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miRNA Roles: Sequence Analyses of Oncogenic and Tumor Suppressive miRNAs

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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 relations between the sequences of cancer-related miRNAs (both oncogenic and tumor suppressive) and those of control miRNAs in human beings are examined from the viewpoint of nucleotide frequencies at individual positions. Oncogenic and tumor suppressive miRNAs are involved in the overexpression/upregulation and underexpression/downregulation of cancers, whereas control miRNAs are not involved in cancer development and progression. The 132 oncogenic, 111 tumor suppressive, and 1610 control miRNA sequences investigated in this work were collected from miRBase on the basis of the relations between miRNAs of *Homo sapiens* and various cancers in the literature. Statistical analyses of the positional nucleotide occurrence features revealed clear differences between the cancer-related and control miRNAs. This indicates that miRNAs can be used as biomarkers in human cancers.

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

Introduction

MicroRNAs (miRNAs) are small (~25 nucleotides) noncoding RNA molecules that regulate gene expression post-transcriptionally by base-pairing to mRNAs [1-5].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 cleaved by the type III RNase Dicer to a ~25-nucleotide product. MicroRNAs regulate the expression levels of other genes by several mechanisms, generally reducing protein levels by cleaving mRNAs or repressing their translation [2].

Many miRNAs have been reported over the past several years to be related to the expression and progression of various cancers, and miRNA profiling has recently been considered a diagnostic and prognostic tool useful for indicating an oncogene or a tumor suppressor. That is, miRNAs may be biomarkers of various cancers [6-8].

In this paper, to investigate the relations between individual miRNAs and various cancers, oncogenic and tumor suppressive miRNA sequences (hereafter called cancer-related miRNA sequences) and control miRNA sequences were collected from miRBase according to the relations between miRNAs and various cancers in the literature [9,10]. Examination of the individual nucleotide frequencies in these sequences at positions 1 to 22 revealed clear differences between the cancer-related and control miRNAs. Consideration of the positional nucleotide occurrences of cancer-related and control miRNAs from the viewpoint of statistical significance also indicates that there are clear differences between them and implies that miRNAs can be used as biomarkers in human cancers.

Sequence Analyses of Oncogenic and Tumor Suppressive miRNAs in Cancers

Relations between Cancer-Related and Control miRNAs

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 [11]. Other studies have also reported that miRNAs play roles in the expression of oncogenes and 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

Table 1. Relations between human cancers and miRNAs.

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,195,138,375,497	hsa-let-7, hsa-miR-15a, 16, 125a, 125b, 127, 145, 204, 146a, 1268
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 multiforme, B-CLL: B cell chromic lymphocytic leukemia
AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia

downregulated in those cancers are listed as suppressive miRNAs [3,7,8,11-44]. It is clear from Table 1 that there are differences in occurrences of the oncogenic and suppressive miRNAs for the individual cancers.

Table 2. Control miRNAs.

chromosome	No. of miRNAs	typical miRNAs
1	133	hsa-miR-137, 760, 761, ...
2	72	hsa-miR-217, 375. 1258, ...
3	80	hsa-miR-198, 711, 1248, ...
4	48	hsa-miR-297, 1243, 1305, ...
5	53	hsa-miR-583, 1244, 1289, ...
6	59	hsa-miR-206, 1202, 1275, ...
7	55	hsa-miR-1200, 1302, 3666, ...
8	65	hsa-miR-1204, 1205, 1206, ...
9	68	hsa-miR-1299, 2861, 3154, ...
10	56	hsa-miR-107, 346, 607, ...
11	98	hsa-miR-326, 1261, 3920, ...
12	61	hsa-miR-492, 920, 1279, ...
13	21	hsa-miR-759, 1267, 1297, ...
14	121	hsa-miR-300, 496, 543, ...
15	51	hsa-miR-184, 1272, 1276, ...
16	65	hsa-miR-762, 1538, 1972, ...
17	90	hsa-miR-1203, 1253, 2909, ...
18	31	hsa-miR-1539, 3929, 4317, ...
19	160	hsa-miR-498, 521, 527, ...
20	37	hsa-miR-298, 1257, 1289, ...
21	17	hsa-miR-802, 3687, 4327, ...
22	43	hsa-miR-1281, 1286, 3909, ...
X	121	hsa-miR-325, 384, 448, ...
Y	2	hsa-miR-3690, 6089

On the other hand, the control miRNAs of *Homo sapiens* can be obtained from miRBase based on considering not related to various cancers. The samples of the obtained control miRNAs are listed in Table 2. The numbers of the oncogenic, tumor suppressive, and control miRNAs were respectively 132, 111, and 1610.

Differences between Cancer-Related and Control miRNAs

To analyze the differences between cancer-related and control miRNAs, the miRNA nucleotide sequences for the oncogenic/tumor suppressive and control miRNAs listed in Tables 1 and 2 were collected from miRBase [9,10].From the obtained miRNA sequences, the frequencies of the four nucleotides (A, G, C, U) at positions from 1 to 22 were determined. These frequencies are listed in Tables 3(A), 3(B), and 3(C). Using Tables 3(A), 3(B), and 3(C), the total frequency ratios of individual nucleotides from positions 1 to 22 were determined for the oncogenic, tumor suppressive and control miRNA sequences. They are listed in Table 4.

