

Original Research Article

MicroRNA Memory I: the positive correlation between synergistic effects of microRNAs in cancer and a novel quantum scoring system

ABSTRACT

Aim: To investigate quantum characters of the microRNA (miRNA) gene as the disease memory device, at the beginning, a novel quantum scoring method was developed and its usability was confirmed by valuating miRNA–miRNA interaction.

Background: In general, activated miRNAs participate in regulation of gene expression via mainly inhibiting translation of messenger RNAs (mRNAs) into proteins. MiRNAs selects their target mRNAs by the degree of sequence complementarity between miRNAs and target sites of mRNA. Therefore, a precious prediction of miRNA–mRNA interaction would be useful for clear up miRNA–protein functions in biological processes. However, although many computer algorithms for miRNA target prediction have been developed and improved, they still have shown many incorrect results. Thus, much more precise prediction tool *in silico* by using computers and databases is necessary.

Methods: First, the miRNA–miRNA synergism was investigated according to modified previous algorism, and some synergistically-working miRNA pairs were collected. Next, we developed two new quantum scores, Static Nexus Score (SNS) and then Dynamic Nexus Score (DNS), and calculated them for each synergistic miRNA pair. SNS and DNS were derived from the miRNA-miRNA matrix that calculates quantum interaction between miRNAs from quantum energy in each single miRNA nucleotide. The synergistic activities of the miRNA functional pairs were evaluated upon the fold changes as synergistic effect (SE).

Results: The relation of SE in miRNA functional activity was subsequently examined with DNSs. As a result, positive correlation ($R = 0.55$, $P < .06$) was observed between DNS and strength of SE of each synergistic miRNA pair which is related to oncogenesis or tumor suppression.

Conclusions: The single miRNA-miRNA synergism would be important to predict biological function of miRNAs in cancer. This is the first report arguing that miRNA synergism could be showed through calculating DNS of each miRNA pair as miRNA memory devise.

33 Keywords: MicroRNA (miRNA), Noncoding RNA, Algorism, Quantum, Synergism, Cancer

34

35 ABBREVIATIONS

36 SE Synergistic Effect

37 SNS Static Nexus Score

38 DNS Dynamic Nexus Score

39 TSS Transcription Start Site

40 FMO Fragment Molecular Orbital method

41

42 1. INTRODUCTION

43 MicroRNAs (miRNAs), which are approximately 20 nucleotides long, belong to the small
44 non-coding RNAs [1]. These RNAs were once considered RNA fragments, but it has recently
45 been revealed that miRNAs are gene, which are able to regulate biological processes in humans
46 and other species, such as cell cycle, apoptosis, cell proliferation, immunoreaction, and
47 tumorigenesis [2–7]. The functional miRNA complex is composed of the Argonaute protein
48 binding to both the mRNA and miRNA, and this complex is able to suppress gene expression
49 post-transcriptionally [8,9]. However, *in vivo* and *in vitro* experiments demonstrated that certain
50 miRNAs may augment gene expression [10]. Individual miRNAs can interact with hundreds of
51 mRNAs, and a single mRNA is controlled by numerous miRNAs; however, the binding affinity is
52 different between each miRNA and mRNA [3,11,12]. Although interactions between miRNA and
53 mRNA can be predicted using the seed theory-based algorithm, *in vitro* or *in vivo* experiments do
54 not always confirm the predicted binding [13].

55

56 Table 1. Existing target prediction services for miRNA

Service Name	Base algorism	Filtering option	References
TargetScan	seed complementarity	conservation, target site abundance	[15,16]
miRanda	complementarity	thermodynamics	[15,16]
miRDB	support vector machine	conservation, expression profile, functional annotation	[14-16]
DIANA-microT	thermodynamics	seed complementarity, conservation, target site abundance	[15-17]
PicTar	complementarity	free energy, co-expression, cross-species comparison	[15,16]
RNAhybrid	thermodynamics	statistical model	[16,18]

57

58 Web services and databases based on seed theory algorithms are available for miRNA target
59 prediction, and these tools enable the comparison of results calculated with various algorithms
60 (Table 1). For instance, the TargetScan, miRanda (miRBase), and miRDB systems are based on
61 sequence complementarity between the miRNA seed region and target site in the mRNA [14–16].
62 DIANA-microT and PicTar use thermodynamics to calculate the stability or free energy of the
63 miRNA–mRNA complex, and RNAhybrid utilizes both a statistical model and thermodynamics for
64 prediction [15–18]. However, these target prediction systems suggest many incorrect
65 candidates such as false positive and incorrect targets [13,19].
66 Original scores are now filtered, thus improving the results of prediction tools (Table 1).
67 However, predictions investigating a one-to-one interaction between a miRNA seed and an
68 mRNA target cannot predict multiple interactions of miRNAs and mRNAs. Therefore, high
69 quality predictions cannot be obtained by improving the filtering alone.
70 Synergies between miRNAs regulate miRNA–mRNA interactions, and in numerous cases, these
71 collaborations caused a more profound effect compared with that caused by a single miRNA
72 [20,21]. Therefore, miRNA–miRNA synergies may have a key function in regulating biological
73 processes because they have potential to reveal the unexplainable target selection mechanisms,
74 such as non-canonical and cell-specific targeting. Indeed, it is reported that interactions
75 between miRNAs have been observed not only in co-target relations but also in multiple mode of
76 function [22]. This suggest that there is room for discussing existence of miRNA–miRNA
77 synergies. Therefore, to improve miRNA target prediction, scoring of miRNA–miRNA
78 interactions would be beneficial, which has been reported in only a few publications [23].
79 In our previous study, to elucidate miRNA biological functions at the nucleotide level, we
80 developed an RNA wave model and annotated the RNA sequences using quantum theory and
81 electrostatic potential according to RNA bases [24,25]. We constructed a matrix for simulating
82 quantum interaction between RNAs and subsequently showed that a miRNA can transform the
83 binary notation with qubit, and miRNA–viral RNA complexes can be expressed using the matrix
84 [26]. Here, we introduce a novel quantum scoring method based on the quantum computing
85 algorithm to investigate single miRNA–miRNA interactions. Using this technique, we found a
86 positive correlation between quantum scoring and synergistic effects (SEs) of miRNAs in
87 malignant diseases. Finally, we discuss the benefits of quantum scores in disease prediction,

