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Binding sites of the miR-1273 family, miR-1285-3p and miR-5684 in human mRNAs

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***Abstract*—**The search of 2578 miRNA binding sites in 13000 mRNAs of human genes using the MirTarget program has been completed. For the binding sites of the miR-1273 family, miR-1285-3p and miR-5684, the hybridization free energies of the bonds are equal to or greater than 90% of the maximum value free energy. Approximately 90-nucleotide regions of mRNAs containing binding sites for the miR-1273 family, miR-1285-3p and miR-5684 were revealed. These regions are located in the 5'UTRs, CDSs and 3'UTRs of the mRNAs and containe between two and six arranged miRNA binding sites. The miR-1273g-3p, miR-1273a, miR-1273c, miR-1285-3p and miR-5684 binding sites are grouped together and located ahead of another group consisting of miR-1273f, miR-1273d, miR-1273e miR-1273g-5p and miR-1273h-5p binding sites. The role of these miRNAs in the regulation of gene expression and its participation in different biological processes will be discussed.

***Keywords*—**apoptosis, cancer, cell cycle, miRNA, mRNA.

I. Introduction

THERE are many unresolved problems in studying the biological role of microRNAs (miRNAs), despite numerous publications in this field [1]. Non-protein-coding miRNAs regulate the expression of protein-coding genes at the post-transcription level [2]. miRNAs participate directly or indirectly in nearly all stages of metazoan development [3]. There are different programs of miRNA prediction of target genes, but many of them generate a large number of false-positive results [4]. This complicates the understanding of connections among miRNAs and target genes participating in different metabolic processes. For a long time, it has been proposed that binding sites can be located only in 3'UTRs [5]; however, several studies have recently reported binding sites in 5'UTRs and CDSs of mRNAs [6, 7]. The largest type of target genes includes genes participating in the development of cancer and other diseases. miRNAs regulate the expression of different genes and participate in many pathological processes [8-10], including carcinogenesis [11-17]. Changes in the concentration of miRNAs were observed in the development of breast cancer [11], lung cancer [12], gastrointestinal cancer [13, 14] and other cancer types [15-17]. Fold changes in miRNA expression have been revealed in the majority of the studies [11-20], although their target genes have been insufficiently investigated. Thus, it is necessary to establish the properties of miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, miR-1273g-3p, miR-1273g-5p, miR-1273h-3p, miR-1273h-5p, miR-1285-3p and miR-5684 binding sites in mRNAs of genes participating in the development of cancer.

II. Material and Methods

The human gene mRNAs were obtained from GenBank (http://www.ncbi.nlm.nih.gov) using Lextractor002 script (http://sites.google.com/site/malaheenee/software), which was written in our laboratory. The miRNA sequences and information regarding their origin was obtained from the miRBase database (http://mirbase.org). The search for target genes of miRNAs was achieved using the MirTarget program, which was written 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'UTRs of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between the miRNAs and mRNAs. The $\Delta G/\Delta G_m$ ratio (%) was determined for each site (ΔG_m equals the free energy of a miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on the mRNAs had $\Delta G/\Delta G_m$

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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. The MirTarget program calculated the interactions between the nucleotides of the miRNAs and those of the mRNA target gene. This program identified hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C [21]. The distance between A-C was same as that as between G-C, A-U, and G-U [22] nucleotides. The number of hydrogen bonds between G-C, A-U, G-U and A-C and the value of their free energy of binding is equal to 3, 2, 1 and 1, respectively.

III. Results

A. Characteristics of the arranged binding sites in 3'UTRs

In this study, 2578 miRNA binding sites in 13000 mRNAs of human genes were identified. The binding sites with equal to or greater than 90% of the maximum free energy were selected and were analyzed. It was revealed that binding sites of the miR-1273 family, miR-1285-3p and miR-5684 are localized side by side in many mRNAs. Therefore we started study them in detail. miR-1273c, miR-1273g-3p and miR-1273f have several hundreds of target genes and are unique miRNAs (umiRNAs) in comparison with others. The mRNAs of genes that have binding sites with two or more studied miRNAs were analyzed. The data regarding the quantity of genes and binding sites of the selected miRNAs are presented in Table 1.