Table 3(A). Nucleotide Frequencies of the 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	41	61	46	40	35	21	47	33	24	43	29	39	49	26	34	44	16	36	19	20	15	37
G	1	34	31	20	49	35	37	17	15	21	34	12	23	43	35	38	42	47	43	50	41	27
C	28	26	33	45	11	38	31	34	20	27	36	45	30	27	28	25	31	36	20	17	22	10
U	62	11	22	27	37	38	17	48	73	41	33	36	30	36	35	25	43	13	50	45	54	43

Table 3(B). Nucleotide Frequencies of the Tumor Suppressive miRNAs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	22	30	48	24	38	9	41	31	22	46	13	30	21	22	26	26	46	17	26	12	16	9
G	10	45	23	47	42	26	20	47	20	17	46	40	14	21	57	16	20	27	42	54	18	22
C	11	16	29	28	11	17	27	9	16	24	31	22	18	12	10	25	17	10	15	20	26	13
U	68	20	11	12	20	59	23	24	53	24	21	19	58	56	18	44	28	57	28	25	50	46

Table 3(C). Nucleotide Frequencies of the Control miRNAs.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A	420	432	412	354	360	359	390	354	320	314	349	355	302	350	317	296	289	293	294	295	271	191
G	240	430	523	587	602	499	446	440	445	502	523	457	515	482	469	421	454	421	402	441	419	276
C	366	377	340	370	312	387	410	416	375	393	388	361	343	350	385	446	448	386	373	320	264	205
U	584	371	335	299	336	365	364	400	470	401	350	437	450	428	439	447	411	449	412	356	332	276

Table 4. Total Frequency Ratios of Individual Nucleotides for Oncogenic, Tumor Suppressive, and Control miRNA Sequences.

nucleotide	oncogenic (%)	tumor suppressive (%)	control (%)
A	26.1	23.8	21.5
G	24.1	27.9	29.4
C	21.5	16.8	23.5
U	28.3	31.6	25.6

As a whole, the ratios of the nucleotides A and U in the oncogenic and tumor suppressive miRNA sequences are higher than those in the control sequences, whereas the ratios of the nucleotides G and C in the cancer miRNA sequences are lower than those in the control sequences.

The ratios of individual nucleotide at positions from 1 to 22 for the oncogenic, tumor suppressive and control miRNA sequences are shown Figures 1(A), 1(B), and 1(C). There are differences in nucleotide distribution ratios at individual positions for oncogenic, tumor suppressive, and control miRNAs.

The relations between individual nucleotides at positions 1 to 22 of oncogenic, tumor suppressive, and control miRNAs were then analyzed as shown in Figures 2(A), 2(B), 2(C), and 2(D).

Although there are differences in individual nucleotides at positions 1 to 22, it is not clear what position and what

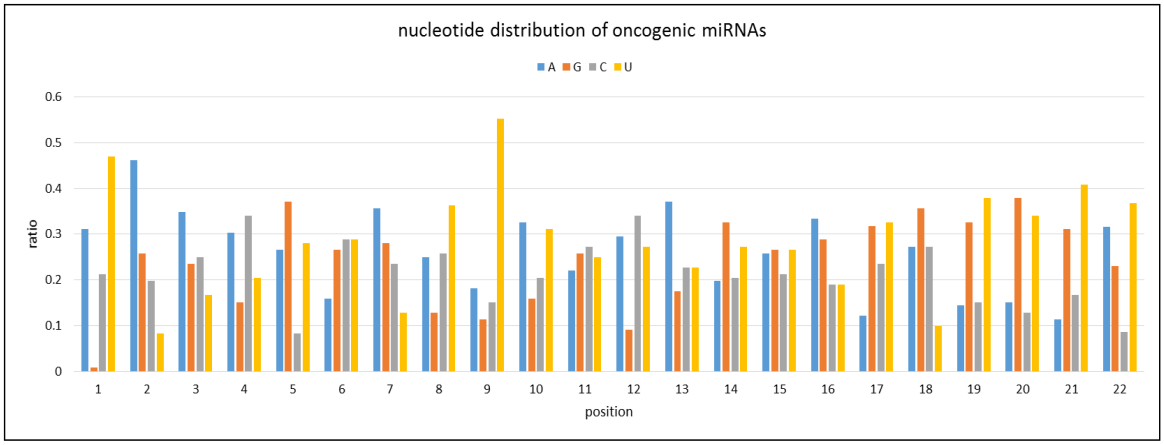

Figure 1(A) Oncogenic nucleotide distribution ratios.