and in interpretation of microRNA as memory device. The quantum superposition and entanglement of miRNAs could record, transmission, and even inherit biological statuses in cells, like a memory devices in computers [25].

2. MATERIAL AND METHODS

2.1 microRNA sequence data

Sequences of mature miRNAs were downloaded as microRNA.dat from miRBase [27]. This data was Release 21 (date: June 2014) and the database contained 28,645 of all mature miRNA sequences. We extracted 2,588, 1,915, 466, 434, and 427 of human, murine, *Drosophila*, *C. elegans* and *Arabidopsis* mature miRNA sequences from above mature miRNAs, respectively.

2.2 Conversion of sequence

All miRNA sequences downloaded from miRBase were converted into respective binary codes (ket alignments). According to the previous report, electrical potential of four basic nucleobases in RNA (Guanine, Adenine, Cytosine, and Uracil) were calculated by referring to FMO of each atom in nucleobase and by integrating them with quantum entanglement [24]. As a result, only G showed positive numeric value and the others showed negative ones, therefore, to consider about the basis state of spanning sets of bits in vector subspace of miR FMOs, the binary transformation was performed into the state 1 and 0. G was transformed as 1 and the rest, U, A, and C as 0 [24].

2.3 The quantum matrix construction

Matrix was made with Microsoft Office Excel 2013 (Microsoft Japan Co., Ltd., Tokyo, Japan) using its macro and function systems. Precisely, matrix is comprised of three parts, one of that is a field for loading miRNA pair sequences (the upper part), another is for arranging sequences (the left and lower parts), and the other is for calculating interactions between loaded miRNA sequences (the central part). MiRNA sequences set in the upper part will be loaded into the left and lower parts with the windows of 3 nucleotides, then interactions between each nucleotide and each its opposite nucleotide will be calculated at intersecting points on the central part. Although the relation of nucleobase binding is flexible phenomenon which occur in sufficiently tiny environments, we have previously reported the existence of quantum energy between or

among miRNAs [24–26]. Original sequences of miRNAs are translated by binary notation based on the quantum energy of nucleotide (Figure 1A), set in upper part of the matrix (Figure 1C), and loaded into left and lower parts of matrix separately by the rule shown in Figure 1B. Then matrix calculated and visualized the quantum state between coupled miRNAs (Figure 1C). The matrix architecture is derived from analogy of quantum computation [28].

124

125 2.4 The quantum calculation

126 Since information is physics and the miRNA gene is information, we here selected the matrix as
127 the Hilbert space, which is a vector space possessing the structure of an inner product, of the
128 Tensor products. The ket modules of two miRNAs in matrix, for instance, A and B are expressed
129 as follows

130

$$131 \quad z(|A\rangle \otimes |B\rangle) = (z|A\rangle \otimes |B\rangle) = (|A\rangle \otimes z|B\rangle) \quad (1, 0)$$

132

$$133 \quad (a \otimes b)(|A\rangle \otimes |B\rangle) = a|A\rangle \otimes b|B\rangle \quad (1, 1)$$

134

135 From (1, 0) and (1, 1), $A \otimes B$ of the inner product spaces are represented the vector space of an
136 intersecting point between $|A\rangle$ and $|B\rangle$ in the matrix.

137

$$138 \quad (\sum a_i |A\rangle \otimes |B_i\rangle, \sum b_j |A_j\rangle \otimes |B_j\rangle) \equiv \sum a_i * B_j \langle A_i | A_j \rangle \langle B_i | B_j \rangle \quad (1, 2)$$

139

140 Since RNA quantum code with the fragment molecular orbital method (FMO) of two miRNAs
141 were calculated according to the binary notation of electro spin as previously described [24].

142 From (1, 2), miRNA–miRNA interaction was calculated in the matrix as a vector space. The
143 coherent of sample miRNA values are applied into a matrix in the order with both rows and
144 columns. When the $f(x)$ is register y for a miRNA and the register x is the other miRNA the
145 values of superpose samples are shown as amplitude vectors of an intersecting point between
146 $|x\rangle$ and $f(x)$ in the matrix. These calculation were performed by the original macro program,
147 MESer [29]. An integer of the amplitude vectors, each superposition (SP) in the intersecting
148 points on the decreasing diagonal of the matrix (1, 3) was summed up as Static Nexus Score
149 (SNS) (1, 4).

150

151 $SP_i = \sum_{j=0}^2 (A_{i+j} \times B_{i+j})$ (1, 3)

152

153 $SNS = 3 \times \sum_{i=0}^{23} (A_i \times B_i)$ (1, 4)

154

155 The RNA wave model 2000 was based on circular miRNA electron spin, therefore two sample
156 miRNAs were moving and shifting one by one and in the counter direction of each other [24–26].
157 Since the torus miRNAs can revolve in the matrix, SNS between two torus miRNAs (A_i , B_j)
158 usually showed different values in each relative location shifting. Subsequently, they were
159 summed up as Dynamic Nexus Score (DNS) for further experiments ($i = 0, \dots, 23$; $j = 0, \dots, 23$) (1,
160 5).

