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Research Article

Sequence Analyses of Oncogenic and Tumor Suppressive miRNAs in Cancers

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Abstract

MicroRNAs (miRNAs) are small (~25 nucleotides) noncoding RNA molecules thought to play an important role in regulating gene expression. Knowledge of the biological functions of most miRNAs is still limited, but these miRNAs are thought to regulate the gene expression in various diseases. In this paper, the sequences of oncogenic and tumor suppressive miRNAs in human cancers are examined from the viewpoint of up- and down-regulation. Oncogenic miRNAs are involved in the overexpression/upregulation of cancers, whereas tumor suppressive miRNAs are involved in the underexpression/downregulation of cancers. The oncogenic and tumor suppressive miRNA sequences were first collected from miRBase based on the relations between miRNAs and various cancers in the literature. Statistical analysis of the positional nucleotide occurrence features of miRNAs revealed differences between the positional nucleotide occurrences of oncogenic and tumor suppressive miRNAs. A miRNA gene-silencing score was then defined on the basis of the higher and lower levels of the statistical significances of positional nucleotides. Since the miRNA scores were closely related to miRNA frequencies, a method using the scores and nucleotide frequencies to distinguish whether a new miRNA is oncogenic or tumor suppressive is proposed.

Keywords: miRNA; Noncoding RNA; Gene Silencing; Cancer; Oncogenic; Tumor Suppressive; Significance Test

MicroRNAs (miRNAs) are small (~25 nucleotides) noncoding RNA molecules that regulate gene expression post-transcriptionally by base-pairing to mRNAs [1-4]. Many miRNAs have recently been identified in various multicellular organisms and are evolutionally conserved. Although knowledge of the biological functions of most miRNAs is still limited, these molecules are thought to regulate the gene expression at various stages in diseases.

Animal microRNAs are typically transcribed as primary transcripts (pri-miRNAs) of varying length that in the nucleus are processed by Drosha into stem-loop precursors consisting of ~70 nucleotides. In the cytoplasm the precursor miRNA (pre-miRNA) is processed by the RNase III type enzyme Dicer to give a mature ~25-nucleotide product. MicroRNAs regulate the expression levels of other genes by several mechanisms, generally reducing protein levels through the cleavage of mRNAs or the repression of their translation.

Over the past several years, it has been reported that many miRNAs are related to various cancer expression and progression. Recently miRNA profiling has been considered as a useful diagnostic and prognostic tool to indicate an oncogene or a tumor suppressor. That is, miRNAs can be considered as biomarkers of various cancers [5-7].

In this paper, to investigate the relations between individual miRNAs and cancers, the oncogenic and tumor suppressive miRNA sequences were first collected from miRBase based on the relations between miRNAs and various cancers in the

literature [8,9]. A miRNA gene-silencing score was then defined on the basis of the higher and lower levels of the statistical significances of miRNA positional nucleotide occurrences. Since the miRNA scores were closely related to miRNA frequencies, a method using the scores and nucleotide frequencies to distinguish whether a new miRNA is oncogenic or tumor suppressive is proposed.

The relations between miRNAs and human cancers

It has been observed that miRNAs contribute to cancer development and progression. Croce and his colleagues analyzed 540 samples of lung, breast, stomach, prostate, colon, and pancreatic tumors and found that miRNAs are differentially expressed in normal tissues and cancers [10]. Other studies have also reported that miRNAs play some roles as oncogenes or tumor suppressor genes. They can therefore be used as biomarkers in human cancers. In Table 1 miRNAs that are upregulated in various cancers are listed as oncogenic miRNAs and miRNAs that are downregulated in those cancers are listed as suppressive miRNAs [6,7,10,11-43]. It is clear from Table 1 that there are differences in occurrences of the oncogenic and suppressive miRNAs for the individual cancers. The related individual miRNA frequencies for more than two cancers are listed in Tables 2(A) and 2(B). More miRNAs are oncogenic than are suppressive. hsa-miR-21, for example, is oncogenic for eleven cancers. Other miRNAs related oncogenic for more than four cancers are hsa-miR-17, -191, -221, -106a, -155, -214, -223, -199a and -92a. In contrast, only hsa-miR-145, let-7, and hsa-miR-143 are suppressive for more than five kinds of cancers. Although there is a possibility that some miRNAs are oncogenic in specific cancers but are tumor suppressive for others, the only miR-32 belongs to this category as listed in Table 1. That is, miR-32 is oncogenic for colorectal, pancreas and prostate gland, and is suppressive for lung. There is therefore little influence on nucleotide features of oncogenic and suppressive miRNAs.

On the other hand, although RNA interference (RNAi) has been widely used for studying gene functions, the gene-silencing effectiveness in mammalian cells depends very much on target sequence positions (sites) selected from the target gene. That is, different short interfering RNAs (siRNAs) from various positions induce different levels of gene-silencing. The gene-silencing of miRNAs may also be considered to depend on the selected target sequence positions. Since the target mRNA sequence is a certain combination of nucleotides, some nucleotide sequences of miRNAs can be considered to play roles in the gene-silencing of the target mRNA.

cancer type	oncogenic miRNAs	suppressive miRNAs
brain, GBM	hsa-miR-21, 221,155,210	hsa-miR-128, 181
breast	hsa-miR-9, 10b, 17, 21, 29b, 34, 146, 155, 181b, 181a,195	hsa-let-7, hsa-miR-15a, 16, 125a, 125b, 127, 145, 204
lung	hsa-miR-17, 21, 24-2, 106a, 128, 146, 150, 155, 191, 192, 197, 199a, 205, 212, 210, 214	hsa-let-7, hsa-miR-9, 26a, 27b, 29b, 32, 33, 30a, 95, 101, 124, 125a, 126, 140, 143, 145, 181c, 192, 198, 199b, 216, 218, 219, 224
esophagus	hsa-miR-21, 93	hsa-miR-203, 205
stomach	hsa-miR-21, 24, 25, 92a, 107, 191, 214, 221, 223,17-5p,106a,106b	hsa-let-7
colorectal	hsa-miR-17, 20a, 21, 24, 29b, 30c, 31, 32, 96, 106a, 107, 128b, 135b, 155, 183, 191, 221, 223,17-3p,92a	hsa-let-7, has-miR-34, 127, 133b, 143, 145
hepatic cell	hsa-miR-15b, 18a, 21, 106b, 221, 222, 224,500	hsa-let-7, has-miR-101, 122, 125a, 195, 199a, 200a
pancreas	hsa-miR-17, 20a, 21, 24, 25, 29b, 30c, 32, 92a, 100, 106a, 107, 125b, 128, 146, 155, 181a, 181b, 191, 196a, 196b, 199a, 214, 221, 223, 301a, 376a ,210	hsa-miR-139, 142, 345, 375
prostate gland	hsa-miR-17, 20a, 21, 25, 30c, 32,92a, 106a, 146a, 181b, 191, 199a, 214, 223	hsa-miR-15a, 16, 143, 145, 218
cervical	hsa-miR-21, 199a	hsa-miR-143, 145
tongue	hsa-miR-184	
ovarian	hsa-miR-21,92a,93,126,29a	
AML,ALL		hsa-miR-92a
B-CLL	hsa-miR-17, 92a-1	hsa-miR-15a, 16, 143, 145, 192, 181a