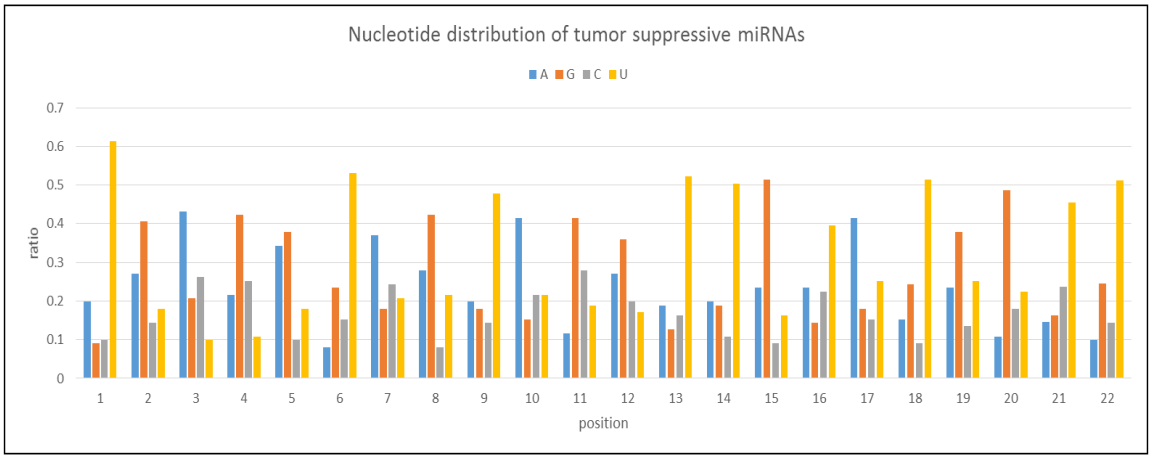

Figure 1(B) Tumor suppressive nucleotide distribution ratios.

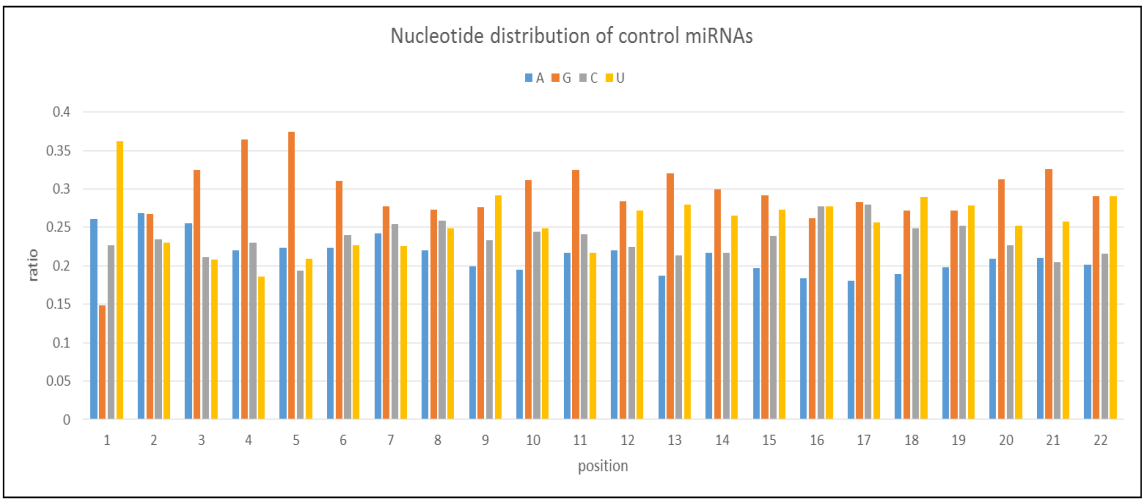

Figure 1(C) Control nucleotide distribution ratios.

Table 5. Distinct Features of Individual Nucleotides among Oncogenic, Tumor Suppressive, and Control miRNAs.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
oncogenic miRNAs	A		high											high									high
	G	low			low																		
	C												high										
	U								high											high	high		
tumor-suppressive miRNAs	A																	high					
	G		high						high						low	high	low	low				low	
	C	low							low						low	low			low				
	U		high				high							high	high				high				high
control miRNAs	A																						
	G										high		high										
	C					high				high										high			
	U																						

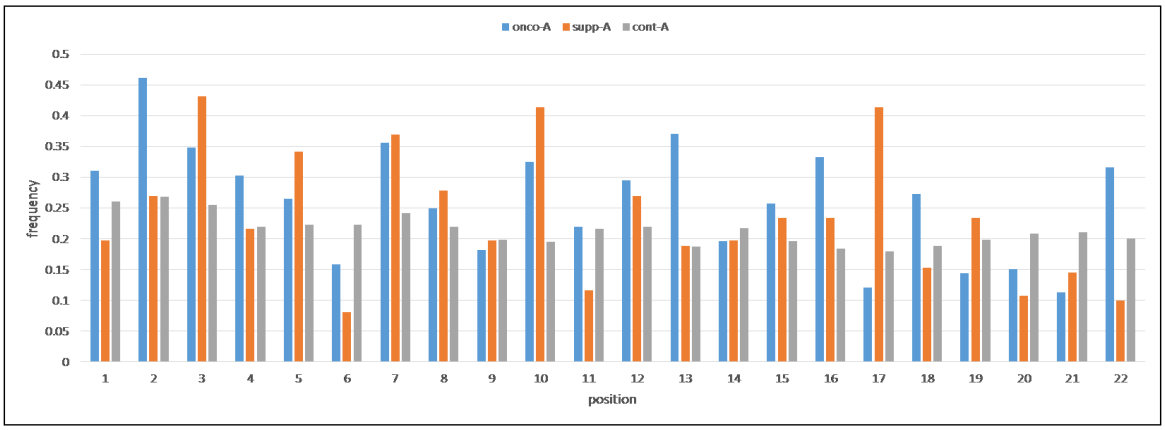

Figure 2(A) Relations between nucleotide A of oncogenic, tumor suppressive and control at individual positions.

