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Intragenic MicroRNAs Binding Sites in MRNAs of Genes Involved in Carcinogenesis

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***Abstract*—**MiRNAs participate in gene regulation of translation. Some studies have investigated the interactions between genes and intragenic miRNAs. It is important to study the miRNA binding sites of genes involved in carcinogenesis. RNAHybrid 2.1 and E-RNAhybrid programmes were used to compute the hybridization free energy of miRNA binding sites. Of these 54 mRNAs, 22.6%, 37.7%, and 39.7% of miRNA binding sites were present in the 5′UTRs, CDSs, and 3′UTRs, respectively. The density of the binding sites for miRNAs in the 5′UTR ranged from 1.6 to 43.2 times and from 1.8 to 8.0 times greater than in the CDS and 3′UTR, respectively. Three types of miRNA interactions with mRNAs have been revealed: 5′-dominant canonical, 3′-compensatory, and complementary binding sites. MiRNAs regulate gene expression, and information on the interactions between miRNAs and mRNAs could be useful in molecular medicine. We recommend that newly described sites undergo validation by experimental investigation.

***Keywords*—**Exon, intron, miRNA, oncogene.

I. Introduction

MicroRNAs (miRNAs) are noncoding RNAs of 22 nucleotides in length that regulate gene translation by binding to target mRNAs [1]. MiRNA can be localised in the intragenic or intergenic regions [2]. Many intragenic miRNAs are localised within the intronic regions of their host genes and others are found within exonic regions [3]. Intronic miRNAs (in-miRNAs) can be located in introns of protein coding genes or in long noncoding RNAs. Some miRNAs are found on the borders of exons and introns [4]. MiRNAs located within the exons (ex-miRNAs) of annotated genes are, usually noncoding transcripts [5]. It is thought that one-third of human genes are regulated by miRNAs [6]. Computational and experimental investigations have established that a single miRNA can regulate the expression of different genes, and the expression of a single gene can be controlled by some miRNAs [7].

More than 50% of miRNA genes are located in fragile sites or in cancer-associated genomic regions [8]. Some miRNAs regulate apoptosis and cell proliferation and can act as oncogenes or tumour suppressors [9]. Changes of miRNA expression have been shown in development of certain cancers, such as breast [10]-[12], oesophageal [13], stomach [14], colon [15], colorectal [16], and lung [17] cancer. Expression of miR-21 was found to be deregulated in most cancers, and miR-21 was therefore classified as an onco-miR [18].

Previously, we carried out searches to identify other such oncomiRs. MiRNAs can act as biomarkers for cancer detection due to their stability in serum and their different expression levels in many different cancers [19], [20]. Some intronic miRNAs can functionally cooperate with their host genes and can silence genes that are functionally antagonistic to their host genes. Many miRNAs are transcribed from their host genes, but approximately half map within the introns of these genes, the significance of which remains hard to understand [21]. Understanding the mechanisms of miRNA regulation is very important for developing novel therapeutic strategies for human disease.

Many researchers have suggested that miRNAs bind with the 3′UTR of mRNAs [22]-[26]; however, some studies have described miRNAs that bind to the 5′-untranslated regions (5′UTRs) and coding sequences (CDSs) of mRNAs [27]-[31]. The data better understanding of miRNA binding sites could shed light on the interactions between miRNAs and mRNAs. The effects of many miRNAs on the expression of mRNAs remain unknown [32]. Previously, it has been shown that 111 intergenic miRNAs bind to 54 mRNAs of genes involved in carcinogenesis. The RNAHybrid 2.1 programme was used to predict strong miRNA binding sites ($p < 0.0005$) in target mRNAs. Among 54 investigated genes, 47 genes were targets for ig-miRNAs [33].

In this study, the binding sites of 686 intronic and 49 exonic miRNAs were investigated within the 5′UTRs, CDSs, and 3′UTRs of mRNAs for 54 human oncogenes. These genes participated in the development of breast, oesophageal, stomach, colon, and colorectal cancer, among others. The aims of our work are as follows: (a) to reveal the characteristics of interactions of intragenic miRNAs with different regions of mRNAs; (b) to identify miRNAs binding with a large number mRNAs of the studied genes; and (c) to establish 3 types of nucleotide interactions during the formation of complexes of intronic and exonic miRNAs with mRNAs.