GBM: glioblastoma maltiforme, B-CLL: B cell chromic lymphocytic leukemia
AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia

Table 1. Relations between human cancers and miRNAs.

miRNA	frequency	cancer type													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
hsa-miR-21	11	O	O	O	O	O	O	O	O	O	O			O	
-17	7		O	O		O	O		O	O		O			
-92a	6					O	O		O	O		O		O	
-191	5			O		O	O		O	O					
-221	5	O				O	O	O	O						
-106a	5			O		O	O		O	O					
-155	4		O	O			O		O						
-214	4			O		O			O	O					
-223	4					O	O		O	O					
-199a	4			O					O	O	O				
-24	3					O	O		O						
-25	3					O			O	O					
-32	3						O		O	O					
-107	3					O	O		O						
-146	3		O	O					O						
-181b	3		O						O	O					
-20a	3						O		O	O					
-29b	3		O				O		O						
-30c	3						O		O	O					
-210	3	O		O					O						
-128	2			O					O						
-181a	2		O						O						
106b	2					O		O							
93	2				O					O					

1: brain, GBM, 2:breast, 3:lung, 4: esophagus, 5: stomach,6: colorectal,
7: hepatic cell, 8: pancreas, 9: prostate gland, 10: cervical, 11: B-CLL,
12: tangue, 13:ovarian,
14: AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia

Table 2(A). miRNA frequencies of the oncogenic miRNAs for various cancers.

miRNA	frequency	cancer type													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
hsa-miR-145	6		○	○			○			○	○	○			
let-7	5		○	○		○	○	○							
hsa-miR-143	5			○			○			○	○	○			
-16	3		○							○		○			
-125a	3		○	○				○							
-15a	3		○							○		○			
-101	2			○				○							
-127	2		○				○								
-192	2			○								○			
-218	2			○						○					

Table 2(B). miRNA frequencies of the suppressive miRNAs for various cancers.

Positional nucleotide features of oncogenic and suppressive miRNAs

To get positional nucleotide features, the nucleotide sequences for the oncogenic and suppressive miRNAs listed in Table 1 were first collected from miRBase [8,9]. The obtained miRNA sequences are listed in Tables 3(A) and 3(B). Then the frequencies of the four nucleotides (A, G, C, U) at positions from 1 to 22 were determined for the pooled sequences. These frequencies are listed in Tables 4(A) and 4(B). As shown in Figures. 1(A) and 1(B), there are differences in nucleotide distribution ratios at individual positions for oncogenic and suppressive miRNAs. (As there are a few nucleotide occurrences at position 23 listed in Tables 3(A) and 3(B), the frequencies of the nucleotides at positions from 1 to 22 are used in Tables 4(A) and 4(B) and Figures. 1(A) and 1(B).)

cancer type	has-miR-	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
brain, GBM	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	-3p	C	A	A	C	A	C	C	A	G	U	C	G	A	U	G	G	G	U	U	G	U		
	221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U	
	155-5p	U	U	A	A	U	G	C	U	A	A	U	C	G	U	G	A	U	A	G	G	G	G	U
	210-3p	C	U	G	U	G	C	G	U	G	U	G	A	C	A	G	C	G	G	C	U	G	A	
breast	9-5p	U	C	U	U	U	G	G	U	U	A	U	C	U	A	G	C	U	G	U	A	U	G	A
	9-3p	A	U	A	A	A	G	C	U	A	G	A	U	A	A	C	C	G	A	A	A	G	U	
	10b-5p	U	A	C	C	C	U	G	U	A	G	A	A	C	C	G	A	A	U	U	U	G	U	G
	10p-3p	A	C	A	G	A	U	U	C	G	A	U	U	C	U	A	G	G	G	G	A	A	U	
	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	21-3p	C	A	A	C	A	C	C	A	G	U	C	G	A	U	G	G	G	C	U	G	U		
	29b-2-5p	C	U	G	G	U	U	U	C	A	C	A	U	G	G	U	G	G	C	U	U	A	G	
	34a-5p	U	G	G	C	A	G	U	G	U	C	U	U	A	G	C	U	G	G	U	U	G	U	
	34a-3p	C	A	A	U	C	A	G	C	A	A	G	U	A	U	A	C	U	G	C	C	C	U	
	34b-5p	U	A	G	G	C	A	G	U	G	U	C	A	U	U	A	G	C	U	G	A	U	U	G
	34b-3p	C	A	A	U	C	A	C	U	A	A	C	U	C	C	A	C	U	G	C	C	A	U	
	34c-5p	A	G	G	C	A	G	U	G	U	A	G	U	U	A	G	C	U	G	A	U	U	G	C
	34c-3p	A	A	U	C	A	C	U	A	A	C	C	A	C	A	C	G	G	C	C	A	G	G	
	146a-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	G	U	U	
	146b-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	C	U	U	
	155-5p	U	U	A	A	U	G	C	U	A	A	U	C	G	U	G	A	U	A	G	G	G	G	U
	181b-5p	A	A	C	A	U	U	C	A	U	U	G	C	U	G	U	C	G	G	U	G	G	G	U
	181a-5p	A	A	C	A	U	U	C	A	A	C	G	C	U	G	U	C	G	G	U	G	A	G	U
	181a-3p	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	U	U	G	U	A	C	C	
	195-5p	U	A	G	C	A	G	C	A	C	A	G	A	A	A	U	A	U	U	G	G	C		
lung	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	24-2-5p	U	G	C	C	U	A	C	U	G	A	G	C	U	G	A	A	A	C	A	C	A	G	
	106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	128-3p	U	C	A	C	A	G	U	G	A	A	C	C	G	G	U	C	U	C	U	U	U		
	146a-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	G	U	U	
	146b-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	C	U	U	
	150-5p	U	C	U	C	C	C	A	A	C	C	C	U	U	G	U	A	C	C	A	G	U	G	
	155-5p	U	U	A	A	U	G	C	U	A	A	U	C	G	U	G	A	U	A	G	G	G	G	U
	191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U	G
	192-5p	C	U	G	A	C	C	U	A	U	G	A	A	U	U	G	A	C	A	G	C	C		
	197-3p	U	U	C	A	C	C	A	C	C	U	U	C	U	C	C	A	C	C	C	A	G	C	
	197-5p	C	G	G	G	U	A	G	A	G	A	G	G	G	C	A	G	U	G	G	G	A	G	G
	199a-5p	C	C	C	A	G	U	G	U	U	C	A	G	A	C	U	A	C	C	U	G	U	U	C
	205-5p	U	C	C	U	U	C	A	U	U	C	C	A	C	C	G	A	G	U	C	U	G		
	212-3p	U	A	A	C	A	G	U	C	U	C	C	A	G	U	C	A	C	G	G	C	C		
	212-5p	A	C	C	U	U	G	G	C	U	C	U	A	G	A	A	U	C	A	G	C	U	A	C	U
	210-3p	C	U	G	U	G	C	G	U	G	U	G	A	C	A	G	C	G	G	C	U	G	A	
	214-3p	A	C	A	G	C	C	A	C	A	G	A	C	A	C	A	G	G	C	A	G	U		
esophagus	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	93-5p	C	A	A	A	G	U	G	C	U	G	U	U	C	G	U	G	C	A	G	G	U	A	G