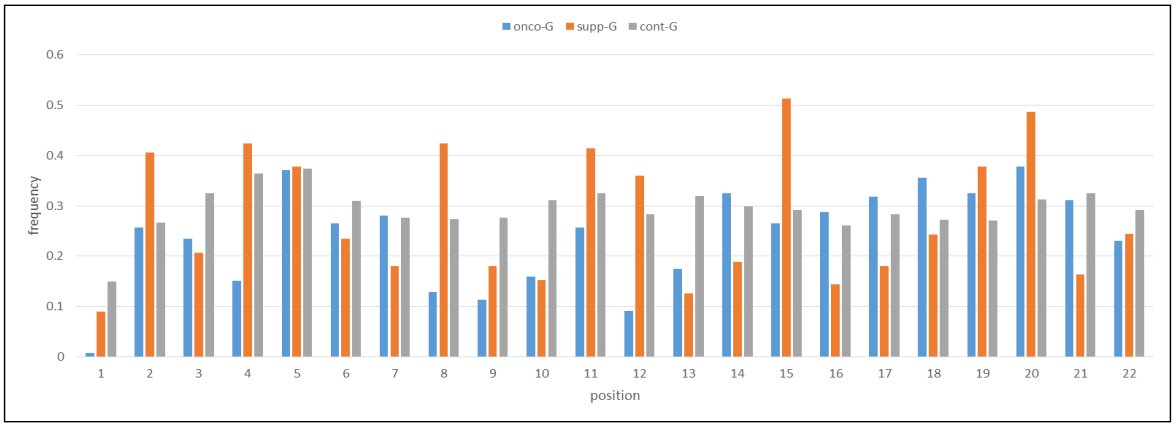

Figure 2(B) Relations between nucleotide G of oncogenic, tumor suppressive and control at individual positions.

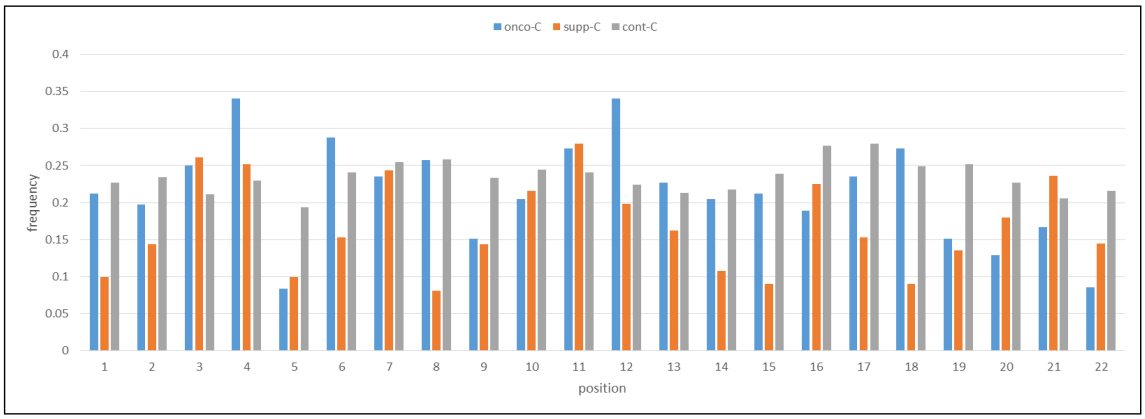

Figure 2(C) Relations between nucleotide C of oncogenic, tumor suppressive and control at individual positions.

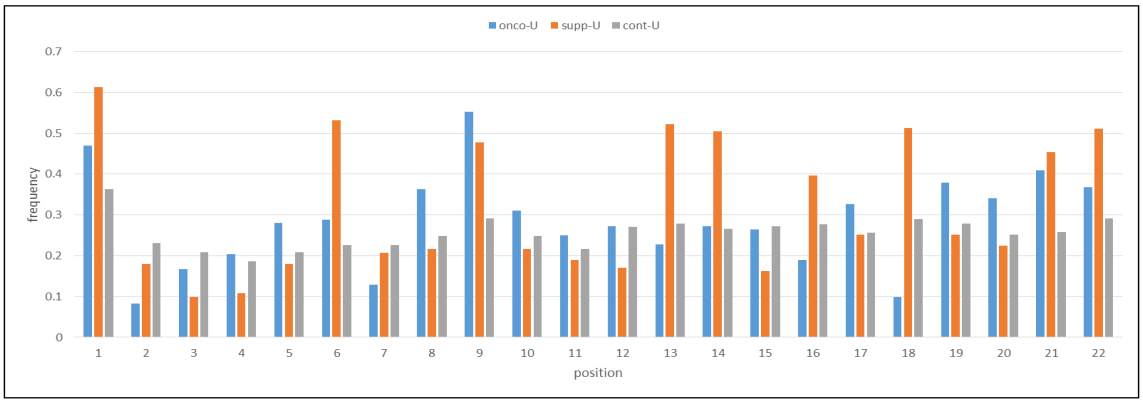

Figure 2(D) Relations between nucleotide U of oncogenic, tumor suppressive and control at individual positions.