Table 1 Number target genes and binding sites of miR-1273 family, miR-1285-3p and miR-5684 in mRNA of human genes.

miRNA	Number genes	Number sites	Number sites in 5'UTR	Number sites in CDS	Number sites in 3'UTR
miR-1273a	145	151	6	2	143
miR-1273c	80	81	7	2	72
miR-1273d	102	104	5	6	93
miR-1273e	399	431	18	9	404
miR-1273f	654	742	30	26	686
miR-1273g-3p	809	945	42	28	875
miR-1273g-5p	32	32	2	5	25
miR-1273h-5p	98	99	6	8	85
miR-1285-3p	127	130	8	2	120
miR-5684	189	200	9	7	184

The miRNAs form two groups with arranged binding sites in 90-nucleotide regions of mRNAs (Fig. 1, A). The arranged binding sites are binding sites of different miRNAs, which have overlapping nucleotide sequences or are located within the same distance in different mRNAs of genes. The region of the nucleotide sequence in the 3'UTRs of the *SLC36A2* gene containing arranged binding sites was selected for comparison with other mRNAs of target genes. The miR-1273g-3p group includes miR-1273a, miR-1273c, miR-1285-3p, and miR-5684 (Fig. 1, A).

The distance between the end of the miR-1273g-3p binding site and the beginning of the miR-1273f binding site is equal to 12 nucleotides (Fig. 1, A). The miR-1273f group includes miR-1273d, miR-1273e, miR-1273g-5p, miR-1273h-5p. There are no arranged miR-1273h-3p binding sites in 90-nucleotide regions of mRNAs with a $\Delta G/\Delta G_m$ ratio equal or greater than 90%. In this study, 2687 arranged binding sites are located in the 90-nucleotide parts of 865 3'UTRs. Six out of ten examined miRNAs are the maximum number of arranged binding sites in two miRNA groups. The aligned nucleotide sequences of the 3'UTR regions with five or six arranged miRNA binding sites are represented in the schematic shown in Fig. 1, A. The nucleotide sequences of the revealed sites of the target genes are highly homologous, which confirms their general origin in the studied genes. The 3'UTRs of 26 genes exhibited arranged miRNA binding sites with a $\Delta G/\Delta G_m$ ratio greater than 96%. Each mRNA of the *CHMP1B, MCTS1, OPRK1, OR7D2, SLC36A2, TAT* and *ZNF527* genes has one binding site with a $\Delta G/\Delta G_m$ ratio greater than 98%. The high $\Delta G/\Delta G_m$ ratio for binding sites of the miRNAs with the RNAs showed that the expression of these genes may be strongly suppressed in conditions of same concentration of mRNAs and miRNAs.

B. Characteristics of the arranged binding sites in 5'UTRs

In this study, 133 arranged binding sites are revealed in the 90-nucleotide parts of 53 5'UTRs (Fig. 1, B). The miRNA binding sites corresponded to one of two groups and are localized in the corresponding arranged nucleotide sequences as well as in 3'UTRs. The *LGMN* mRNA has miR-1273e, miR-1273f, miR-1273g-3p, miR-1285-3p and miR-5684 binding sites. The *KCNJ11, RGS12, TMC1* and *ZNF527* mRNAs have four arranged miRNA sites. In addition, 17 mRNAs have three binding sites, and 30 mRNAs have two sites. Moreover, the 5'UTRs of 12 genes have miRNA binding sites with a ratio $\Delta G/\Delta G_m$ greater than 96%. The mRNA of the *CD59, FAIM* and *TMC1* genes have one binding site with a $\Delta G/\Delta G_m$ ratio greater than 98%. Thus, the expression of these genes will be strongly suppressed in conditions of comparable concentrations of mRNAs and miRNAs. The homology degree of nucleotide sequences in 5'UTR regions of the genes is high, which confirms the important role of these binding sites in the biological function of these genes (Fig. 1, B). The binding sites of the studied miRNAs are distributed in the 5'UTR as well as in 3'UTR; thus, it is possible that these sites have one general precursor.