161

162 $DNS = 3 \times \sum_{i=0}^{23} (A_i \times \sum_{j=0}^{23} B_j) = 3 \times \sum_{i=0}^{23} A_i \times \sum_{j=0}^{23} B_j$ (1, 5)

163

164 In the case of different nucleotide length of two miRNAs, the surplus space of 3' end region of
165 miRNA was filled up with zeros.

166

167 2.5 Evaluation of synergistic effects of miRNAs

168 The biological activity of synergistic effects was investigated from previous reports about miRNA
169 synergism. When a specific numerical value was shown in above reports as miRNA synergism,
170 we directly applied the specific value to the comparison to the miRNA-miRNA quantum scores.
171 Otherwise, the single miRNA activity unit (E) and synergistic miRNA activity unit (S) were
172 calculated as follows,

173

$$\text{Single microRNA activity unit (E)} = \frac{\text{The single miRNA activity}}{\text{Total amounts of single miRNA}}$$

174

$$\text{Synergistic microRNA activity unit (S)} = \frac{\text{The synergistic miRNA activity}}{\text{Total amounts of synergistic miRNA}}$$

175

176 Total amounts means an amount of miRNA that is applied to sample in each researches, such as
177 ng and mol.

178 A synergistic effect (SE) between miRNA_a and miRNA_b was calculated as follows,

179

$$\text{Synergistic Effect (SE)} = \frac{S \text{ of miRNA}_a \text{ and miRNA}_b}{E \text{ of miRNA}_a + E' \text{ of miRNA}_b}$$

180

181 When a single miRNA pair has multiple synergistic activities, these synergistic effects were

182 averaged as multiple SE unit (SEU) ($i = 1, 2, \dots, n$),

183

$$\text{Multiple SE unit (SEU)} = \frac{\sum_i^n \text{SE}}{n}$$

184

185 **3. RESULTS**

186 **3.1 MicroRNAs with the binary quantum code**

187 According to a previous report that compared electrical states among the four types of nucleic

188 acid bases, only G has a positive electrical **potential**; the others have a negative one. We

189 performed a new RNA evaluation by transforming the original RNA sequences into binary codes

190 substituting Gs with 1 and the others with 0. Using this quantum theory, we transformed the

191 hsa-let-7b and hsa-miR-34a miRNA sequences into a quantum code as a sample and defined

192 these quantum codes as ket alignment according to the quantum mechanics field. Ket

193 alignments of hsa-let-7b and hsa-miR-34a were represented with the binary code (Figure 1A).