II. Materials and Methods

Nucleotide sequences of mRNAs of 54 human genes (*Homo sapiens*, Genome build 37.2.) were obtained from Genbank (http://www.ncbi.nlm.nih.gov). These genes were tumour suppressor genes and oncogenes according to a literature review [31]. Nucleotide sequences of in-miRNAs and ex-

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miRNAs were obtained from the miRBase database (http://www.mirbase.org). The programme Finder 2.2 (http://sites.google.com/site/malaheenee/software/) was used to find in-miRNAs and ex-miRNAs. All miRNA:mRNA pairs were found using the RNAHybrid 2.1 programme, which provides the position of potential binding sites, the free energy value (ΔG) of hybridization, and interaction schemes.

The selection criteria for miRNA sites were as follows: (a) to select only those binding sites of miRNAs that have no more than one internal loop whose size is less than or equal to a predetermined maximal size, and no more than one bulge loop whose size is less than or equal to a predetermined maximal size; and (b) to select only 1 miRNA site with the lowest energy of hybridization (highest negative energy). The E-RNAhybrid programme was used to compute the ratio $\Delta G/\Delta G_m$, (http://sites.google.com/site/malaheenee/software/) equalising coefficient, *p*-value, and type of corresponding region (5′UTR, CDS, or 3′UTR) where the miRNA binds. To achieve this, a quantitative criterion was defined. The ratio the $\Delta G/\Delta G_m$ value (%), where ΔG_m equals the binding energy for miRNA with a perfectly complementary nucleotide sequence was calculated.

The number of binding sites in the 5′UTR, CDS, and 3′UTR was computed as the number of sites divided by the nucleotide length of the region and multiplied by 10^3 (s/l), which was calculated per 1000 nucleotides. The significance degree (*p*-value) was estimated by relying on the ΔG value and its standard deviation. The threshold significance was set to $p < 0.0005$.

The binding energy of binding site paired miRNA:mRNA depends on the number of complementary nucleotide pairs. Thus, miRNA length was used in the selection of miRNA binding sites. MiRNA length varies from 16 to 27 nucleotides. If the miRNA length is shorter, the probability of receiving false positive results is higher, so an equalising coefficient was entered. According to studies of the distribution of miRNA lengths, the majority of miRNAs have 21 nucleotides, and this was taken as a basic value. Values of equalising coefficients were calculated in the search of all studied miRNA sites on random nucleotide sequences that have the average length of mRNAs. If the miRNA length was more than 21 nucleotides, the value of threshold significance (p) was divided by the equalising coefficient, thus increasing reliability. In cases in which the miRNA length was less than 21 nucleotides, the value of threshold significance (p) was multiplied by the corresponding coefficient, thus reducing reliability.

Supplementary Tables I-IV are given on site https://sites.google.com/site/malaheenee/software/other.

III. Results and Discussion

MiRNA binding sites of 54 mRNAs with intragenic miRNAs have been investigated (Tables I, II). To find locations of miRNA in introns or exons, 1424 pre-miRNAs were studied using the programme Finder 1.2. Thirteen pre-miRNAs in 3′UTRs, 76 pre-miRNAs in introns of 3′UTRs, 19 pre-miRNAs in 5′UTRs, 140 pre-miRNAs in introns of 5′UTRs, 18 pre-miRNAs in exons of pre-miRNAs, 460 pre-miRNAs in introns of pre-mRNAs, and 21 pre-miRNAs on boundaries of exons and introns were found. Intragenic miRNAs were conditionally divided into 2 groups: 49 exonic and 686 intronic miRNAs. Some miRNAs had a different origin (within exons and introns of mRNA) that depended on the isoform of the gene which encoded its pre-miRNA.