nucleotide is clearly different from other ones. Therefore the distinct features of individual nucleotides among the oncogenic, tumor suppressive and control miRNAs were summarized as "high" and "low" (Table 5). It is clear that there are distinct different features of individual nucleotides among oncogenic, tumor suppressive, and control miRNAs.

In addition, the differences of individual nucleotides between oncogenic and control miRNAs, the differences of individual nucleotides between tumor suppressive and control miRNAs, and the differences of individual nucleotides between oncogenic and tumor suppressive miRNAs were examined based on Figures 2(A), 2(B), 2(C), and 2(D). They are summarized as follows:

Differences between Oncogenic and Control miRNAs

Nucleotide A: As a whole, the frequency of A in the oncogenic miRNAs is 4.6% higher than that in the control miRNAs. As shown in Figure 2(A), the especially higher positions are 2, 10, 13, 16, and 22.

Nucleotide G: As a whole, the frequency of G in the oncogenic miRNAs is 5.3% lower than that in the control miRNAs. As shown in Figure 2(B), the especially lower positions are 1, 4, 8, 9, 10, 12, and 13.

Nucleotide C: On the average, the frequency of C in the oncogenic miRNAs is 2% lower than that in the control miRNAs. As shown in Figure 2(C), however, there are clearly two kinds of positions with regard to the relative C frequency in the oncogenic miRNAs: the higher positions (i.e., positions 4 and 12) and the lower positions (i.e., positions 5, 9, 19, 20, and 22).

Nucleotide U: On the average, the frequency of U in the oncogenic miRNAs is 2.7% higher than that in the control miRNAs. As shown in Figure 2(D), there are two kinds of positions with regard to the relative U frequency in the oncogenic miRNAs: the higher positions (positions 9 and 21) and the lower positions (positions 2 and 18).

Differences between Tumor Suppressive and Control miRNAs

Nucleotide A: On the average, the frequency of A in the tumor suppressive miRNAs is 2.3% higher than that in the control miRNAs. As shown in Figure 2(A), however, there are clearly two kinds of positions with regard to the relative A frequency in the tumor suppressive miRNAs: the higher positions (positions 3, 5, 7, 10, and 17) and the lower positions (positions 6, 11, 20, and 22).

Nucleotide G: On the average, the frequency of G in the tumor suppressive miRNAs is 1.5% lower than that in the control miRNAs. As shown in Figure 2(B), however, again there are two kinds of positions with regard to the relative G frequency: the higher positions (2, 8, 15, and 20) and the lower positions (3, 10, 13, 14, 16, and 21).

Nucleotide C: As a whole, the frequency of C in the tumor suppressive miRNAs is 5.7% lower than that in the control miRNAs. As shown in Figure 2(C), the lower positions are positions 1, 5, 6, 8, 9, 14, 15, 17, 18, and 19.

Nucleotide U: As a whole, the nucleotide frequency of the tumor suppressive miRNAs is 6% higher than that of the control miRNAs. As shown in Figure 2(D), the higher positions are 1, 6, 9, 13, 14, 18, 21, and 22.

Differences between Oncogenic and Tumor Suppressive miRNAs

Nucleotide A: On the average, the frequency of A in the oncogenic miRNAs is 2.3% higher than that in the tumor suppressive miRNAs. As shown in Figure 2(A), the higher positions are 1, 2, 6, 11, 13, 16, 18, and 22, whereas the lower positions are 3, 5, 10, 17, and 19.

Nucleotide G: As a whole, the frequency of G in the oncogenic miRNAs is 3.8% lower than that in the tumor suppressive miRNAs. As shown in Figure 2(B), the lower positions are 1, 2, 4, 8, 11, 12, 15, and 20, whereas the higher positions are 7, 14, 16, 17, 18, and 21.

Nucleotide C: As a whole, the frequency of C in the oncogenic miRNAs is 4.7% higher than that in the tumor suppressive miRNAs. As shown in Figure 2(C), the higher positions are 1, 4, 6, 8, 12, 14, 15, 17, and 18, whereas the lower positions are 21 and 22.

Nucleotide U: On the average, the frequency of U in the oncogenic miRNAs is 3.3% lower than that in the tumor suppressive miRNAs. As shown in Figure 2(D), the lower positions are 1, 6, 13, 14, 16, 18, and 22, whereas the higher positions are 4, 5, 8, 10, 12, 15, 19, and 20.