stomach	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	24-3p	U	G	G	C	U	C	A	G	U	U	C	A	G	C	A	G	G	A	A	C	A	G	
	25-5p	A	G	G	C	G	G	A	G	A	C	U	U	G	G	G	C	A	A	U	U	G		
	25-3p	C	A	U	U	G	C	A	C	U	U	G	U	C	U	C	G	G	U	C	U	G	A	
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
	107	A	G	C	A	G	C	A	U	U	G	U	A	C	A	G	G	G	C	U	A	U	C	A
	191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U	G
	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	106b-5p	U	A	A	A	G	U	G	C	U	G	A	C	A	G	U	G	C	A	G	A	U		
	214-3p	A	C	A	G	C	A	G	G	C	A	C	A	G	A	C	A	G	G	C	A	G	U	
	221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U	
	221-3p	A	G	C	U	A	C	A	U	U	G	U	C	U	G	C	U	G	G	G	U	U	U	C
	223-3p	U	G	U	C	A	G	U	U	U	G	U	C	A	A	A	U	A	C	C	C	C	A	
colorectal	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	20a-5p	U	A	A	A	G	U	G	C	U	U	A	U	A	G	U	G	C	A	G	G	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	24-3p	U	G	G	C	U	C	A	G	U	U	C	A	G	C	A	G	G	A	A	C	A	G	
	29b-3p	U	A	G	C	A	C	C	A	U	U	U	G	A	A	A	U	C	A	G	U	G	U	U
	30c-5p	U	G	U	A	A	A	C	A	U	C	C	U	A	C	A	C	U	C	U	C	A	G	C
	31-5p	A	G	G	C	A	A	G	A	U	G	C	U	G	G	C	A	U	A	G	C	U		
	32-5p	U	A	U	U	G	C	A	C	A	U	U	A	C	U	A	A	G	U	U	G	C	A	
	96-5p	U	U	U	G	G	C	A	C	U	A	G	C	A	C	A	U	U	U	U	U	G	C	U
	106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	107	A	G	C	A	G	C	A	U	U	G	U	A	C	A	G	G	G	C	U	A	U	C	A
	128-3p	U	C	A	C	A	G	U	G	A	A	C	C	G	G	U	C	U	C	U	U	U		
	135b-5p	U	A	U	G	G	C	U	U	U	U	C	A	U	U	C	C	U	A	U	G	U	G	A
	155-5p	U	U	A	A	U	G	C	U	A	A	U	C	G	U	G	A	U	A	G	G	G	G	U
	183-5p	U	A	U	G	G	C	A	C	U	G	G	U	A	G	A	A	U	U	C	A	C	U	
	191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U	G
	221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U	
	221-3p	A	G	C	U	A	C	A	U	U	G	U	C	U	G	C	U	G	G	G	U	U	U	C
	223-3p	U	G	U	C	A	G	U	U	U	G	U	C	A	A	A	U	A	C	C	C	C	A	
	17-3p	A	C	U	G	C	A	G	U	G	A	A	G	G	C	A	C	U	U	G	U	A	G	
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
hepatic cell	15b-5p	U	A	G	C	A	G	C	A	C	A	U	C	A	U	G	G	U	U	U	A	C	A	
	18a-5p	U	A	A	G	G	U	G	C	A	U	C	U	A	G	U	G	C	A	G	A	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	106b-5p	U	A	A	A	G	U	G	C	U	G	A	C	A	G	U	G	C	A	G	A	U		
	106b-3p	C	C	G	C	A	C	U	G	U	G	G	G	U	A	C	U	U	G	C	U	G	C	
	221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U	
	221-3p	A	G	C	U	A	C	A	U	U	G	U	C	U	G	C	U	G	G	G	U	U	U	C
	222-3p	A	G	C	U	A	C	A	U	C	U	G	G	C	U	A	C	U	G	G	G	U		
	224-5p	C	A	A	G	U	C	A	C	U	U	A	G	U	G	G	U	U	C	C	G	U	U	
	500a-3p	A	U	G	C	A	C	C	U	G	G	G	C	A	A	G	G	A	A	U	U	C	U	G
pancreas	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	20a-5p	U	A	A	A	G	U	G	C	U	U	A	U	A	G	U	G	C	A	G	G	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	24-3p	U	G	G	C	U	C	A	G	U	U	C	A	G	C	A	G	G	A	A	C	A	G	
	25-5p	A	G	G	C	G	G	A	G	A	C	U	U	G	G	G	C	A	A	U	U	G		
	29b-3p	U	A	G	C	A	C	C	A	U	U	U	G	A	A	A	U	C	A	G	U	G	U	U
	30c-5p	U	G	U	A	A	A	C	A	U	C	C	U	A	C	A	C	U	C	U	C	A	G	C
	32-5p	U	A	U	U	G	C	A	C	A	U	U	A	C	U	A	A	G	U	U	G	C	A	
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
	100-5p	A	A	C	C	C	G	U	A	G	A	U	C	C	G	A	A	C	U	U	G	U	G	
	106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	107	A	G	C	A	G	C	A	U	U	G	U	A	C	A	G	G	G	C	U	A	U	C	A
	125b-5p	U	C	C	C	U	G	A	G	A	C	C	C	U	A	A	C	A	C	U	U	G	U	G	A
	128-3p	U	C	A	C	A	G	U	G	A	A	C	C	G	G	U	C	U	C	U	U	U		
	146a-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	G	U	U	
	155-5p	U	U	A	A	U	G	C	U	A	A	U	C	G	U	G	A	U	A	G	G	G	G	U
	181a-5p	A	A	C	A	U	U	C	A	A	C	G	C	U	G	U	C	G	G	U	G	A	G	U
	181a-3p	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	U	U	G	U	A	C	C	
	181b-5p	A	A	C	A	U	U	C	A	U	U	G	C	U	G	U	C	G	G	U	G	G	G	U
	191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U	G
	196a-5p	U	A	G	G	U	A	G	U	U	U	C	A	U	G	U	U	G	U	U	G	G	G	
	199a-5p	C	C	C	A	G	U	G	U	U	C	A	G	A	C	U	A	C	C	U	G	U	U	C
	199a-3p	A	C	A	G	U	A	G	U	C	U	G	C	A	C	A	U	U	G	G	U	U	A	
	214-3p	A	C	A	G	C	A	G	G	C	A	C	A	G	A	C	A	G	G	C	A	G	U	
	221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U	
	221-3p	A	G	C	U	A	C	A	U	U	G	U	C	U	G	C	U	G	G	G	U	U	U	C
	223-3p	U	G	U	C	A	G	U	U	U	G	U	C	A	A	A	U	A	C	C	C	C	A	
	301a-5p	G	C	U	C	U	G	A	C	U	U	U	A	U	U	G	C	A	C	U	A	C	U	
	301a-3p	C	A	G	U	G	C	A	A	U	A	G	U	A	U	U	G	U	C	A	A	A	G	C
	376a-3p	A	U	C	A	U	A	G	A	G	G	A	A	A	A	U	C	C	A	C	G	U		
	210-3p	C	U	G	U	G	C	G	U	G	U	G	A	C	A	G	C	G	G	C	U	G	A	
prostate gland	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	20a-5p	U	A	A	A	G	U	G	C	U	U	A	U	A	G	U	G	C	A	G	G	U	A	G
	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	25-5p	A	G	G	C	G	G	A	G	A	C	U	U	G	G	G	C	A	A	U	U	G		
	30c-5p	U	G	U	A	A	A	C	A	U	C	C	U	A	C	A	C	U	C	U	C	A	G	C
	32-5p	U	A	U	U	G	C	A	C	A	U	U	A	C	U	A	A	G	U	U	G	C	A	
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
	106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	146a-5p	U	G	A	G	A	A	C	U	G	A	A	U	U	C	C	A	U	G	G	G	U	U	
	181b-5p	A	A	C	A	U	U	C	A	U	U	G	C	U	G	U	C	G	G	U	G	G	G	U
	191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U	G
	199a-5p	C	C	C	A	G	U	G	U	U	C	A	G	A	C	U	A	C	C	U	G	U	U	C
	221-3p	A	G	C	U	A	C	A	U	U	G	U	C	U	G	C	U	G	G	G	U	U	U	C
	223-3p	U	G	U	C	A	G	U	U	U	G	U	C	A	A	A	U	A	C	C	C	C	A	
cervical	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	199a-5p	C	C	C	A	G	U	G	U	U	C	A	G	A	C	U	A	C	C	U	G	U	U	C
B-CELL	17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A	G
	92a-1-5p	A	G	G	U	U	G	G	G	A	U	C	G	G	U	U	G	C	A	A	U	G	C	U
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
tangue	184	U	G	G	A	C	G	G	A	G	A	A	C	U	G	A	U	A	A	G	G	G	U	
ovarian	21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A	
	92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U	
	93-5p	C	A	A	A	G	U	G	C	U	G	U	U	C	G	U	G	C	A	G	G	U	A	G
	126-5p	C	A	U	U	A	U	U	A	C	U	U	U	U	G	U	A	C	G	C	G			
	126-3p	U	C	G	U	A	C	C	G	U	G	A	G	U	A	A	U	A	A	U	G	C	G	
	29a-3p	U	A	G	C	A	C	C	A	U	C	U	G	A	A	A	U	C	G	G	U	U	A	