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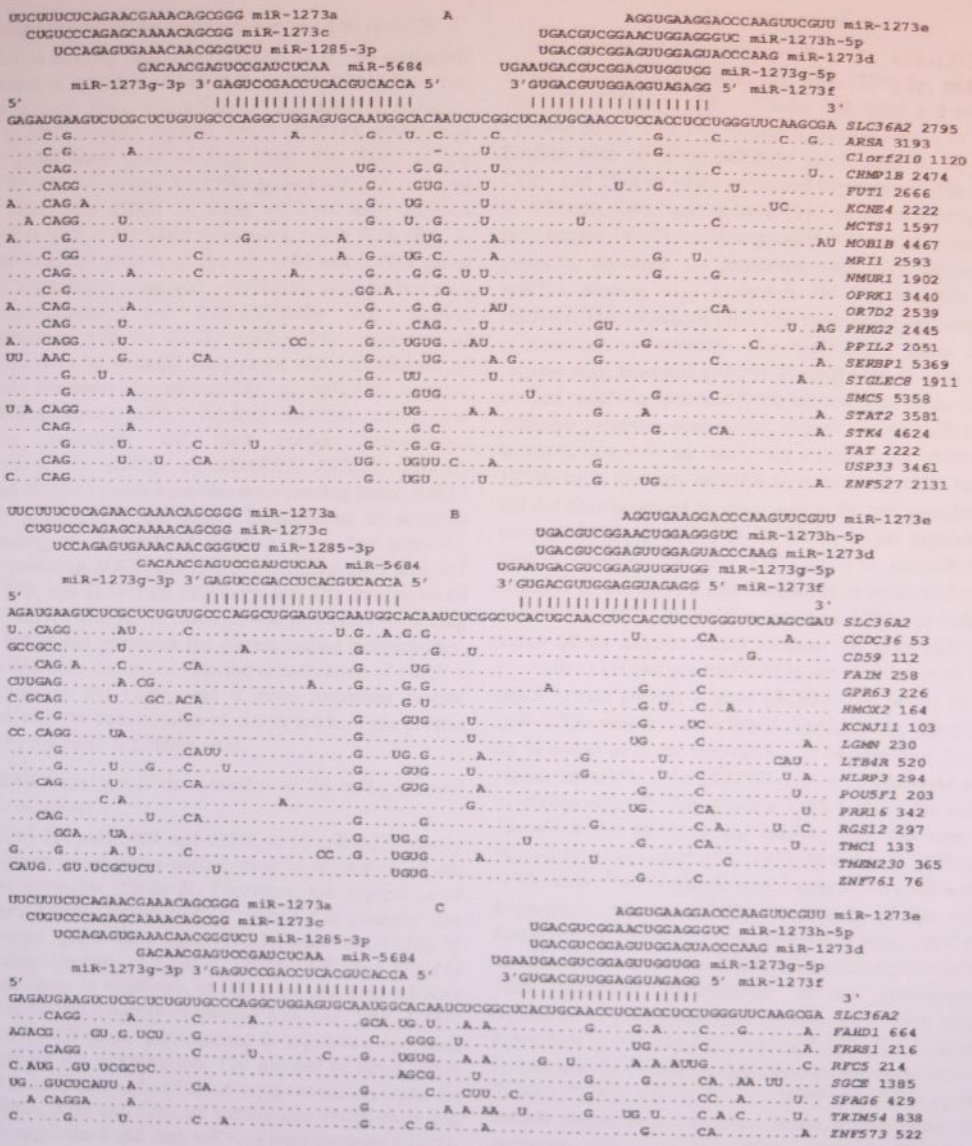

Fig. 1 the localizations of the aligned nucleotide sequences of five and six arranged miR-1273 family, miR-1285-3p and miR-5684 binding sites in the 3'UTR (A), 5'UTR (B) and CDS (C).