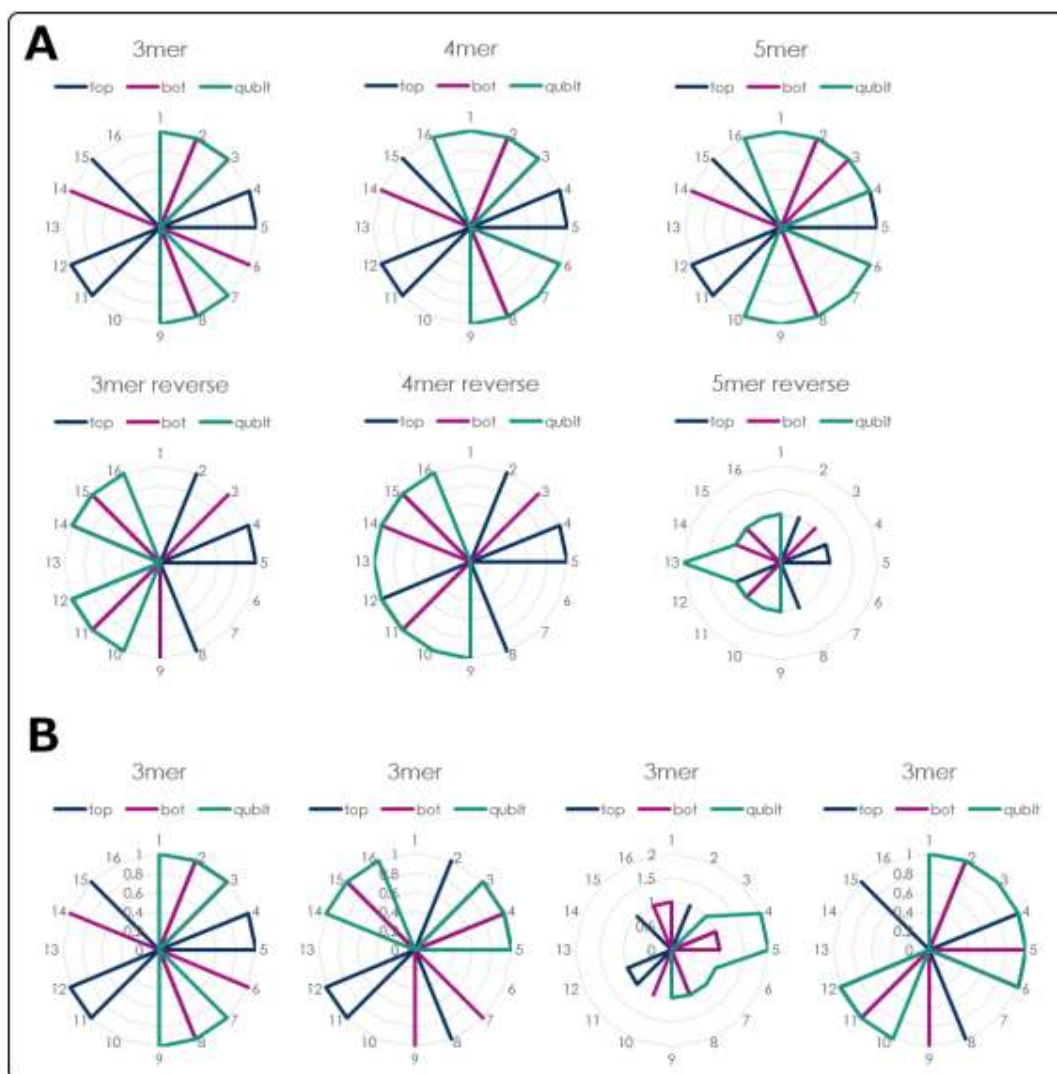
194

195 **Figure 1. The matrix for quantum calculation.**

215 them had same value but showed different SP shapes). This indicates that SNS was not
 216 constant for hsa-let-7b and hsa-miR-34a. To improve the results, we calculated the DNS value
 217 using the RNA wave 2000 model, which enables miRNAs to form a torus, and therefore, the
 218 relative location of nucleic acid sequences can be shifted. DNS is generated from the sum of all
 219 SNS values of the shifted miRNA pairs. DNS was a constant value either after the parallel or
 220 antiparallel shifting between the two miRNAs.

221

222 **Figure 2. Transition of SNS value in single miRNA pair.**



223

224 *Top: ket alignment of the miRNA on top side. Bot: ket alignment of the mRNA on bottom side. SP:*

225 *entanglement status between top and bot.*

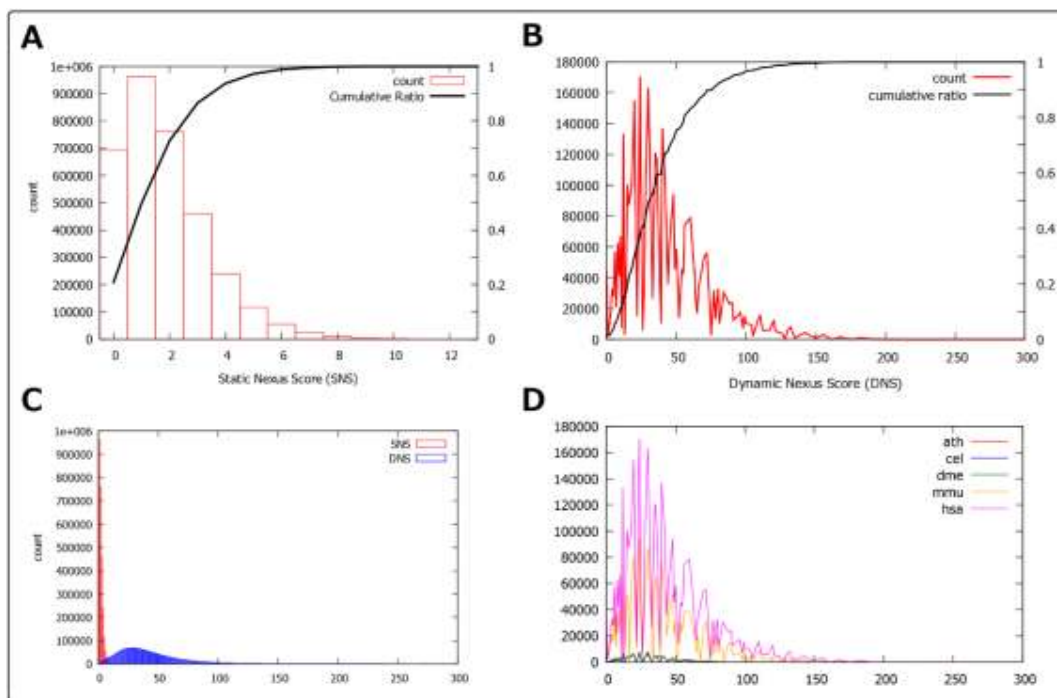
226

227 3.3 Evaluation of miRNA–miRNA interactions

228 Firstly, miRNA ket alignment homology was investigated among 2,588 mature human miRNAs.
229 Complete ket homologies of miRNAs were detected in a total of 107 alignments containing 259
230 miRNAs, which belong to the same family, subtype, or isomiR. Since 2,426 of 2,588 (93.7%)
231 miRNA kets displayed different individual characteristics, it is obvious that most of miRNA kets
232 are heterogeneous. SNS and DNS values were calculated for different miRNA pairs to evaluate
233 miRNA–miRNA interactions. Human mature miRNA sequences (2,588) were extracted from
234 miRBase, and SNS and DNS general distributions of 3,347,578 miRNA–miRNA pairs were
235 computed. In this experiment, SNS was calculated based on the basic state shown in Figure
236 1C. Because all SNS and DNS values were in multiples of 3, we divided them by 3 in order to
237 draw a graph. Human miRNA SNS values ranged from 0 to 14 and peaked at 1 (Figure 3A),
238 and human miRNA DNS values ranged from 0 to 288 and peaked at 29 (Figure 3B). To
239 compare the distributions of SNS and DNS, they were plotted on the same graph, which
240 revealed a much wider range for DNA than for SNS (Figure 3C). This result indicates that DNS
241 values are more suitable for evaluating miRNA–miRNA interactions. Furthermore, DNS
242 distributions of murine, *Drosophila*, *Caenorhabditis elegans*, and *Arabidopsis* miRNA–miRNA
243 interactions were investigated individually, and then were compared to each other (Figure 3D).
244 In murine, *Drosophila*, *C. elegans*, and *Arabidopsis* species, the maximum values of DNS were
245 272, 147, 172, and 168, respectively, and DNS peaked at 24 in all species, except for *C. elegans*
246 (30) (Table 2). Although dominant ranges are similar to each other in all 5 species, proportions
247 of miRNA pairs in dominant range are greatly different. Intriguingly, all of them showed a similar
248 distribution in spite of their various sample sizes (Figure 4). This indicates that RNA language
249 may be common among plural species.