Table 3(A). Nucleotide sequences of oncogenic miRNAs for various cancers.

cancer type	has-miR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
brain, GBM	128-1-5p	C	G	G	G	G	C	C	G	U	A	G	C	A	C	U	G	U	C	U	G	A	G	A	
	128-1-3p	U	C	A	C	A	G	U	G	A	A	C	C	G	G	U	C	U	C	U	U	U			
	181a-5p	A	A	C	A	U	U	C	A	A	C	G	U	U	G	U	C	G	G	U	G	A	G	U	
	181a-3p	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	U	U	G	U	A	C	C		
	181b-5p	A	A	C	A	U	U	C	A	U	U	G	C	U	G	U	C	G	G	U	G	G	G	U	
	181b-3p	C	U	C	A	C	U	G	A	A	C	A	A	U	G	A	A	U	G	C	A	A			
	181c-5p	A	A	C	A	U	U	C	A	A	C	C	U	G	U	C	G	G	U	G	A	G	U		
	181c-3p	A	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	G	U	G	G	A	C		
	181d-5p	A	A	C	A	U	U	C	A	U	U	G	U	U	G	U	C	G	G	U	G	G	G	U	
breast	let-7a-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7b-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	G	G	U	U		
	let-7c-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U		
	let-7d-5p	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U		
	let-7e-5p	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7f-5p	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U		
	15a-5p	U	A	G	C	A	G	C	A	C	A	U	A	A	U	G	G	U	U	U	G	U	G		
	16-5p	U	A	G	C	A	G	C	A	C	G	U	A	A	A	U	A	U	U	G	G	C	G		
	125a-5p	U	C	C	C	U	G	A	G	A	C	C	C	U	U	U	A	A	C	C	U	G	U	G	A
	125b-5p	U	C	C	C	U	G	A	G	A	C	C	C	U	A	A	C	U	U	G	U	G	A		
	127-5p	C	U	G	A	A	G	C	U	C	A	G	A	G	G	G	C	U	C	U	G	A	U		
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
	204-5p	U	U	C	C	C	U	U	U	G	U	C	A	U	C	C	U	A	U	C	G	C	U		
lung	let-7a-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7b-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	G	G	U	U		
	let-7c-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U		
	let-7d-5p	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U		
	let-7e-5p	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7f-5p	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U		
	9-5p	U	C	U	U	U	G	G	U	U	A	U	C	U	A	G	C	U	G	U	A	U	G	A	
	9-3p	A	U	A	A	A	G	C	U	A	G	A	U	A	A	C	C	G	A	A	A	G	U		
	26a-5p	U	U	C	A	A	G	U	A	A	U	C	C	A	G	G	A	U	A	G	G	C	U		
	27b-5p	A	G	A	G	C	U	U	A	G	C	U	G	A	U	U	G	G	U	G	A	A	C		
	29b-3p	U	A	G	C	A	C	C	A	U	U	U	G	A	A	A	U	C	A	G	U	G	U	U	
	32-5p	U	A	U	U	G	C	A	C	A	U	U	A	C	U	A	A	G	U	U	G	C	A		
	33a-5p	G	U	G	C	A	U	U	G	U	A	G	U	U	G	C	A	U	U	G	C	A			
	30a-5p	U	G	U	A	A	A	C	A	U	C	C	U	C	G	A	C	U	G	G	A	A	G		
	95-3p	U	U	C	A	A	C	G	G	G	U	A	U	U	U	A	U	U	G	A	G	C	A		
	101-3p	U	A	C	A	G	U	A	C	U	G	U	G	A	U	A	A	C	U	G	A	A			
	124-3p	U	A	A	G	G	C	A	C	G	C	G	G	U	G	A	A	U	G	C	C				
	125a-5p	U	C	C	C	U	G	A	G	A	C	C	C	U	U	U	A	A	C	C	U	G	U	G	A
	126-5p	C	A	U	U	A	U	U	A	C	U	U	U	U	G	U	A	C	G	C	G				
	126-3p	U	C	G	U	A	C	C	G	U	G	A	G	U	A	A	U	A	A	U	G	C	G		
	140-5p	C	A	G	U	G	G	U	U	U	U	A	C	C	C	U	A	U	G	G	U	A	G		
	140-3p	U	A	C	C	A	C	A	G	G	G	U	A	G	A	A	C	C	A	C	G	G			
	143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C			
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
	181c-5p	A	A	C	A	U	U	C	A	A	C	C	U	G	U	C	G	G	U	G	A	G	U		
	181c-3p	A	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	G	U	G	G	A	C		
	198	G	G	U	C	C	A	G	A	G	G	G	G	A	G	A	U	A	G	G	U	U	C		
	192-5p	C	U	G	A	C	C	U	A	U	G	A	A	U	U	G	A	C	A	G	C	C			
	199b-3p	A	C	A	G	U	A	G	U	C	U	G	C	A	C	A	U	U	G	G	U	U	A		
	216a-5p	U	A	A	U	C	U	C	A	G	C	U	G	G	C	A	A	C	U	G	U	G	A		
	216b-5p	A	A	A	U	C	U	C	U	G	C	A	G	G	C	A	A	A	U	G	U	G	A		
	218-5p	U	U	G	U	G	C	U	U	G	A	U	C	U	A	A	C	C	A	U	G	U			
	219a-5p	U	G	A	U	U	G	U	C	C	A	A	A	C	G	C	A	A	U	U	C	U	U		
	219a-1-3p	A	G	A	G	U	U	G	A	G	U	C	U	G	G	A	C	G	U	C	C	C	G		
	224-5p	C	A	A	G	U	C	A	C	U	A	G	U	G	G	U	U	C	C	G	U	U			
esophagus	203a	G	U	G	A	A	A	U	G	U	U	U	A	G	G	A	C	C	A	C	U	A	G		
	205-5p	U	C	C	U	U	C	A	U	U	C	C	A	C	C	G	G	A	G	U	C	U	G		
stomach	let-7a-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7b-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	G	G	U	U		
	let-7c-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U		
