containing arranged umiRNA sites. However, in another group of mRNAs, the beginnings of the miR-619-5p binding sites are located at distances of 7 nucleotides upstream of the miR-5585-3p binding sites Fig. 3. There is another group of genes in which the mir-619-5p binding sites are downstream of the miR-5095 sites and upstream of the miR-5585-3p sites. The distances between the positions of the two miR-619-5p binding sites in the mRNAs of these genes are constant at 112 nt. The nucleotide sequences of the mRNAs with miR-619-5p, miR-5095 and miR-5585-3p binding sites are highly homologous, which testifies to the strength of the selection pressure on these nucleotide sequences.

E. Connection of umiRNAs with mRNA of tumor suppressor genes

The miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites with mRNA of 455 tumor suppressor genes participating in breast cancer and lung cancer were predicted (Table 1). Free energy of the umiRNA:mRNA is equaled 95% - 100%. In this case, the umiRNAs have similar features as well as siRNAs, that is lead to mRNA degradation. Therefore, it is possible to assume that suppression of target gene expression via miR-619-5p, miR-5095, miR-5096 and miR-5585-3p can lead to tumorigenesis. For example, Reshmi et al. [21] established that in normal level of miR-5095 and miR-5096 concentration is much less, than in cancer cells.

IV. Discussion

In this study, it was established that miR-619-5p, miR-5095, miR-5096 and miR-5585 can bind to the mRNAs of the 1215, 832, 725 and 655 genes, respectively. The nucleotide sequences of these miRNAs form hydrogen bonds with the mRNAs, and the free energy of these bonds is equal to or greater than 90% of the maximum possible free energy. The miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites are generally located in the 3'UTRs of target genes. Obviously, maintaining nucleotide sequences for the binding site of one umiRNA in the CDSs of such a high number of genes is complicated. Approximately 180 nucleotides of the mRNAs of many target genes containing binding sites for the miRNAs and the placement of these nucleotide sequences for the binding sites of two and more miRNA are highly conserved. The miRNA binding sites are located in the 5'UTRs of some genes; however, the number of such genes is small.

The mRNAs of some genes have multiple miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites. It is possible that the identification of large number miRNA binding sites in the mRNAs of some genes will be necessary for reliable control of gene expression.

Some groups of genes with different patterns of localization of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites were established in this work. First, the strict order of the binding sites was established based on the origin of the general nucleotide sequences (these data are not described here). Secondly, it is necessary to control the expression of the corresponding gene complexes that are functionally associated with the miRNAs.

The detection of a large number miRNA binding sites in the mRNAs of the genes studied here presumably indicates to new functional opportunities. It is possible that these umiRNAs are coordinators of gene expression that participate in many major biological processes. The influences of miRNAs on the expression of genes that code for transcription factors [10, 11] and proteins that participate in the cell cycle [3, 12-14], apoptosis [4, 15-17], stress responses, etc. [18] have previously been shown. If these proteins define the limiting stages of multistage processes, these proteins will need to be controlled to manage multistage processes. Specifically, an appreciable portion of the targets of miR-5095 and miR-5096 are genes for transcription factors. One or several umiRNAs regulating the expression of several hundreds of genes will create a system of interconnected processes in cells and organisms. Such role for these miRNAs is quite possible because these miRNAs circulate in the blood and nearly all cells of an organism are available to them [19, 20]. The normal functioning of the system of the interconnected processes in which the umiRNAs participate is maintained because insignificant deviations in the expression of protein-coding genes or typical miRNAs cannot significantly alter the function of the system. On the other hand, the system is also vulnerable because it can be broken by changes in umiRNA expression. For example, it have been established that the basal expression of miR-5096 in normal cells is low [21], but, in tumor cells, the expression of miR-5096 repeatedly elevated. These elevations result in suppression of the expression of many target genes and unbalanced and uncontrollable cell functioning. Some interconnected umiRNAs have to function in the cell and the organism to minimize the consequences of such events. These interactions can be carried out via the general target genes of miRNAs. Thus, the loss or augmentation of the influence of one component (miRNA) in the regulatory system will have less influence on the functioning of the entire system.

The present results provide the basis to study the systemic roles of unique and typical miRNAs in the regulation of gene expression in human cells based on new ideas of miRNA properties.

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