Interactions between 686 intronic miRNAs and the mRNAs of 54 protein-coding human genes were examined. Forty-five out of the 54 genes were targets of in-miRNAs, and 9 genes (*ABCB1*, *ABCG2*, *ADAM29*, *BAX*, *MLH1*, *MLH3*, *PIK3CA*, *PROM1*, and *TNFSF10*) have no binding sites for in-miRNAs according to established criteria ($p < 0.0005$). Table I shows the sites of in-miRNA interactions with mRNAs for which the ΔG/ΔGm value was greater than or equal to 75%. Only 88 of 686 in-miRNAs strongly regulated the 45 genes (Supplementary Table I).

Studied in-miRNAs differed significantly in terms of the number of connections with mRNAs. For example, *EP300*, *FLCN*, *MTHFR*, *PTEN*, and *SMAD4* mRNAs bound to 6–8 in-miRNAs. These numbers were greater than the average number of miRNAs associating with a single mRNA, which was 2.0 for the 45 mRNAs. Only 1 miRNA out of 88 (miR-574-5p) had 2 interaction sites in a single mRNA (the *EP300* mRNA). There was no significant correlation between the length of the target mRNA and the number of miRNA binding sites (correlation coefficient, 0.023). Of the in-miRNA binding sites, 21.1%, 37.5%, and 41.4% were located within the 5′UTRs, CDSs, and 3′UTRs of the 45 mRNAs, respectively (Table III). The densities of the binding sites varied from 0.09 s/l for *APC3* mRNA to 4.12 s/l for *BAD* mRNA and were an average of 0.76 s/l for the 45 mRNAs (Supplementary Table II). *BAD* mRNA had the highest interaction site density, which demonstrated the important role of miRNAs in the regulation of *BAD* gene expression.

The 5′UTRs, CDSs, and 3′UTRs of these mRNAs differed in their miRNA binding abilities. *PTEN* and *GNAS* mRNAs had 6 and 4 miRNA binding sites in their 5′UTRs, respectively. The mRNA of *SMAD4* bound to 8 miRNAs in its 3′UTR. The average densities of the binding sites for miRNAs in the 5′UTRs, CDSs, and 3′UTRs of the 45 mRNAs were 1.91 s/l, 0.50 s/l, and 0.63 s/l, respectively. The average binding site density in the 5′UTR was 3.8 times more than that in the CDS and 3.0 times more than that in the 3′UTR. This data demonstrated that miRNAs can bind to mRNAs at the 5′UTR, CDS, and 3′UTR. Intronic miRNA binding sites are presented in Table III. For example, 5′-dominant canonical sites were miR-511 and miR-1913 with *PTEN* mRNA; 3’-compensatory sites were miR-4296 with *MSH6* mRNA, miR-553 with *FLCN* mRNA, and miR-1273f with *MET* mRNA; and the complementary site was miR-593 with *MTHFR* mRNA.

TABLE I
CHARACTERISTICS OF MRNAS BINDING WITH INTRONIC MIRNAS

Gene: in-miRNA, ΔG/ΔGm, %

5'UTR

ALCAM: miR-885-3p, 77.0. ***APC-1:*** miR-942, 80.5. ***AXIN1:*** miR-1268b, 77.4. ***AXIN2:*** miR-3181, 79.8. ***BAD:*** miR-1910, 76.0. ***BRCA2:*** miR-224, 78.0. ***CD44:*** miR-1268b, 81.5. ***CTNNB1:*** miR-1236, 76.5. ***ENG:*** miR-4436b-3p, 75.5. ***GNAS:*** miR-885-3p, 77.4, miR-1268b, 85.1, miR-3178, 88.6, miR-4651, 81.4. ***KRAS:*** miR-1913, 76.0. ***MMP2:*** miR-1913, 81.9, miR-3130-5p, 76.0. ***MYC:*** miR-1268b, 77.0, miR-3196, 80.6. ***PMS1:*** miR-548l, 75.6, miR-3620, 78.0. ***PTEN:*** miR-346, 76.9, miR-511, 83.2, miR-593, 87.9, miR-885-3p, 76.0, miR-1249, 75.7, miR-1913, 88.3. ***PTPN12:*** miR-1238, 79.6.