*Note: | symbol shows the presence of hydrogen bond between the miRNA and mRNA nucleotides; * after the name of the gene given the start of the binding site of miR-1273g-3p.*

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C. Characteristics of the arranged binding sites in CDSs

95 arranged binding sites were revealed in the 90-nucleotide parts located in 42 CDSs. The CDSs of 26 mRNAs have two arranged binding sites. The CDSs of the *ARGFX, FAHD1, FRRS1, GINS3, MKNK1, PRR16, RFC5, RNF135, SPAG6* and *ZNF573* genes have three binding sites. The CDSs of the *SGCE, TRIM54, NEK4* and *ADARB1* genes have four arranged binding sites. The CDSs of eight mRNAs have miRNA binding sites with a ΔG/ΔGm ratio greater than 96% (Fig. 1, C). In addition, the mRNAs of the *ADARB1* and *GINS3* genes have miRNA binding sites with a ΔG/ΔGm ratio greater than 98%. Furthermore, the miR-1273h-5p has perfect complementation with the *GINS3* mRNA. The expression of these genes may be strongly suppressed under conditions of comparable concentrations of mRNAs and miRNAs. Regions of the CDS containing miRNA binding sites have homologous nucleotide sequences, and they encode homologous oligopeptides. The mRNA of *ADARB1, FAHD1, FRRS1, SGCE* and *ZNF573* genes have one open reading frame (ORF) and their polypeptides are highly homologous in domains corresponding to the miRNA binding sites. For example, regions containing SGCE and ZNF573 proteins have identical AQAGVQW and SLQPPPP oligopeptides. The translation of *SPAG6, TRIM54* and *RFC5* mRNAs was processed according to other ORFs and generates the corresponding polypeptides. The obtained data confirmed that the conservation of miRNA binding sites in the CDSs was a more important feature compared with the conservation of amino acids in the protein encoded by the miRNA binding sites.

D. miRNA target genes associated with the cell cycle and apoptosis at cancer

miR-1285-3p, miR-5684 and miRNAs of miR-1273 family have common target genes participating in regulation of cell cycle and apoptosis (Table 2). Therefore, the expression of these genes is under strong miRNA control. For example, five of six target genes have miR-1285-3p and miR-1273g-3p binding sites. Proteins of *CLSPN, MDM2, NF2* and *TRIM13* genes participate in regulation of cell cycle and their mRNAs are targets for the majority of studied miRNAs. *SPN, CASP, STK4* and *DFFB* genes participating in regulation apoptosis are targets for the miRNAs too. The number of target genes involved in apoptosis and cell cycle is approximately equal for each miRNA. *ATM* and *VHL* are participants of cell cycle and apoptosis regulations and their mRNAs have common miRNA binding sites (Table 2). Therefore, these genes more than others can define speed ratio between cell cycle and apoptosis.

IV. Discussion

As a result of our study, miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, miR-1273g-3p, miR-1273g-5p, miR-1273h-3p, miR-1273h-5p miR-5684 and miR-1285-3p have from 32 to 945 arranged binding sites. The arranged binding sites of these miRNAs are located in the 5′UTRs, CDSs and 3′UTRs of all examined target genes. The miRNAs in different combinations have 2915 arranged binding sites in the examined mRNAs. Management of the expression of target genes is achieved via important regulatory interactions of miRNA binding sites located in the 90-nucleotide parts of mRNAs. The examined miRNAs have a different origin and it is necessary to establish why their target genes contained regions with arranged miRNA binding sites. Precursors of the studied miRNAs (pre-miRNAs) are encoded in the introns of different protein-coding genes, except the pre-miR-1273h-3p, which is coded in an intergenic sequence. In addition, miR-1273h-3p is the only miRNA from the miR-1273 family that did not have arranged binding sites in the two revealed groups. pre-miR-1273a is coded in an intron of the regulator of G-protein signaling 22 gene (*RGS22*). The pre-miR-1273c is coded in an intron of T cell lymphoma invasion and metastasis 2 gene (*TIAM2*). In addition pre-miR-1273d is coded in an intron of the kinesin family member 1B gene (*KIF1B*). The pre-miR-1273f and pre-miR-1273g are encoded in an intron of sterol carrier protein 2 gene (*SCP2*). The origin of the pre-miR-1273e was not established. Furthermore, pre-miR-1285-3p is coded as a pre-mir-1285-1 in an intron of the ankyrin repeat containing gene (*KRIT1*) and in an intergenic sequence of chromosome 2. The pre-mir-1285-1 codes for miR-1285-5p, which did not have any binding sites that were closely located to the arranged miR-1273g-3p and miR-1273f groups.