250

251 **Figure 3. Contrasts between SNS and DNS, or Human and other species.**



252

253

254

255 **Table 2. Profiles of miRNA count and DNS distribution in 5 species.**

	Human	Mouse	Fly	Nematode	Plant
Counts of miRNAs	2588	1915	466	434	427
Counts of miRNA pairs	3321753	1832655	108345	93961	90951
Peak DNS value	24	24	24	30	24
(its count)	(170397)	(94858)	(7905)	(6717)	(6150)
Min-max of range	0 – 290	0 – 272	0 – 147	0 – 172	0 – 168
Dominant range of DNS	20 – 40	12 – 40	12 – 40	12 – 42	12 – 42
Counts of miRNA pairs	1291767	941590	68793	67438	63936
included in dominant range	(38.6%)	(51.4%)	(63.5%)	(71.8%)	(70.3%)

256 *Dominant range means the extent between minimum and maximum DNS values which occupy more than*

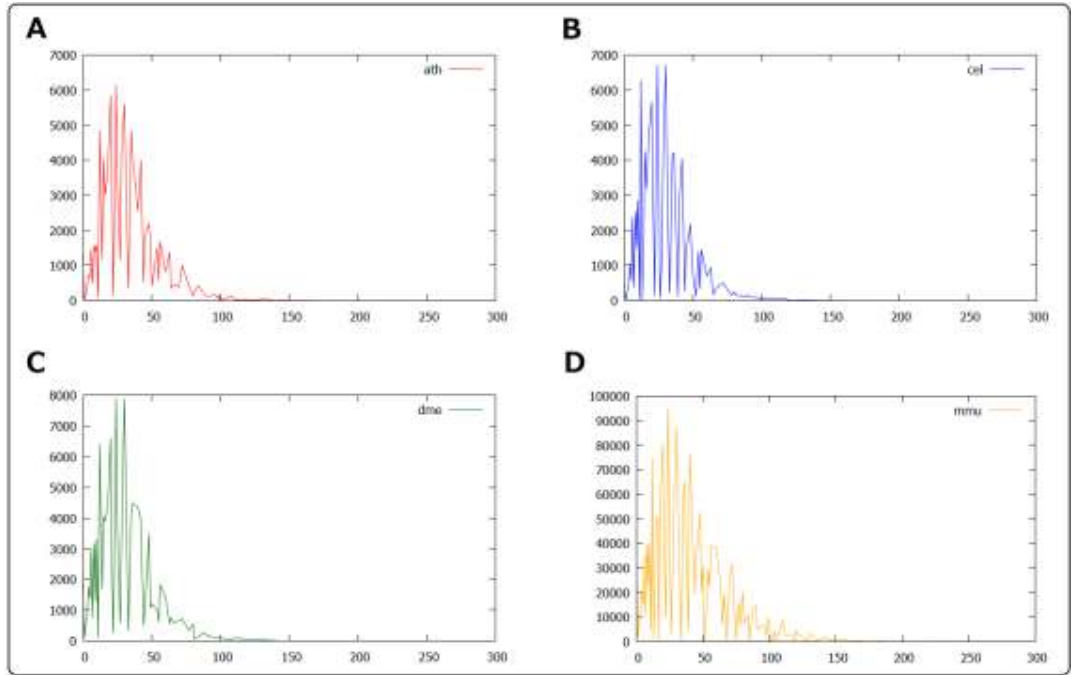
257 *4% count in that of whole DNS.*

258

259

260

261 **Figure 4. DNS distributions of 4 different species.**



262
263 *X axis: value of DNS, Y axis: count*

264

265 **3.4 Relationship between DNS and SE**

266 We collected scientific papers which report miRNA SEs, then extracted 18 reports that describe
267 about miRNA-miRNA SE. Fifteen reports were related to cancer, 13 of the 18 reports were
268 chosen as samples to examine synergisms between miRNAs (Table 3) [22,27–38]. We built ket
269 alignments of all miRNAs present in these reports, then calculated DNS of these miRNA
270 combinations (Table 4). Finally, the strength of SE for all miRNA pairs were calculated, and 31
271 DNS and 11 SE values were obtained (Table 5). We observed a positive correlation between 11
272 of the DNSs and SEs related to malignant diseases ($R = 0.55$, $P < .06$, $R^2 = 0.2982$) (Figure 5A).
273 For SNS, 31 data were obtained, but no correlation was observed between SNSs and SEs
274 (Table 6 and Figure 5B).