The above analyses revealed that there are differences in individual nucleotide occurrences at positions among oncogenic, tumor suppressive, and control miRNAs. Although there were differences in nucleotide distribution ratios, it is not clear that how nucleotide differences are significant statistically.

Positional Nucleotide Features of Cancer-Related and Control miRNAs

To analyze the significance of the nucleotide frequency differences statistically, two-sample population testing (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.

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

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).

Nucleotide frequencies at the individual positions listed in Tables 3(A), 3(B), and 3(C) were analyzed by using Eq. (1), and many higher-significance and lower-significance nucleotides were obtained. They are listed in Tables 6(A), 6(B), and 6(C).

Table 6(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*		5.4	2.09				1.9						2.7									1.9
	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		3.86	2.1										
C	*HN*				3.1		2.4					3.4											
	LN					3.5															2.3		3.9
U	*HN*	4.8							2	6.4										1.8	1.7	3	1.7
	LN		4.7	2.8	2.01			4.2									2.2		4.5				

HN: Higher nuleotide, *LN*: Lower nucleotide

Table 6(B). Values of Higher- and Lower-Significance Nucleotides in Tumor Suppressive 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*			4.23		2.3		3.1			3.81							3.81					
	LN						3.69					2.8							2		2.96	2.1	3.2
G	*HN*		2.7		3.03	2.3			3.03			3	1.7			4.89				2.1	4.3		
	LN	4.3						2.17		2.17	2.8			3.4	2		2.8	2				2.4	
C	*HN*			2.1	1.9			1.7				2.5											
	LN	1.9				1.9			2.1							1.9			1.9				
U	*HN*	6.2					4.41			3.5				4.2	3.8				4.01			2.78	4.2
	LN		3	4.6	4.4	3		2.4	2.2		2	2.8	3			3.38					1.8		

HN: Higher nuleotide, *LN*: Lower nucleotide

Table 6(C). Values of Higher- and Lower-Significance Nucleotides in Control 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	4.6	3.6				2.4															
	LN													2.4			2.8	3.1	2.3				
G	*HN*			2.5	5.8	6.5						2.5		2.1								2.6	
	LN	12.1	2.2														2.7						
C	*HN*							1.98									3.59	3.84					
	LN			2.09		3.58																2.61	
U	*HN*	8.89								3.02				2.02					2.85				2.94
	LN		2.21	4.09	6	4	2.46	2.55				3.32											

HN: Higher nuleotide, *LN*: Lower nucleotide

Higher- and Lower-Level Nucleotides of Oncogenic, Suppressive, and Control miRNAs

Higher- and lower-level nucleotides at individual positions in oncogenic and control miRNAs and in suppressive and control miRNAs are listed in Tables 7(A) and 7(B). Since the higher- and lower-level nucleotides at individual positions are those that have a larger influence on the upregulation or downregulation due to miRNAs, the coincidences between higher- and lower-level nucleotides of oncogenic and control miRNAs and the coincidences between higher- and lower-level nucleotides of suppressive and control miRNAs were examined. The coincidences between individual nucleotides of oncogenic and control miRNAs and the coincidences between individual nucleotides of suppressive and control miRNAs at individual positions are listed in Table 8. As the degrees of coincidences of each distinct nucleotide are not clear in the tables, they are shown in Figures 3(A) and 3(B). Although one can infer from Tables 7 and 8 and Figures 3(A) and 3(B) that there are differences in the coincidences between individual nucleotides of oncogenic/suppressive and control miRNAs, how many nucleotides are totally different in oncogenic/suppressive and control miRNAs is not clear. To clarify the differences quantitatively, the number of same and different nucleotides at individual positions were examined. The results are listed in Table 9. In the higher oncogenic miRNAs, the number of the same nucleotides is 7, whereas the number of the different nucleotides is 19. This means that the different nucleotides are 2.71 times more likely than the same nucleotides in the comparison with the oncogenic and control miRNAs. Similarly, in the lower oncogenic miRNAs, the different nucleotides are 3.14 times more likely than the same nucleotides. There are similar tendencies in the higher and lower suppressive miRNAs, i.e., the different nucleotides are respectively 2.11 times and 2.56 times more frequently than the same nucleotides. From the nucleotide position point of view, there is a tendency that there are many different nucleotides from positions 12 to 22 (Table 9).

Table 7(A). Higher- and Lower-Level Nucleotides in Oncogenic and Control 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	cancer	U	A	A	C	G	X	A	X	U	X	X	C	A	G	X	X	X	G	G	G	U	U
																				U			
	control	U	A	A	G	G	X	A	C	U	X	G	X	G	X	X	C	C	U	X	X	G	U
		A	G	G										U									
Lower	cancer	G	U	U	G	C	A	U	G	G	G	X	G	X	X	X	U	A	U	A	A	A	C
					U					A											C		
	control	G	U	U	U	U	U	U	X	X	X	U	X	A	X	X	A	A	A	X	X	C	X
			G	C		C											G						

X: unspecified nucleotide (i.e., X=A, G, C, or U)