CDS

ABCC2: miR-618, 75.6. ***ALCAM:*** miR-590-3p, 76.4, miR-4677-5p, 77.3. ***APC-1:*** miR-576-5p, 79.5. ***APC-2*** miR-576-5p, 79.5. ***APC-3:*** miR-576-5p, 79.5. ***AXIN1:*** miR-1203, 77, miR-4524*, 76.3. ***BAD:*** miR-211, 81.1, miR-609, 77.8. ***BRAF:*** miR-149, 74.4. ***BRCA1:*** miR-3121-5p, 75.3, ***BUB1:*** miR-500a*, 77.0, miR-502-3p, 76.0, miR-4287, 80.6, miR-4799-3p, 81.2. ***DLC1:*** miR-3691-5p, 75.4, miR-4506, 79.2. ***ENG:*** miR-1273g, 82.6, miR-2467-5p, 78.8, miR-3621, 77.2. ***EP300:*** miR-511, 76.0, miR-4436b-3p, 82.4, miR-4439, 76.0, miR-4668-3p, 76.0. ***FLCN:*** miR-640, 76.8, miR-1272, 77.9. ***FZD7:*** miR-502-5p, 78.5. ***KIT:*** miR-4748, 76.6. ***MET:*** miR-23b*, 79.4, miR-3657, 80.0. ***MMP2:*** miR-502-5p, 77.0. ***MMP9:*** miR-1236, 76.5. ***MSH2:*** miR-4668-3p, 77.1. ***MSH6:*** miR-4296, 85.0. ***MTHFR:*** miR-593, 87.0. ***MUTYH-α:*** miR-608, 72.6, miR-1273f, 78.1, miR-3115, 80.5. ***MYC:*** miR-4258, 83.0, miR-4726-5p, 75.8. ***PMS1:*** miR-9, 75.6. ***PMS2:*** miR-3682-5p, 77.5. ***PTPN12:*** miR-548j, 80.3. ***SNAI1:*** miR-1224-5p, 80.9. ***TGFBR2:*** miR-511, 76.5. ***VDR:*** miR-2278, 77.0. ***ZEB1:*** miR-548ah , 79.2.

3'UTR

AXIN1: miR-3684, 78.6, ***AXIN2:*** miR-339-5p, 75.5. ***BAD:*** miR-1915, 79. ***BRCA1:*** miR-5095, 79.2. ***BRCA2:*** miR-5095, 76.9. ***CCND1:*** miR-574-5p, 79.0, miR-877*, 81.3, miR-1236, 77.0. ***CDH1:*** miR-548ah, 78.6, miR-1273, 82.4, miR-1285, 78.8. ***CD44:*** miR-766, 77.5. ***DLC1:*** miR-4766-3p, 76.7. ***EP300:*** miR-574-5p, 82.8; 79.0. ***FLCN:*** miR-553, 84.4, miR-1273d, 77.2, miR-1273f, 83.4, miR-1285, 78.1, miR-3646, 75.0. ***FZD7:*** let-7g*, 84.1, miR-598, 75.8. ***KLF12:*** miR-574-5p, 75.0, miR-4799-3p, 78.0. ***MET:*** miR-553, 76.4, miR-1273f, 91.1. ***MMP2:*** miR-3202, 76.9. ***MSH3:*** miR-5096, 76.4. ***MTHFR:*** miR-1289, 83.3, miR-1976, 81.0, miR-4296, 82.8, miR-4540, 78.1, miR-5095, 79.2, miR-5096, 79.2. ***SMAD4:*** miR-566, 78.1, miR-574-5p, 85.3; 79.7, miR-644, 78.4, miR-1273, 78.4, miR-1273f, 82.4, miR-1285, 77.0, miR-4799-3p, 77.8. ***SNAI1:*** miR-1228*, 77.8. ***SRC:*** miR-320b, 85.5, miR-3162-3p, 76.8, miR-4748, 81.1. ***TGFBR2:*** miR-511, 80.8. ***TP53:*** miR-1273, 73, miR-1273g, 81.8, miR-3192, 77. ***VDR:*** miR-4312, 78.7, miR-4646-3p, 80.5, miR-5096, 84.3. ***ZEB1:*** miR-574-5p, 79.0.