The miR-5684 is coded in an intron of the hook homolog 2 *Drosophila* gene (*HOOK2*). Precursors of the miR-1273 families were found in a large number of introns and exons in lncRNAs, protein-coding RNAs and repeating sequences [23]. Thus, miRNAs participating in the post-transcription regulation of gene expression are intronic in most cases. The expression of 949 target genes of miR-1273 family, miR-1285-3p and miR-5684 may be dependent on the expression of host *RGS22, TIAM2, KIF1B, KRIT1, SCP2* and *HOOK2* genes that encode intronic miRNAs. It is possible, if the intronic miRNAs coexpress with their host genes. Because proteins of the studied target genes participate in different metabolic processes, the estimated regulation of their expression via the interaction with miRNAs has an important biological value and is not casual.

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Table 2 miRNA target genes participating in cell cycle and apoptosis at cancer

miRNA	Cell cycle genes	Apoptosis genes
mir-1273a	*ATM*, 11054, 90; *EIF2AK2*, 2445, 90; *NF2*, 4324, 90; *TRIM13*, 2364, 92.	*ATM*, 11054, 90; *CASP2*, 2804, 92; *EIF2AK2*, 2445, 90; *SPN*, 1484, 90.
mir-1273c	*LZTS1*, 3474, 95; *RBBP4*, 6770, 91; *TP53*, 2297, 91.	*CASP2*, 2806, 93; *SPN*, 1484, 91; *TP53*, 2297, 91.
mir-1273d		*SPN*, 4944, 91.
miR-1273e	*ATM*, 11119, 93; *CLSPN*, 5984, 93; *FLCN*, 3179, 93; *MDM2*, 2521, 93; *NF2*, 3646, 95; *NF2*, 5139, 91.	*ATM*, 11119, 93; *SPN*, 5693, 91; *STK4*, 3865, 93; *TNFRSF10B*, 3661, 95.
mir-1273f	*CLSPN*, 5974, 92; *E2F2*, 4161, 92, *FLCN*, 3169, 92; *HECA*, 3460, 92; *KRAS*, 3209, 100; *MCC*, 5239, 92; *MDM2*, 6772, 92; *NF2*, 3636, 98; *NF2*, 4379, 96; *NF2*, 5129, 94; *RBBP4*, 5439, 92; *SASH1*, 5544, 92; *TRIM13*, 2419, 92; *VHL*, 1857, 92.	*CTSB*, 2449, 92; *DFFB*, 2243, 92; *SPN*, 1536, 92; *STK4*, 3855, 96; *STK4*, 4657, 96; *TP63*, 1695, 96; *VHL*, 1857, 92.
mir-1273g-3p	*ATM*, 11076, 96; *CLSPN*, 4911, 91; *E2F2*, 4128, 96; *FLCN*, 3136, 93; *HECA*, 3427, 95; *KRAS*, 3176, 93; *LZTS1*, 3326, 96; *MCC*, 5205, 91; *MDM2*, 2117, 96; *MDM2*, 2486, 91; *MDM2*, 6739, 96; *NF2*, 4346, 91; *NF2*, 5096, 93; *PPM1D*, 3509, 95; *RASSF2*, 4504, 98; *SASH1*, 5511, 93; *TADA3*, 1927, 96; *TP53*, 2317, 91; *TRIM13*, 2386, 95; *VHL*, 3423, 98; *VHL*, 1824, 96.	*AIFM2*, 2010, 96; *APAF1*, 4933, 91; *APAF1*, 5231, 96; *ATM*, 11076, 96; *CASP10*, 2589, 93; *CASP2*, 2826, 93; *CASP8*, 1256, 96; *CFLAR*, 3667, 96; *CTSB*, 2416, 96; *DFFB*, 1566, 93; *DFFB*, 2210, 96; *SPN*, 5650, 93; *STK4*, 4624, 96; *TNFRSF10B*, 2050, 95; *TNFRSF10B*, 3618, 93; *TP53*, 3217, 91; *VHL*, 1824, 96; *VHL*, 3423, 98.