275

276 **Table 3. Synergy-associated miRNA combinations.**

Biofunctions	Common targets	miRNAs	miRBase ID	Sequences	References
lung cancer	C-MYC	let-7b-5p	MIMAT0000063	UGAGGUAGUAGGUUGUAUAGUU	[30]
		miR-34a-5p	MIMAT0000255	UGGCAGUGUCUUAGCUGGUUGU	

MDSC induction	SHIP-1	miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	[31]
		miR-155-5p	MIMAT0000646	UUA AUGCUAAUCGUGAUAGGGGU	
proliferation / invasion	MMP-2, EGFR, PDCD4	miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	[32]
		miR-10b-5p	MIMAT0000254	UACCCUGUAGAACCGAAUUUGUG	
colon cancer	-	miR-491-5p	MIMAT0002807	AGUGGGGAACCCUCCAUGAGG	[33]
		miR-221-3p	MIMAT0000278	AGCUACAUUGUCUGCUGGGUUUC	
		miR-342-3p	MIMAT0000753	UCUCACACAGAAUCCGACCCGU	
proliferation / survive	WEE1	miR-381-3p	MIMAT0000736	UAUACAAGGGCAAGCUCUCUGU	[34]
		miR-424-5p	MIMAT0001341	CAGCAGCAAUUCAUGUUUUGAA	
cell viability	-	miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	[23]
		miR-1-3p	MIMAT0000416	UGGAAUGUAAAGAAGUAUGUAU	
tumor growth	EphA2	miR-520d-3p	MIMAT0002856	AAAGUGCUUCUCUUUGGUGGGU	[35]
		si-Eph2A-2	(Eph2A: NM_004431.3)	CCAUCAAGAUGCAGCAGUA	
p63 cell cycle	Cyclin D1, E2, CDK4, 6, E2F3, Bcl2, etc.	miR-34a-5p	MIMAT0000255	UGGCAGUGUCUUAGCUGGUUGU	[36]
		miR-34c-5p	MIMAT0000686	AGGCAGUGUAGUUAGCUGAUUGC	
glioma	-	miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	[37]
		miR-23b-5p	MIMAT0004587	UGGGUUCCUGGCAUGCUGAUUU	
		miR-181d-5p	MIMAT0002821	AACAUUCAUUGCUGUCGGUGGGU	
milk fat synthesis	-	miR-23a-3p	MIMAT0000078	AUCACAUUGCCAGGGAUUUCC	[38]
		miR-27b-3p	MIMAT0000419	UUCACAGUGGCUAAGUUCUGC	
		miR-103-3p	MIMAT0000101	AGCAGCAUUGUACAGGGCUAUGA	
		miR-200a-3p	MIMAT0000682	UAACACUGUCUGGUAACGAUGU	
tumor growth	PDCD4	miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	[39]
	BTG2	miR-23a-3p	MIMAT0000078	AUCACAUUGCCAGGGAUUUCC	
	NEDD4L	miR-27a-3p	MIMAT0000084	UUCACAGUGGCUAAGUCCG	
proliferation	-	miR-19b-3p	MIMAT0000074	UGUGCAAAUCCAUGCAAAACUGA	[40]
		miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	
		miR-148a-3p	MIMAT0000243	UCAGUGCACUACAGAACUUUGU	
Carcinoma	E2F5	miR-196a-5p	MIMAT0000226	UAGGUAGUUUCAUGUUGUUGGG	[41]
		miR-337-3p	MIMAT0000754	CUCCUAUAUGAUGCCUUUCUUC	

	MCM4	let-7c-5p	MIMAT0000064	UGAGGUAGUAGGUUGUAUGGUU	
		miR-196a-5p	MIMAT0000226	UAGGUAGUUUCAUGUUGUUGGG	
		miR-337-3p	MIMAT0000754	CUCCUAUAUGAUGCCUUUCUUC	
		miR-99a-3p	MIMAT0004511	CAAGCUCGCUUCUAUGGGUCUG	
	TP53	miR-328-3p	MIMAT0000752	CUGGCCCUCUCUGCCCUUCCGU	
		let-7c-5p	MIMAT0000064	UGAGGUAGUAGGUUGUAUGGUU	
	SKP2	miR-337-3p	MIMAT0000754	CUCCUAUAUGAUGCCUUUCUUC	
		let-7c-5p	MIMAT0000064	UGAGGUAGUAGGUUGUAUGGUU	
	CDK1	miR-196a-5p	MIMAT0000226	UAGGUAGUUUCAUGUUGUUGGG	
		miR-337-3p	MIMAT0000754	CUCCUAUAUGAUGCCUUUCUUC	

277

278 **Table 4. miRNA kets and its combination and DNS**

Biofunctions	miRNAs	Ket alignments	Combinations	DNSs
lung cancer	let-7b	010110010011001000010000 >	let-7b / miR-34	64
	miR-34a	011001010000010011001000 >		
MDSC induction	miR-21	001000000010001001001000 >	miR-21 / miR-155	35
	miR-155	000001000000101000111100 >		
proliferation / invasion	miR-21	001000000010001001001000 >	miR-21 / miR-10b	25
	miR-10b	000000100100001000001010 >		
colon cancer	miR-491-5p	010111100000000000101100 >	miR-491 / miR-221	48
	miR-221-3p	010000000100010011100000 >	miR-491 / miR-342	24
	miR-342-3p	000000000100000100000100 >	miR-342 / miR-221	18
proliferation / survive	miR-381	000000011100010000001000 >	miR-381 / miR-424	25
	miR-424	001001000000001000010000 >		
cell viability	miR-21	001000000010001001001000 >	miR-21 / miR-1	35
	miR-1	011000100001001000100000 >		
tumor growth	miR-520d-3p	000101000000000110111000 >	miR-520d-3p / siRNA	35
	Eph2A-siRNA	000000010010010010000000 >		
p63 cell cycle	miR-34a	011001010000010011001000 >	miR-34a / miR-34c	64
	miR-34c	011001010010001001000100 >		
glioma	miR-21	001000000010001001001000 >	miR-21 / miR-23b	35