TABLE II
CHARACTERISTICS OF MRNAS BINDING WITH EXONIC MIRNAS

Gene: ex-miRNA, ΔG/ΔGm, %

5'UTR

AXIN1: miR-1306, 88.6, miR-4315, 81.1. ***MMP2:*** miR-935, 74.8, miR-1306, 85.5. ***PMS1:*** miR-4687-5p, 75.7. ***PTPN12:*** miR-4800-3p, 80.5.

CDS

ABCB1: miR-4724-3p, 78.5. ***AXIN1:*** miR-1306, 89.4. ***AXIN2:*** miR-1306, 86.5. ***ENG:*** miR-3652, 82.9. ***EP300:*** miR-4800-3p, 79.1. ***KIT:*** miR-4709-5p, 78.7. ***MSH3:***miR-3652, 81.2.

3'UTR

CCND1: miR-4687-5p, 80.0. ***CD44:*** miR-4775, 80.7. ***MTHFR:*** miR-4751, 73.5. ***SNAI1:*** miR-4687-3p, 77.1.

Interactions between 49 exonic miRNAs and 54 protein-coding human genes were investigated, and it was revealed that 14 genes were targets for ex-miRNAs (Table II). Eleven out of 49 ex-miRNAs were found to interact with these 14 genes. The number of binding sites for ex-miRNAs differed among mRNAs.

For example, *AXIN1* and *MMP2* each interacted with 2 ex-miRNAs. MiR-1306 bound to 4 sites in 3 target mRNAs, and miR-3652, miR-4687-5p, and miR-4800-3p each bound to 2 mRNAs (Supplementary Table III). There was no significant relationship between the length of the target mRNA and the number of miRNA binding sites (correlation coefficient, -0.46 ($p < 0.09$]). Of the binding sites for ex-miRNAs, 35.3%, 41.2%, and 23.5% were located in the 5′UTRs, CDSs, and 3′UTRs of the 14 target mRNAs, respectively. The densities of the binding sites varied from 0.11 s/l in the *EP300* mRNA to 0.82 s/l in the *AXIN1* mRNA and were an average of 0.40 s/l for all 14 mRNAs (Supplementary Table IV). *AXIN1* mRNA had the highest density of interaction sites, demonstrating that ex-miRNAs are important in the regulation of this gene's expression The 5′UTRs, CDSs, and 3′UTRs of these genes differed in their ability to bind ex-miRNAs. The average binding site densities for ex-miRNAs in the 5′UTR, CDS, and 3′UTR were 1.75, 0.17, and 0.32 s/l, respectively. The average binding site density for ex-miRNAs in the 5′UTR was 10.3 times more than that in the CDS and 5.5 times more than that in the 3′UTR. The mRNAs for *AXIN1* and MMP2 each had 2 binding sites for ex-miRNAs in their 5′UTRs. *CCND1*, *CD44*, *MTHFR*, and *SNAI1* mRNAs bound to miRNAs in the 3′UTR. These findings demonstrated that ex-miRNAs can bind to mRNAs at the 5′UTR, CDS, and 3′UTR.

Exonic miRNA binding sites are presented in Table IV. For example, 5′-dominant canonical sites included miR-4775 with *CD44* mRNA, miR-4687 with *CCND1* mRNA, and miR-1306 with *MMP* mRNA; 3′-compensatory sites were miR-1306 with *AXIN1* mRNA and miR-1306 with *AXIN2* mRNA. The primary contribution to energy included all regions of the miRNA binding site, not only the 5′ seed.

The number of miRNAs found in humans is gradually increasing, and currently approximately 2000 miRNAs are known. Most studies use hundreds of well-described miRNAs, but only some researchers have studies intragenic and intergenic miRNAs separately. Our investigation investigated 735 intragenic miRNAs and their binding sites within 54 target genes. Each miRNA was able to bind with mRNA, accomplishing different functions in cells. The majority of human genes are regulated by one and/or several miRNAs.