	let-7d-5p	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U		
	let-7e-5p	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7f-5p	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U		
colorectal	let-7a-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7b-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	G	G	U	U		
	let-7c-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U		
	let-7d-5p	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U		
	let-7e-5p	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7f-5p	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U		
	34a-5p	U	G	G	C	A	G	U	G	U	C	U	U	A	G	C	U	G	G	U	U	G	U		
	34b-5p	U	A	G	G	C	A	G	U	G	U	C	A	U	U	A	G	C	U	G	A	U	U	G	
	34b-3p	C	A	A	U	C	A	C	U	A	A	C	U	C	C	A	C	U	G	C	C	A	U		
	34c-5p	A	G	G	C	A	G	U	G	U	U	A	G	U	U	A	G	C	U	G	A	U	U	G	C
	127-3p	U	C	G	G	A	U	C	C	G	U	C	U	G	A	G	C	U	U	G	G	C	U		
	133b	U	U	U	G	G	U	C	C	C	C	U	U	C	A	A	C	C	A	G	C	U	A		
	143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C			
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
hepatic cell	let-7a-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7b-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	G	U	G	G	U	U		
	let-7c-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U		
	let-7d-5p	A	G	A	G	G	U	A	G	U	A	G	G	U	U	G	C	A	U	A	G	U	U		
	let-7e-5p	U	G	A	G	G	U	A	G	G	A	G	G	U	U	G	U	A	U	A	G	U	U		
	let-7f-5p	U	G	A	G	G	U	A	G	U	A	G	A	U	U	G	U	A	U	A	G	U	U		
	101-3p	U	A	C	A	G	U	A	C	U	G	U	G	A	U	A	A	C	U	G	A	A			
	122-5p	U	G	G	A	G	U	G	U	G	A	C	A	A	U	G	G	U	G	U	U	U	G		
	125a-5p	U	C	C	C	U	G	A	G	A	C	C	C	U	U	U	A	A	C	C	U	G	U	G	A
	195-5p	U	A	G	C	A	G	C	A	C	A	G	A	A	A	U	A	U	U	G	G	C			
	199a-5p	C	C	A	G	U	G	U	U	C	A	G	A	C	U	A	C	C	U	G	U	U	C		
	199a-3p	A	C	A	G	U	A	G	U	C	U	G	C	A	C	A	U	U	G	G	U	U	A		
	200a-3p	U	A	A	C	A	C	U	G	U	C	U	G	G	U	A	A	C	G	A	U	G	U		
pancreas	139-5p	U	C	U	A	C	A	G	U	G	C	A	C	G	U	G	U	C	U	C	C	A	G	U	
	142-5p	C	A	U	A	A	A	G	U	A	G	A	A	A	G	C	A	C	U	A	C	U			
	142-3p	U	G	U	A	G	U	G	U	U	U	C	C	U	A	C	U	U	U	A	U	G	G	A	
	345-5p	G	C	U	G	A	C	U	C	C	U	A	G	U	C	C	A	G	G	C	U	C			
	375	U	U	U	G	U	U	C	G	U	U	C	G	G	C	U	C	G	C	G	U	G	A		
prostate gland	15a-5p	U	A	G	C	A	G	C	A	C	A	U	A	A	U	G	G	U	U	U	G	U	G		
	16-5p	U	A	G	C	A	G	C	A	C	G	U	A	A	A	U	A	U	U	G	G	C	G		
	143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C			
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
	218-5p	U	U	G	U	G	C	U	U	G	A	U	C	U	A	A	C	C	A	U	G	U			
cervical	143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C			
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
B-CELL	15a-5p	U	A	G	C	A	G	C	A	C	A	U	A	A	U	G	G	U	U	U	G	U	G		
	16-5p	U	A	G	C	A	G	C	A	C	G	U	A	A	A	U	A	U	U	G	G	C	G		
	143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C			
	145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C	U	
	192-5p	C	U	G	A	C	C	U	A	U	G	A	A	U	U	G	A	C	A	G	C	C			
	181a-5p	A	A	C	A	U	U	C	A	A	C	G	U	U	G	U	C	G	G	U	G	A	G	U	
	181a-3p	A	C	C	A	U	C	G	A	C	C	G	U	U	G	A	U	U	G	U	A	C	C		
AML,ALL	184	U	G	G	A	C	G	G	A	G	A	A	C	U	G	A	U	A	A	G	G	G	U		

Table 3(B). Nucleotide sequences of suppressive miRNAs for various cancers.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	44	71	51	46	40	22	50	37	25	48	35	43	55	32	38	47	20	43	19	21	16	44
G	1	35	39	21	57	38	45	18	20	25	41	15	25	49	41	45	46	51	53	57	49	31
C	33	28	33	49	13	45	36	40	23	28	36	51	36	30	30	28	36	39	24	19	24	10
U	71	15	26	33	39	44	18	54	81	48	37	40	33	38	40	29	47	16	53	52	60	46

Table 4(A). Nucleotide frequencies at positions in the oncogenic miRNAs.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	22	31	48	24	38	9	42	31	22	46	13	30	21	22	26	26	46	17	26	12	16	9
G	10	45	23	47	43	26	20	47	20	17	47	40	14	21	57	17	21	27	42	54	19	22
C	11	16	29	28	11	18	27	10	16	24	31	22	19	13	11	25	17	11	16	20	26	13
U	69	20	12	13	20	59	23	24	54	25	21	20	58	56	18	44	28	57	28	26	50	47

Table 4(B). Nucleotide frequencies at positions in the suppressive miRNAs.