Table 7(B). Higher- and Lower-Level Nucleotides in Suppressive and Control 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	cancer	U	G	A	G	A	U	A	G	U	A	G	X	U	U	G	X	A	U	G	G	U	U
				C	C	G						C											
	control	U	A	A	G	G	X	A	C	U	X	G	X	G	X	X	C	C	U	X	X	G	U
		A	G	G										U									
Lower	cancer	G	U	U	U	U	A	G	C	G	G	A	U	G	X	U	G	G	A	X	A	C	A
								U	U		U	U				C			C			A	
	control	G	U	U	U	U	U	U	X	X	X	U	X	A	X	X	A	A	A	X	X	C	X
			G	C		C											G						

X: unspecified nucleotide (i.e., X=A, G, C, or U)

Table 8. Coincidences between Individual Nucleotides of Cancer and Control miRNAs.

		position	1				2				3				4				5				6				7				8				9				10				11			
		nucleotide	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U
oncogenic	higher	cancer					○	○			○	○					○		○						○						○						○										
		control	○				○	○	○			○	○				○								○						○						○									
		difference	×						×		×				×	×																	×													
	lower	cancer		○						○				○		○	○			○			○					○			○			○	○				○							
		control		○				○		○			○	○			○	○				○				○				○																
		difference						×				×				×				×	×					×	×									×	×				×					
suppressive	higher	cancer				○		○			○		○				○	○			○	○					○	○				○					○	○								
		control	○				○	○	○			○	○				○								○						○						○									
		difference	×					×				×	×				×																×													
	lower	cancer		○					○			○		○				○	○				○				○	○			○	○			○				○		○					
		control	○				○		○		○	○			○			○				○				○				○															
		difference						×				×				×	×				×	×					×	×					×	×				×								

		position	12				13				14				15				16				17				18				19				20				21				22			
		nucleotide	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U	A	G	C	U
oncogenic	higher	cancer			○		○																				○				○		○		○					○							○
		control						○											○				○					○				○						○								○
		difference		×			×	×												×				×				×	×			×	×					×	×							
	lower	cancer	○																○	○															○	○										○	
		control																			○	○			○				○											○						
		difference	×																		×	×			×				×	×										×	×					
suppressive	higher	cancer							○						○				○				○				○				○				○										○
		control					○	○											○				○				○											○								○
		difference					×								×				×				×	×				×			×								×	×						
	lower	cancer			○				○							○	○		○				○				○	○							○	○	○									
		control							○							○	○				○				○													○								
		difference				×		×	×					×	×	×					×	×				×				×							×								

Table 9. The Relations Between Same and Different Nucleotides in Oncogenic/Suppressive and Control miRNAs.

		position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	total
oncogenic	higher	No. of same nucleotides	1	1	1		1		1		1													1	7
		No. of different nucleotides	1	2	1	2				1				1	2			1	1	2	2	1	2		19
	lower	No. of same nucleotides	1	1	1	1	1		1										1						7
		No. of different nucleotides		1	1	1	1	2		1	2	1		1				3		2	1	2	2	1	22
suppressive	higher	No. of same nucleotides	1	1	1	1	1		1		1				1						1				9
		No. of different nucleotides	1	1	2	1	1	1		2		1			1		1	1	2		1	1	2		19
	lower	No. of same nucleotides	1	1	1	1	1		1									1		1			1		9
		No. of different nucleotides		1	1		1	2	1	2	1	2		1	2		2	1	2	1		1	1	1	23