Our results showed that many mRNAs were targets for intragenic miRNAs. In the 54 mRNAs, 22.6%, 37.7%, and 39.7% of the miRNAs bound within the 5′UTRs, CDSs, and 3′UTRs of their target genes, respectively (Tables I, II). When considered miRNA sites only in the 3′UTR, we lose 1.5 times more sites in the 5′UTR and CDS. Some mRNAs were strongly regulated by in-miRNAs. The mRNAs of *PTEN* and *MTHFR* each contained 6 in-miRNA binding sites, while the mRNA of *SMAD4* contained 7 in-miRNA binding sites.

TABLE III
SCHEMES OF INTERACTION OF IN-MIRNAS WITH SOME MRNAS

Region mRNA	site,nt	ΔG,kcal/mol	ΔG/ΔG$_m$,%
3'UTR, 3277nt, ΔG=-115kcal/mol, ΔG/ΔG$_m$=84%, 3'compn.			
mRNA ***FLCN***	5' A	G	3'
	GAGACGGGGUUUCACCGU		
	UUUUGUUUUAGAGUGGCA		
miR-553	3'	AAA	5'
CDS, 1726nt, ΔG=-151kcal/mol, ΔG/ΔG$_m$=87%, compl.			
mRNA ***MTHFR***	5' U	U	3'
	GGGGCCCCAGCGGGGGC		
	CUUUGGGGUCGUCUCUG		
miR-593	3' U	U	5'
5'UTR, 904nt, ΔG=-147kcal/mol, ΔG/ΔG$_m$=83%, 5'-d/c			
mRNA ***PTEN***	5' C G	C	3'
	GGC GCAGAGCGAGGGGCAU		
	CUG CGUCUCGUUUUCUGUG		
miR-511	3' A A	5'	
3'UTR, 4993nt, ΔG=-159kcal/mol, ΔG/ΔG$_m$=91%, 3'compn.			
mRNA ***MET***	5' U	C C	3'
	CACUGCAACCUCCA CUCC		
	GUGACGUUGGAGGU GAGG		
miR-1273f	3'	A	5'
CDS, 310nt, ΔG=-142kcal/mol, ΔG/ΔG$_m$=85%, 3'compn.			
mRNA ***MSH6***	5' C	G C	3'
	UGGGCCUGGGCCCA G		
	ACUCGGACUCGGGU U		
miR-4296	3'	G A	5'
5'UTR, 82nt, ΔG=-212kcal/mol, ΔG/ΔG$_m$=88%, 5'-d/c			
mRNA ***PTEN***	5' C		3'
	G GCGGCGGCGGAGGGGGCGGG		
	C CGUCGUCGCCUCCCCCGUCU		
miR-1913	3' A		5'

Types of binding sites: 5'-d/c – 5'-dominant canonical, 3'compn. – 3'-compensatory, compl. – complementary site.

According to our data, the human genome contained many strong miRNA binding sites in mRNAs of different genes. The miR-1913*:PTEN* mRNA complex had a ΔG/ΔGm of 88.3%; the miR-593*:MTHFR* mRNA had a ΔG/ΔGm of 87.0%; and the miR-574-5p:*SMAD4* complex had a ΔG/ΔGm of 85.3% (further information can be found in Tables I, II). Each of these sites had about 15–20 complementary base pairs. Table III shows interaction schemes for miRNA binding sites with 14–20 complementary base pairs. *GNAS*, *CD44*, *AXIN1*, and *MYC* mRNAs contained strong binding sites for intronic miR-1268b. Intronic miR-1273f had binding sites with *MET*, *FLCN*, *SMAD4*, and *MUTYH-alpha5* mRNAs. Additionally, *PTEN* and *EP300* mRNAs had 1 site, and *TGFBR* mRNA had 2 strong binding sites with intronic miR-511. *SMAD4*, *EP300*, *SMAD4*, *CCND1*, *EP300*, *ZEB1*, and *KLF12* mRNAs contained strong binding sites for intronic miR-574-5p. Some intragenic miRNAs had 2 or 3 sites in several mRNAs of these 54 oncogenes. Ji and colleagues revealed that miR-574-5p negatively regulates Qki6/7 to impact β-catenin/Wnt signalling and the development of colorectal cancer [35]. Additionally Foss and colleagues revealed that serum miR-574-5p is differentially expressed in patients with early-stage non-small cell lung cancer and healthy controls [36]. Using quantitative real-time PCR, Wang and colleagues found that miR-511 was downregulated in hepatocellular carcinoma [37].