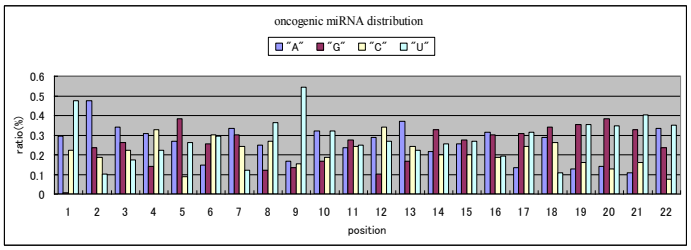

Figure 1(A). Oncogenic miRNA nucleotide distribution ratios.

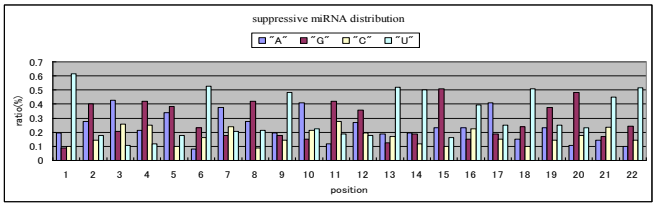

Figure 1(B). Suppressive miRNA nucleotide distribution ratios.

To analyze the differences statistically, two sample population ratio test (not in pairs) was used for the number of nucleotides at individual positions and the total number of nucleotides in all positions. The following significance test was carried out.

$$Z_s = \frac{|p_{as} - p_b|}{\sqrt{P(1-P)\left(1/n_{as} + 1/n_b\right)}}, \qquad \cdots\cdots\cdots (1)$$

where s is the site (position) 1 to 22, p_{as} is the probability of each nucleotide a occurring at each site s (a = A, G, C, or U), p_b is the occurrence probability of each nucleotide averaged over the entire target sequence population, P is the arithmetic mean of p_{as} and p_b, n_{as} is the number of nucleotides at position s, and n_b is the total number of nucleotides in all positions. As the two-sided statistical test has two types of significance values, higher (upper) and lower levels of significance, they are expressed as follows:

Higher-significance nucleotide (HN_s^v) and

Lower-significance nucleotide (LN_s^v),

where H denotes higher, L denotes lower, and N is a nucleotide,

v: 95-significance probability is 95% (level of significance = 0.05),

99-significance probability is 99% (level of significance = 0.01), and

s: nucleotide position (site) (i.e., 1–22).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	*HN*		5.4	2.1										2.7									
	LN						2.9			2.4								3.3		3.5	3.1	4	
G	*HN*					3.5									2.1				2.5	2.8	3.5	2.1	
	LN	6.6			2.8				3.3	3	2.1		3.9	2.1									
C	*HN*				3.1		2.4						3.4										
	LN					3.5															2.3		3.9
U	*HN*	4.8							2	6.4												3	
	LN		4.7	2.8	2			4.2									2.2		4.5				

HN: Higher nuleotide, *LN*: Lower nucleotide

Table 5(A). Values of higher- and lower-significance nucleotides in oncogenic miRNAs.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	*HN*			4.2		2.3		3.1			3.8							3.8					
	LN						3.7					2.8							2		3	2.1	3.2
G	*HN*		2.7		3	2.3			3			3				4.9				2.1	4.3		
	LN	4.3						2.2		2.2	2.8			3.4	2		2.8	2				2.4	
C	*HN*			2.1								2.5											
	LN								2.1														
U	*HN*	6.2					4.4			3.5				4.2	3.8				4			2.8	4.2
	LN		3	4.6	4.4	3		2.4	2.2		2	2.8	3			3.4							

HN: Higher nuleotide, *LN*: Lower nucleotide

Table 5(B). Values of higher- and lower-significance nucleotides in suppressive miRNAs.

miRNA gene-silencing score

Nucleotide frequencies at the individual positions listed in Tables 4(A) and 4(B) were analyzed by using Eq. (1), and many higher-significance and lower-significance nucleotides were obtained. They are listed in Tables 5(A) and 5(B). A miRNA sequence including many higher-significance nucleotides and a few lower-significance nucleotides could be inferred to be a highly effective gene silencer. A miRNA gene-silencing score S based on this idea is therefore defined as the sum of the higher-significance nucleotides in the sequence minus the sum of the lower-significance nucleotides in the sequence. That is,

$$S = \sum HN_s^v - \sum LN_s^v , \qquad \cdots\cdots\cdots\cdots (2)$$

where (HN_s^v) and (LN_s^v) are higher-significance and lower-significance nucleotides, respectively. The larger S is, the greater the likelihood that the sequence is effective for gene silencing. miRNA scores for the oncogenic and suppressive miRNAs listed in Tables 3(A) and 3(B) were calculated using Eq. (2). Their sums and averages for the individual cancers are listed in Table 6. One sees in Table 6 and Fig. 2 that overall there is a tendency that the average scores of suppressive miRNAs are greater than those of oncogenic ones. This dependency may be due to the big role that let-7 miRNAs play as suppressive miRNAs.

	oncogenic miRNAs		suppressive miRNAs	
	sum	average	sum	average
brain, GBM	68.2	13.64	64.97	7.22
breast	189.6	9.03	530.6	40.82
lung	215.3	11.33	611.09	17.46
esophagus	55.8	27.90	-4.23	-2.115
stomach	255.7	18.26	421.36	70.23
colorectal	350.2	16.68	519.05	37.08
hepatic cell	157.8	15.78	536.28	41.25
pancreas	446.3	14.40	-18.6	-3.72
prostage gland	309.2	22.09	78.35	15.67
cervical	47.9	23.95	33.44	16.72
B-CELL	44.6	14.87	74.08	10.58
tangue	8.4	8.4		
ovarian	109.2	18.20		
AML,ALL			15.83	15.83
Total	2258.2	15.16	2862.22	24.89

Table 6. Sums and averages of oncogenic and suppressive miRNAs for various cancers.

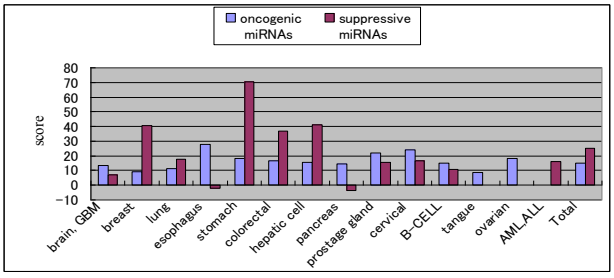

Figure 2. Relations between average scores of oncogenic and suppressive miRNAs for various cancers.