mir-1273h-3p		*IRAK1*, 2896, 93.
mir-1273h-5p	*TP53*, 2351, 91; *TRIM13*, 2420, 93.	*CASP10*, 2234, 91; *CASP10*, 2623, 100; *TP53*, 2351, 91.
mir-1285-3p	*AURKA*, 352, 91; *EIF2AK2*, 2450, 91; *MAPK1*, 3078, 93; *MDM2*, 3218, 91; *RBBP4*, 6773, 93; *TP53*, 2301, 95; *VHL*, 3407, 91.	*CASP10*, 2357, 91; *DFFB*, 2193, 91; *EIF2AK2*, 2450, 91; *STK4*, 4607, 93; *TP53*, 2301, 95; *VHL*, 3406, 91.
mir-1285-5p	*BRCA2*, 10821, 91; *CLSPN*, 7586, 91; *GTSE1*, 2757, 91; *IL2RA*, 2322, 92; *IRF1*, 2899, 94; *TADA3*, 2317, 91; *VHL*, 4140, 92; *VHL*, 4291, 92.	*CFLAR*, 5666, 91; *CFLAR*, 6570, 91; *DFFA*, 2039, 91; *DFFA*, 2985, 96; *DNASE*, 1325, 91; *IL2RA*, 2322, 91; *NAIP*, 6164, 91; *SPN*, 2752, 93; *SPN*, 5497, 94; *SPN*, 6578, 91; *VHL*, 4140, 93; *VHL*, 4291, 93.
mir-5684	*CLSPN*, 6633, 90; *E2F2*, 4122, 92; *EIF2AK2*, 2461, 92; *MDM2*, 2480, 90; *MDM2*, 6733, 90; *NF2*, 4340, 90; *TRIM13*, 2380, 90; *VHL*, 3417, 92.	*CASP8*, 1850, 90; *CFLAR*, 3661, 90; *DFFB*, 2204, 92; *EIF2AK2*, 2461, 92; *FOXO1*, 461, 90; *STK4*, 4618, 92; *VHL*, 3417, 92.

*Note. * given gene name, the start of the binding site of miRNA (nt) and the value $\Delta G/\Delta G_m$ ratio (%).*

The proteins of the majority of studied target genes participate in different cellular processes, including apoptosis and cell cycle. In addition, they define the development of many pathologies. Other genes are oncogenes, tumor

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suppressors, and transcription factors, among others. The genes were selected according to their participation in the development of diseases, including lung cancer, breast cancer, gastrointestinal cancer and cancers of other tissues. Thus, the change in the regulation of target gene expression via these miRNAs might be the reason underlying many diseases. If the concentration of miR-1273 family, miR-1285-3p and miR-5684 is lower, than their target mRNAs, these miRNAs will poorly suppress cell cycle and apoptosis. There are different speed ratios between apoptosis and cell cycle in the cases when miRNAs super express in comparison with mRNAs of the target genes. It was shown that concentration of miR-1273 in tumour cells was significant increased [24]. Unfortunately, there is not enough experimental studies proved the participation of miR-1273 family, miR-1285-3p and miR-5684 in tumorigenesis. However, all target genes of studied miRNAs participate in development of breast cancer, lung cancer and etc. [24].

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