	miR-23b	011100000110001001000000 >	miR-21 / miR-181d	35
	miR-181d	000000000010010011011100 >	miR-23b / miR-181d	49
milk fat synthesis	miR-23a	000000001000111000000000 >	miR-23a / miR-27b	24
	miR-27b-3p	000000101100001000010000 >	miR-103 / miR-200a	35
	miR-103-3p	010010000100001110000100 >	miR-27b / miR-200a	40
	miR-200a	000000010001100001001000 >		
tumor growth	miR-21	001000000010001001001000 >	miR-21 / miR-23a	20
	miR-23a-3p	000000001000111000000000 >	miR-21 / miR-27a	25
	miR-27a-3p	000000101100001000010000 >	miR-23a / miR-27a	20
proliferation	miR-19b-3p	010100000000010000000100 >	miR-19b / miR-21	20
	miR-21	001000000010001001001000 >	miR-19b / miR-148a	16
	miR-148a	000101000000010000001000 >	miR-21 / miR-148a	20
Carcinoma	miR-196a-5p	001100100000010010011100 >	miR-196a / miR-337	16
	miR-337-3p	000000000100100000000000 >	miR-196a / let-7c	72
	let-7c-5p	010110010011001000110000 >	let-7c / miR-337	18
	miR-196a-5p	001100100000010010011100 >	miR-196a / miR-99a	48
	miR-337-3p	000000000100100000000000 >	miR-337 / miR-99a	12
	miR-99a-3p	000100010000000111000100 >	miR-196a / miR-337	16
	miR-328-3p	001100000000100000001000 >	miR-328 / let-7c	36
	let-7c-5p	010110010011001000110000 >		
	miR-337-3p	000000000100100000000000 >	miR-337 / let-7c	18
	let-7c-5p	010110010011001000110000 >		
	miR-196a-5p	001100100000010010011100 >	miR-196a / miR-337	16
	miR-337-3p	000000000100100000000000 >		

279

280 **Table 5. DNS values and SE strengths.**

Biofunctions	Combinations	DNSs	SEs
lung cancer	let-7b / miR-34a	64	1.85
MDSC induction	miR-21 / miR-155	35	NA
proliferation / invasion	miR-21 / miR-10b	25	1.65
colon cancer	miR-491 / miR-221	48	1.45

colon cancer	miR-491 / miR-342	24	1.45
colon cancer	miR-342 / miR-221	18	1.45
proliferation / survive	miR-381 / miR-424	25	1.86
cell viability	miR-21 / miR-1	35	2.2
tumor growth	miR-520d-3p / siRNA	35	1.8
p63 cell cycle	miR-34a / miR-34c	64	2
glioma	miR-21 / miR-23b	35	NA
glioma	miR-21 / miR-181d	35	NA
glioma	miR-23b / miR-181d	49	NA
milk fat synthesis	miR-23a / miR-27b	24	NA
milk fat synthesis	miR-103 / miR-200a	35	NA
milk fat synthesis	miR-27b / miR-200a	30	NA
tumor growth	miR-21 / miR-23a	20	1
tumor growth	miR-21 / miR-27a	25	1
tumor growth	miR-23a / miR-27a	20	1.28
proliferation	miR-19b / miR-21	20	NA
proliferation	miR-19b / miR-148a	16	NA
proliferation	miR-21 / miR-148a	20	NA
Carcinoma	miR-196a / miR-337	16	NA
Carcinoma	miR-196a / let-7c	72	NA
Carcinoma	let-7c / miR-337	18	NA
Carcinoma	miR-196a / miR-99a	48	NA
Carcinoma	miR-337 / miR-99a	12	NA
Carcinoma	miR-196a / miR-337	16	NA
Carcinoma	miR-328 / let-7c	36	NA
Carcinoma	miR-337 / let-7c	18	NA
Carcinoma	miR-196a / miR-337	48	NA

281 NA means that any synergistic effect was not obtained as specific value.

282

283 **Table 6. SNS values and SE strengths.**

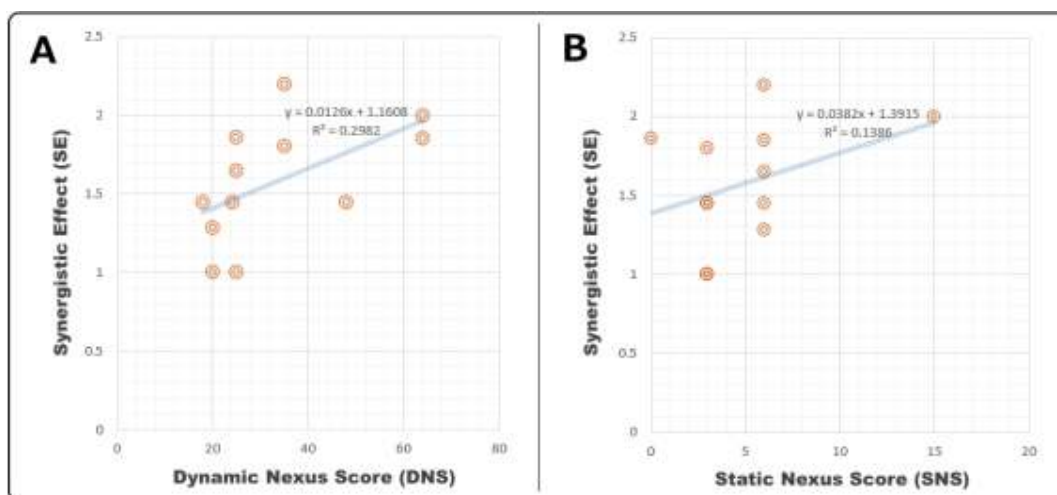
Biofunctions	Combinations	SNSs	SEs
lung cancer	let-7b / miR-34a	2	1.85
MDSC induction	miR-21 / miR-155	2	NA
oncogene	miR-21 / miR-10b	2	1.65
colon cancer	miR-491 / miR-221	2	1.45