Higher- and lower-level nucleotides of oncogenic and suppressive miRNAs

Higher- and lower-level nucleotides at individual positions in oncogenic and suppressive miRNAs are listed in Tables 7(A) and 7(B). Since the higher-level nucleotides at individual positions are those that have a larger influence on the upregulation or down regulation due to the miRNAs, the miRNA sequences including many higher-level nucleotides may have a big impact on the target mRNA silencing. With this in mind, the coincidences between highly frequent miRNA sequences and higher-level nucleotides were examined for oncogenic and suppressive miRNAs of individual cancers. The results are listed in Tables 8(A) and 8(B). Eleven of the kinds of cancers listed in Table 2(A) are stimulated by hsa-miR-21, but one sees in Table 8(A) that there are only six nucleotide coincidences between the miR-21 sequence and higher-level nucleotides. On the other hand, although only seven kinds of cancers are stimulated by hsa-miR-17 and only five kinds are stimulated by hsa-mir-191 and hsa-miR-106a, the numbers of nucleotide coincidences between miR-17, -191, and -106a sequences and higher-level nucleotides are respectively ten, eight, and ten. This implies that the miR-17, -191, and -106a sequences influence the target mRNA regulations more than the miR-21 sequence does. In contrast, hsa-miR-145 suppresses six kinds of cancers and has six coincident nucleotides listed in Table 8(B), whereas although both let-7 and hsa-miR-143 suppress five kinds of cancers, their nucleotide coincidences are respectively twenty and ten as listed in Table 8(B). This also implies that the influences of let-7 and miR-143 are greater than the influence of miR-145.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Higher	U	A	A	C	G	C	X	U	U	X	X	C	A	G	X	X	X	G	G	G	U	X
																					G	
Lower	G	U	U	G	C	A	U	G	G	G	X	G	G	X	X	U	A	U	A	A	A	C
				U					A											C		

X: not specified nucleotide, i.e., X=A, G, C, or U

Table 7(A). Higher- and lower-level nucleotides in oncogenic miRNAs.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Higher	U	G	A	G	A	U	A	G	U	A	G	X	U	U	G	X	A	U	G	G	U	U
			C		G						C											
Lower	G	U	U	U	U	A	U	U	G	G	A	U	G	G	U	G	G	A	X	A	G	A
							G	C		U	U										A	

X: not specified nucleotide, i.e., X=A,G,C, or U

Table 7(B). Higher- and lower-level nucleotides in suppressive miRNAs.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Higher nucleotides	U	A	A	C	G	C	X	U	U	X	X	C	A	G	X	X	X	G	G	G	U	X
																					G	
miR-21-5p	U	A	G	C	U	U	A	U	C	A	G	A	C	U	G	A	U	G	U	U	G	A
	O	O		O				O										O			O	
miR-17-5p	C	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A
		O	O		O				O			O	O	O					O	O	O	
miR-92a-3p	U	A	U	U	G	C	A	C	U	U	G	U	C	C	C	G	G	C	C	U	G	U
	O	O			O	O			O												O	
miR-191-5p	C	A	A	C	G	G	A	A	U	C	C	C	A	A	A	A	G	C	A	G	C	U
		O	O	O	O				O			O	O							O		
miR-221-5p	A	C	C	U	G	G	C	A	U	A	C	A	A	U	G	U	A	G	A	U	U	U
					O				O				O					O			O	
miR-106a-5p	A	A	A	A	G	U	G	C	U	U	A	C	A	G	U	G	C	A	G	G	U	A
		O	O		O				O			O	O	O					O	O	O	

X: not specified nucleotide, i.e., X=A, G, C, or U

Table 8(A). Relations between higher-level nucleotides and highly frequent oncogenic miRNAs.

position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Higher nucleotides	U	G	A	G	A	U	A	G	U	A	G	X	U	U	G	X	A	U	G	G	U	U
			C		G						C											
miR-145-5p	G	U	C	C	A	G	U	U	U	U	C	C	C	A	G	G	A	A	U	C	C	C
			O		O				O		O				O		O					
let-7e-5p	U	G	A	G	G	U	A	G	U	A	G	G	U	U	G	U	A	U	G	G	U	U
	O	O	O	O	O	O	O	O	O	O	O		O	O	O		O	O	O	O	O	O
miR-143-3p	U	G	A	G	A	U	G	A	A	G	C	A	C	U	G	U	A	G	C	U	C	
	O	O	O	O	O	O					O			O	O		O					

X: not specified nucleotide, i.e., X=A, G, C, or U

Table 8(B). Relations between higher-level nucleotides and highly frequent suppressive miRNAs.

Relations between miRNA scores and nucleotide coincidences

Then the relations between miRNA scores and the number of coincident nucleotides of oncogenic and suppressive miRNA sequences were examined. As miRNA scores indicated different ranges from low to high for individual miRNA sequences, the score ranges were used to examine the relations between the scores and nucleotide coincidences of the higher and lower significance levels. In addition, the average coincident nucleotides in the score ranges were used at the higher and lower levels. Total coincident nucleotides were calculated as the number of coincident nucleotides at the higher level minus the number of coincident nucleotides at the lower level. The relations between miRNA scores and the number of coincident nucleotides of oncogenic and suppressive miRNAs are shown in Tables 9(A) and 9(B) and in Figures. 3(A) and 3(B). It is clear that the number of coincident nucleotides at the higher level for oncogenic miRNAs decreases gradually as the score ranges decrease, whereas the number of coincident nucleotides at the lower level increases as the score ranges decrease; and the total number of coincident nucleotides decreases distinctly as shown in Figure 3(A). This tendency is similar to the tendency in Figure 3(B).

score range	Higher	Lower	Total
31 ～	10.12	0.35	9.76
26 ～ 30	6.91	0.48	6.43
21 ～ 25	6.82	2.24	4.59
16 ～ 20	6.72	3.18	3.55
11 ～ 15	5.96	3.26	2.7
6 ～ 10	6	4.87	1.13
1 ～ 5	4.19	4.31	-0.13
-5 ～ 0	3.93	5.14	-1.21
-10 ～ -6	3	5.8	-2.8
～ -11	1.33	7.33	-6

Higher: No. of average coincident nucleotides at higher level
Lower: No. of average coincident nucleotides at lower level
Total: Higher - Lower

Table 9(A). Relations between miRNA scores and the number of co-incident nucleotides in oncogenic miRNAs.

score range	Higher	Lower	Total
71 ～	19.33	0	19.33
61 ～ 70	18.33	0.67	17.67
31 ～ 60	11	2.71	8.29
26 ～ 30	8	2	6
21 ～ 25	9	3.25	5.75
16 ～ 20	8.33	4.78	3.56
11 ～ 15	7	4.4	2.6
6 ～ 10	6.42	6	0.43
1 ～ 5	5.71	6.33	-0.63
-5 ～ 0	4.75	6.25	-1.5
-10 ～ -6	5	7.6	-2.6
～ -11	3.25	8.5	-5.25

Higher: No. of average coincident nucleotides at higher level
Lower: No. of average coincident nucleotides at lower level
Total: Higher - Lower

Table 9(B). Relations between miRNA scores and the number of co-incident nucleotides in suppressive miRNAs.