TABLE IV
SCHEMES OF INTERACTION OF EX-MIRNAS WITH SOME MRNAS

Region mRNA	site,nt	ΔG,kcal/mol	ΔG/ΔG$_m$,%
3'UTR, 3641nt, ΔG=-108kcal/mol, ΔG/ΔG$_m$=81%, 5'd/c			
mRNA ***CD44***	5' A	U	3'
	GGCUGAGACAGGAGGUUA		
	CUGGCUUUGUUUUUUAAU		
miR-4775	3' CA	U	5'
CDS, 1330nt, ΔG=-144kcal/mol, ΔG/ΔG$_m$=89%, 3'compn.			
mRNA ***AXIN1***	5' A U	A	3'
	GCCACCAG GCCAACG		
	UGGUGGUC CGGUUGC		
miR-1306	3' U	A	5'
5'UTR, 43nt, ΔG=-143kcal/mol, ΔG/ΔG$_m$=89%, 3'compn.			
mRNA ***AXIN1***	5' C	C	3'
	GCCGCCAGAGCCGGC		
	UGGUGGUCUCGGUUG		
miR-1306	3'	CA	5'
3'UTR, 1349nt, ΔG=-177kcal/mol, ΔG/ΔG$_m$=80%, 5'd/c			
mRNA ***CCND1***	5' A C G	U	3'
	G GGUG GGGAGGAGGGUUG		
	C CCAC CCCUCCUCCCGAC		
miR-4687	3' AA G		5'
CDS, 1167nt, ΔG=-140kcal/mol, ΔG/ΔG$_m$=86%, 3'compn.			
mRNA ***AXIN2***	5' A C	A	3'
	GCCACCAG GCCAACG		
	UGGUGGUC CGGUUGC		
miR-1306	3' U	A	5'
5'UTR, 183nt, ΔG=-138kcal/mol, ΔG/ΔG$_m$=85%, 5'-d/c			
mRNA ***MMP2***	5' G	G	3'
	GCCACC GAGCCAGCG		
	UGGUGG CUCGGUUGC		
miR-1306	3' U	A	5'

Types of binding sites: 5'-d/c – 5'-dominant canonical, 3'compn. – 3'-compensatory.

Tserel and colleagues predicted that miRNA-511 targeted several genes with known immune functions [38], and Kim

and colleagues revealed that miR-511 was expressed at lower levels in malignant ovarian epithelial tumours [39].

Three types of binding sites are thought to exist: 5′-dominant canonical, 5′-dominant seed only, and 3′-compensatory [34]. The canonical site exhibited almost perfect complementarity at both the 3′- and 5′-ends of the miRNA with a specific bulge in the middle. Dominant sites exhibited perfect seed 5′-complementarity to the miRNA but poor 3′-complementarity. The compensatory site contained a mismatch (wobble) in the 5′-seed region, but compensated through excellent complementarity at the 3′-end of miRNA. 5′-Dominant canonical and 3′-compensatory types of binding sites were found in the obtained data. There was no 5′-dominant seed only type of binding site because such sites have low binding energies and are not reliable according to our criteria. In addition, complementary type miRNA binding sites were found after the analysis of our results. These sites had perfect complementarity beginning with second nucleotide and ending with the next-to-last nucleotide of miRNA in the binding site. There was no a bugle in the middle (Table III, IV). Such binding sites had high binding energies and were reliable according to our criteria.

IV. Conclusion

These data suggested that the miRNAs described here may regulate gene expression more effectively than miRNAs studied in experimental investigations. MiR-1268b, miR-1273f, miR-511, and miR-574-5p had several oncogenic mRNA targets. We recommend that newly described sites should be validated by experimental investigations. Several miRNAs affect the mRNAs of genes that participate in oncogenic diseases, and this information could be useful in molecular medicine.

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