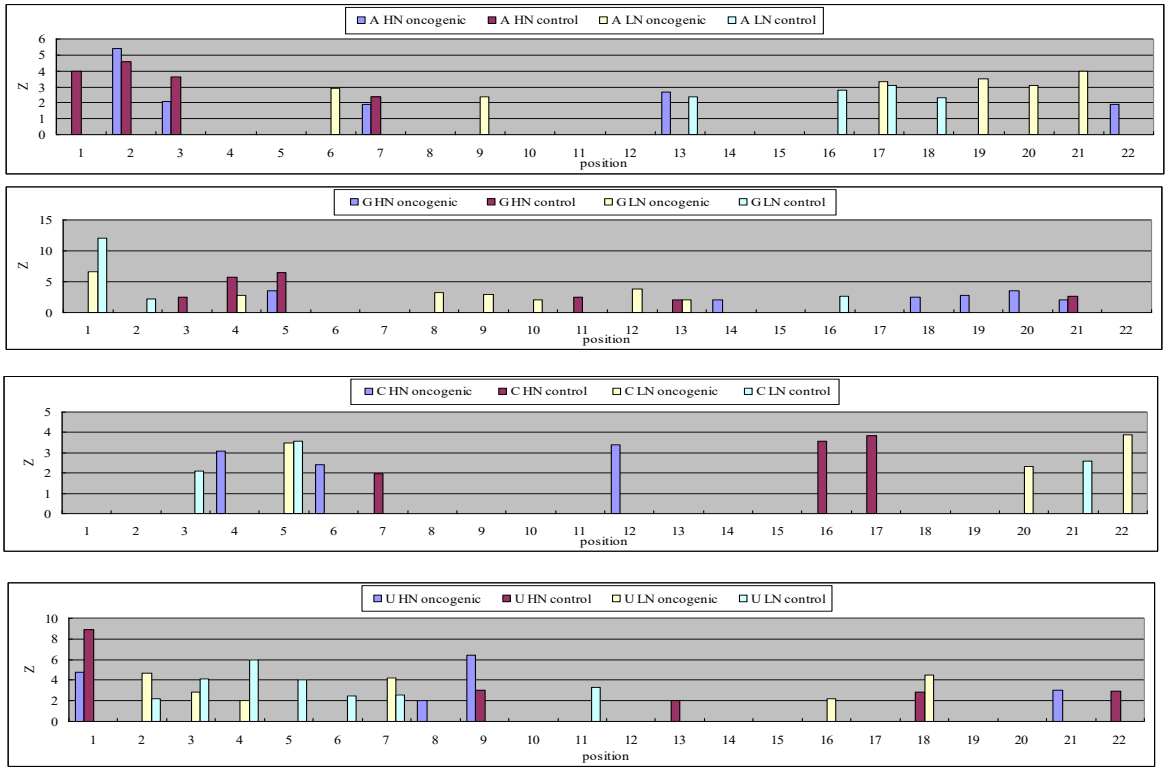

Figure 3(A) Degrees of coincidences between individual nucleotides of oncogenic and control miRNAs.

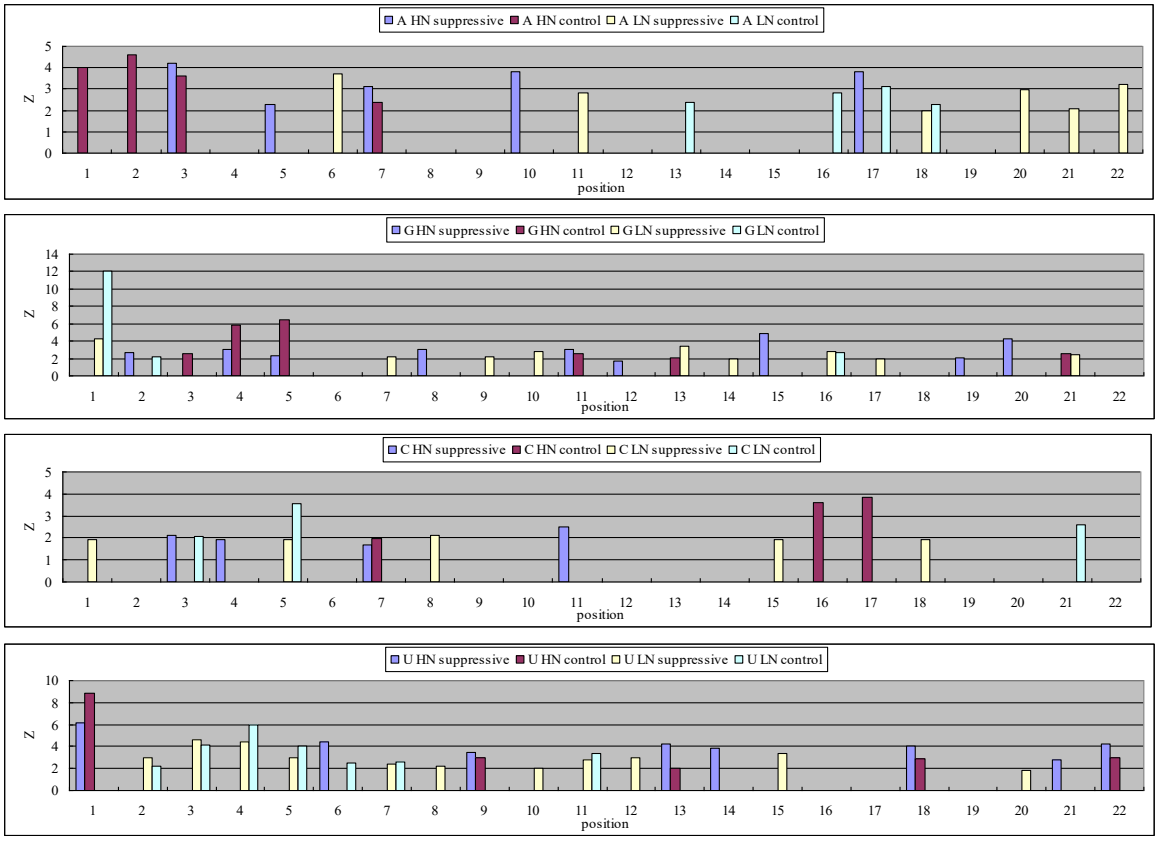

Figure 3(B) Degrees of coincidences between individual nucleotides of suppressive and control miRNAs.

Conclusion

In this paper the cancer-related (oncogenic and tumor suppressive) and control miRNAs of human being were analyzed and clear differences between the positional occurrences of cancer-related and control miRNAs were shown. This paper also considered the positional nucleotide occurrences of cancer-related and control miRNAs from the viewpoint of statistical significance. This result also indicates that there are clear differences between them and implies that miRNAs can be used as biomarkers in human cancers.

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