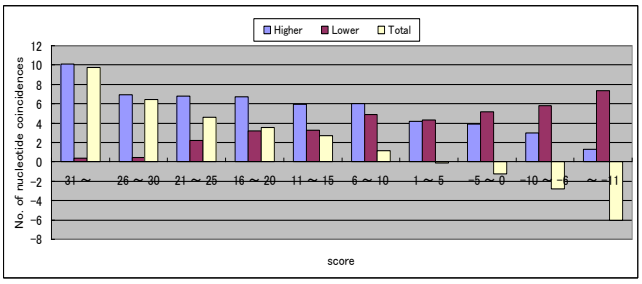

Figure 3(A). Relations between miRNA scores and the number of co-incident nucleotides in oncogenic miRNAs.

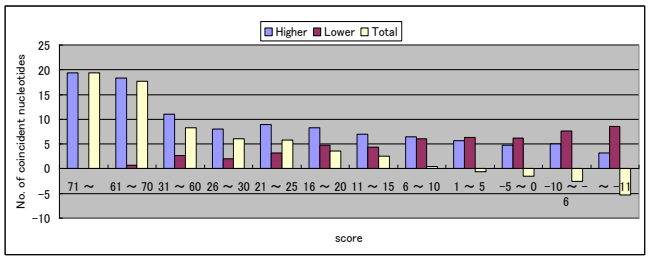

Figure 3(B). Relations between miRNA scores and the number of co-incident nucleotides in suppressive miRNAs.

Comparing Table 7(A) with Table 7(B), it become clear what nucleotides occur coincidentally in oncogenic and suppressive miRNA sequences at higher and lower significance levels. In case of the higher level, the nucleotides U, A, G, U, G, G, and U respectively occurred at positions 1, 3, 5, 9, 19, 20, and 21 in both oncogenic and suppressive miRNAs. On the other hand, in case of the lower level, the nucleotides G, U, U, U, A, U, G, G, G, A, and A respectively occurred at positions 1, 2, 3, 4, 6, 7, 9, 10,13, 20, and 21. These nucleotide coincidences imply that it is difficult to tell from only the higher- and lower-level nucleotides whether a miRNA is an oncogenic or suppressive one.

Frequencies of individual nucleotides in miRNAs

Frequencies of individual nucleotides were also examined for oncogenic and suppressive miRNAs in both the higher and lower significances as shown in Figure 4. It is clear that the most significant nucleotides as a whole are G and U. They play an especially important role in suppressive miRNAs. The least frequently occurring nucleotide is C. The occurrences of U at positions 2 to 12 in the lower-level rows of Table 7(B) indicate that this nucleotide has strong influences on the suppressive miRNAs.

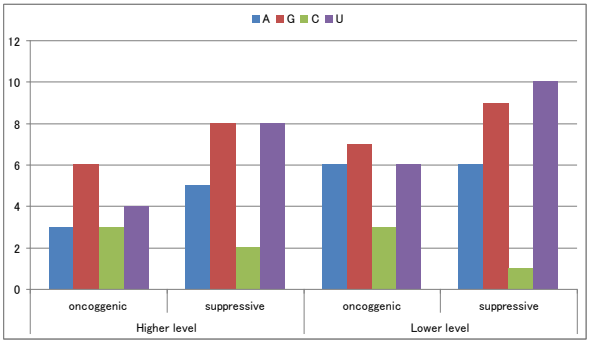

Figure 4. Frequencies of individual nucleotides at higher and lower levels.

It has been believed that nucleotides 2 to 8 in the 5' portion of miRNAs are most important for target recognition by vertebrate miRNAs. The higher- and lower-level nucleotides in oncogenic and suppressive miRNAs listed in Tables 7(A) and 7(B) were examined from that point of view. The nucleotide coincidences of the higher levels between oncogenic and suppressive miRNAs are two (i.e., at positions 3 – A and 5 – G), whereas those of the lower level are five (at positions 2 – U, 3 – U, 4 – U, 6 – A, and 7 – U). At the higher level the most frequently occurring nucleotides in positions 2 to 8 are A (five) and G (five), whereas at the lower level the most frequently occurring nucleotide is U (ten) and the second-most frequently occurring one is G (three).

Distinguishing whether a new miRNA is oncogenic or suppressive

It is important to know what function a new miRNA has, especially whether the miRNA is oncogenic or suppressive. A new method to distinguish the function of a new miRNA can be based on the higher-level nucleotides and miRNA scores and is shown in Figure 5. The determination processes are as follows:

Step 1: Examine the possibility that it is an oncogenic miRNA.

- Count the numbers of higher- and lower-level nucleotides in it according to the nucleotides listed in Table 7(A).
- Calculate the score of the new miRNA by using Eq. (2) and Table 5(A).

Step 2: Examine the possibility that it is a suppressive miRNA.

- Count the numbers of higher- and lower-level nucleotides in it according to the nucleotides listed in Table 7(B).
- Calculate the score of the new miRNA by using Eq. (2) and Table 5(B).

Step 3: Distinguish whether the new miRNA is an oncogenic one or a suppressive one.

-Whether the new miRNA is oncogenic or suppressive is determined on the basis of Figs. 3(A) and 3(B) and Tables 7(A) and 7(B). If the numbers of the higher- and lower-level nucleotides of the new miRNA are respectively greater than ten and less than two (i.e., the total number is greater than eight) and the score based on Fig. 3(A) and Table 7(A) is greater than 30, the new miRNA is likely to be oncogenic. If, on the other hand, the total number is greater than 17 and the score based on Fig. 3(B) and Table 7(B) is greater than 61, the new miRNA is likely to be suppressive. If neither of these conditions applies to the new miRNA, the possibility of cancer regulations by the new miRNA is estimated to be low.

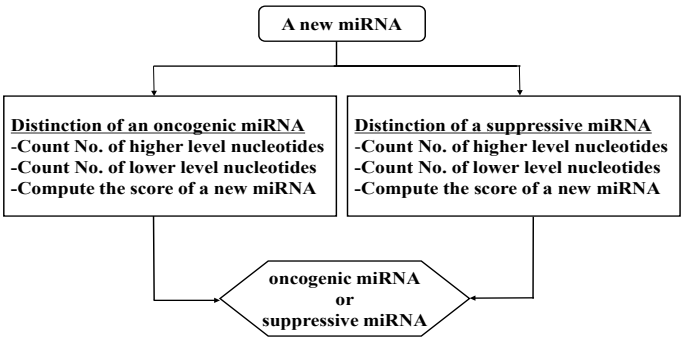

Figure 5. Distinguishing whether a new miRNA is oncogenic or suppressive.

Conclusion

In this paper, the sequences of oncogenic and tumor suppressive miRNAs in human cancers were analyzed from the viewpoint of up- and down-regulation. As a result, the statistical analysis of the positional nucleotide occurrence features of miRNAs revealed differences between the positional nucleotide occurrences of oncogenic and suppressive miRNAs. A miRNA gene-silencing score was then defined on the basis of the higher and lower levels of the statistical significances of positional nucleotides. Since the miRNA scores were closely related to miRNA frequencies, a method using the scores and nucleotide frequencies to distinguish whether a new miRNA is oncogenic or suppressive was proposed.

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