colon cancer	miR-491 / miR-342	1	1.45
colon cancer	miR-342 / miR-221	1	1.45
proliferation / survive	miR-381 / miR-424	0	1.86
cell viability	miR-21 / miR-1	2	2.2
tumor growth	miR-520d-3p / siRNA	1	1.8
p63 cell cycle	miR-34a / miR-34c	5	2
glioma	miR-21 / miR-23b	4	NA
glioma	miR-21 / miR-181d	3	NA
glioma	miR-23b / miR-181d	2	NA
milk fat synthesis	miR-23a / miR-27b	2	NA
milk fat synthesis	miR-103 / miR-200a	0	NA
milk fat synthesis	miR-27b / miR-200a	0	NA
tumor growth	miR-21 / miR-23a	1	1
tumor growth	miR-21 / miR-27a	1	1
tumor growth	miR-23a / miR-27a	2	1.28
proliferation	miR-19b / miR-21	0	NA
proliferation	miR-19b / miR-148a	2	NA
proliferation	miR-21 / miR-148a	1	NA
Carcinoma	miR-196a / miR-337	0	NA
Carcinoma	miR-196a / let-7c	2	NA
Carcinoma	let-7c / miR-337	0	NA
Carcinoma	miR-196a / miR-99a	3	NA
Carcinoma	miR-337 / miR-99a	0	NA
Carcinoma	miR-196a / miR-337	0	NA
Carcinoma	miR-328 / let-7c	1	NA
Carcinoma	miR-337 / let-7c	0	NA
Carcinoma	miR-196a / miR-337	0	NA

284

285

286 **Figure 5. DNS and SE correlate to each other.**



4. Discussion

There is an increasing need for the prediction of miRNA function and synergy. Many current studies explore miRNA targets using computing methods, and similarly, these approaches are used for detecting novel miRNAs and predicting miRNA functions or synergisms. These methods are based on the mRNA–miRNA interaction theory and various miRNA target databases (Table 1) [15,16]. TargetScan, miRanda, and miRDB are the most frequently used miRNA target prediction tools, and they are primarily based on sequence complementarity, and thus binding strength and stability between the mRNA target site and the miRNA or its seed region [14,42,43]. DIANA-microT, PicTar, and RNAhybrid are other common miRNA target prediction tools, but they are based on thermodynamic data of the mRNA–miRNA complex such as stability and free energy state [17,18,42–44]. Ordinarily, differential theory-based algorithms are used in combination to improve the precision of predictions [45,46].

Although these computational techniques have been improved and now produce more accurate results, false positive results are still obtained. Prediction algorithms have a technical limitation whereby it is difficult to improve prediction accuracy by regarding only complementarity, homology, and thermodynamics. Therefore, novel approaches are necessary to improve and calibrate prediction algorithms.

To improve the accuracy of miRNA target and synergy prediction algorithms, contribution of new factors, such as miRNA clusters, conservations, and transcription start sites (TSSs), are now

310 considered [7,47–49]. MicroTSS integrates RNA sequence data, active transcription marks,
311 and DNase I hypersensitive site sequencing results, and this concept enabled the identification
312 of various tissue-specific pri-miRNAs and intergenic miRNAs [49]. Furthermore, miRNAs can
313 interact with themselves, for example, via miRNA–miRNA bindings [50,51]. Certain miRNAs
314 form homologous or heterologous miRNA pairs, which suggests that miRNA synergism is caused
315 not only by co-expression and co-target relationships but also by direct interactions. We also
316 tried to add novel factors to generate a more accurate computation method by adopting the RNA
317 wave 2000 model, which includes numerous new factors, such as quantum energy, torus,
318 miRNA pairs, and G potential.

319
320 Among the four types of nucleotides, G is the only one that has a positive electric charge [24].
321 This specific characteristic is linked to certain clinical and technical aspects. G is a prominent
322 target for mutations in cancer and inherited diseases [52], and G quartets enable the
323 ultrasensitive detection of miRNAs [53]. Additionally, G bases may have unknown roles in gene
324 regulation, and they may be a critical factor for the *bona fide* construction of miRNA–miRNA,
325 mRNA–miRNA, or DNA–miRNA interaction models [54].

326
327 Preliminarily, we examined the context + score values of all co-target genes present in Table 3
328 using TargetScan. Only 3 mRNAs, RAS, CDK, and Rho were found, and their context+ score
329 value was –0.35 (let-7b), –0.25 (miR-491), and –0.22 (miR-21), respectively. Curiously, DNS of
330 let-7b/miR-34a, miR-491/miR-221, and miR-21/miR-10b, is 64, 48, and 25, respectively;
331 therefore, these context+ scores were directly proportional to the DNS values, even though the
332 context + score is based on mRNA–miRNA interactions and DNS is based on miRNA–miRNA
333 interactions. This implies that miRNA synergism and mRNA targeting may be coupled.
334 Context+ score is associated with human diseases according to the polymiRTS database [55],
335 and miRNA-related single nucleotide polymorphisms are scored by the context+ score.
336 Therefore, further investigations using DNS are crucial for building an integrated model of
337 miRNA–miRNA interactions as miRNA memory device and to identify mRNA–miRNA interactions
338 based on human disease databases.

339
340

341 **5. CONCLUSION**

342 In our analysis, we could utilize only a very limited sample size because there are still few reports
343 about miRNA synergisms. However, we found a synergistic effect in miRNA–miRNA
344 interactions from the perspective of quantum energy as miRNA memory. We suggest that more
345 precise cancer-related prediction could be achieved using specific G-based DNS analysis in
346 addition to conventional mRNA-miRNA interaction factors such as thermodynamic stability.

347

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