Features of miR-574-5p and miR-574-3p binding sites in mRNA of target genes

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miRNAs function as key regulators of developmental timing, and are highly conserved. They have specific nucleotide sequences and participate in post-transcriptional regulation of their target genes [1]. miRNAs with high affinity to mRNAs of several hundred genes were recently identified [2-4]. Their binding sites are located in 5′ UTRs, CDSs, and 3′ UTRs. These miRNAs have unique properties; for example, some miRNAs have specific binding site locations at a certain distance in mRNAs of different genes [5]. Other miRNAs have multiple binding site locations within several tens of nucleotides [2-4]. Such unique miRNAs have hundreds of target genes that regulate several key biological processes. Therefore, it is important to understand both the characteristics of the binding sites in the mRNAs of target genes as well as the biological processes they regulate. Specifically, miR-574-5p is a unique miRNA that has multiple binding sites in mRNAs of target genes, and their specific functions need to be extensively investigated. While there is some data regarding the function of miR-574-5p in different processes and pathologies, further research in this regard is warranted. The present work examined the interaction of miR-574 with the mRNAs of its target genes as well as analysed the functions of these genes.

Materials and Methods. Human mRNAs were obtained from GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of human mature miRNAs were downloaded from the miRBase database (http://mirbase.org). Target genes for miRNAs were determined using the MirTarget program [5]. This program defines the following features of binding sites: the start position of an miRNA binding site with respect to the mRNA sequence, the localisation of miRNA binding sites in the 5′UTRs, CDSs and 3'UTRs of genes, the free energy of hybridisation (ΔG, kJ/mole), and the schemes of nucleotide interactions between miRNAs and mRNAs. The ΔG/ΔGm (%) ratio was estimated for each binding site, where ΔGm is equal to the free energy value of miRNA binding to its perfect complementary nucleotide sequence. The miRNA binding sites identified had ΔG/ΔGm more than 90%.

Results and Discussion. Pre-miR-574 consists of miR-574-3p and miR-574-5p, and is encoded in an intron of a family with sequence similarity 114 member A1 gene (*FAM114A1*) located on chromosome 4.

Table 1 Characteristics of multiple miR-574-5p binding sites in 3'UTR of mRNA

Gene	Binding site position, nt.	Gene	Binding site position, nt.	Gene	Binding site position, nt.
ACVR2B	10963-11013 (26)	*GFRA1*	8453-8481 (15)	*RAB3IP*	4086-4100 (7)
ADAT2	3307-3329 (12)	*GLI2*	6124-6148 (13)	*RAB7A*	1021-1031 (6)
ADRBK2	3880-3896 (8)	*GLP1R*	1546-1562 (9)	*RABGAP1L*	34-66 (16)
AFF3	4208-4224 (7)	*GLRA2*	2526-2568 (20)	*RALGAPB*	7474-7488 (7)
AMOTL1	6023-6037 (8)	*GLYR1*	1965-1979 (8)	*REEP5*	1478-1496 (10)
ANKRD42	1888-1908 (10)	*GRIA3*	3351-3363 (7)	*RPH3A*	2859-2869 (6)
ANO8	4031-4041 (6)	*GRIA4*	5084-5098 (8)	*RUNX1T1*	3269-3301 (17)
ARHGAP35	7726-7740 (8)	*HPS3*	3253-3267 (8)	*SAMD9L*	6484-6508 (12)
ARHGEF9	2537-2549 (7)	*HS6ST3*	3847-3855 (5)	*SBK1*	2321-2362 (21)
ARRB1	6399-6417 (10)	*IFFO2*	4395-4407 (6)	*SDK2*	7582-8030 (7)
ATMIN	4182-4192 (6)	*IGF1*	4042-4062 (10)	*SEPT6*	4493-4521 (15)
BDH1	2611-2710 (35)	*INHBA*	2041-2053 (7)	*SH3TC2*	20874-20898 (13)
BTBD9	5797-5825 (15)	*ITGA11*	4598-4638 (20)	*SHB*	3405-3481 (9)
C10orf71	4716-4735 (10)	*KATNAL1*	4198-4526 (30)	*SLC31A1*	867-879 (7)
C15orf57	862-904 (22)	*KCNIP3*	1007-1021 (8)	*SLITRK3*	4415-4437 (11)
CAMK2N1	1850-1864 (8)	*KCNK10*	6170-6178 (5)	*SMAD4*	7742-7756 (7)
CARNS1	3225-3235 (6)	*KCNQ3*	3560-3576 (9)	*SNAP29*	1361-1385 (12)
CD22	2733-2753 (9)	*KCNQ5*	5370-5412 (22)	*SNX2*	1737-1753 (8)
CD40LG	1549-1579 (16)	*KIAA0895L*	2882-2928 (23)	*SPATA6*	3213-3231 (10)
CD93	3489-3499 (6)	*KIAA1549L*	9102-9118 (9)	*SRD5A3*	1415-1433 (10)
CDH6	4747-4759 (7)	*KIAA2018*	9953-9965 (6)	*SSX1*	1066-1080 (8)
CHRDL1	2247-2265 (9)	*KIF1B*	5813-5823 (6)	*SSX2B*	1275-1306 (15)
CHST11	5219-5233 (8)	*LDB3*	4421-4447 (14)	*SSX2*	1102-1129 (13)
CLIC6	2573-2587 (8)	*LEPREL1*	2947-2963 (9)	*SSX5*	1199-1217 (10)
CNGA4	2098-2120 (12)	*LHFPL5*	1461-1531 (13)	*STXBP6*	2559-2573 (8)
CPPED1	5408-5422 (8)	*LHFP*	1397-1426 (15)	*SYNPO*	3601-3623 (12)
CREB3L2	6066-6082 (9)	*LRRTM2*	2949-2973 (12)	*TCTE1*	2238-2266 (15)
CYP4V2	2906-2916 (6)	*LYRM7*	5255-5279 (13)	*TMEM130*	2003-2026 (11)
DNAJC15	1131-1141 (6)	*MAF*	2106-2127 (10)	*TNS4*	3471-3511 (21)
DNAJC6	5549-5563 (8)	*MAP2*	8797-8809 (7)	*TREML2*	2350-2380 (16)
DOK6	7831-7851 (11)	*MARCH4*	3767-3785 (10)	*TRIOBP*	7488-7575 (8)
DPYSL5	2933-2949 (9)	*MCM8*	2994-3020 (13)	*UBLCP1*	1550-1574 (13)
EHD3	2274-2284 (6)	*MNT*	4275-4289 (7)	*VAMP4*	1890-1900 (6)
EOGT	2684-2694 (6)	*NAV1*	7555-7579 (13)	*VSNL1*	1023-1045 (12)
EP300	8566-8584 (10)	*NCDN*	3557-3587 (15)	*WNT4*	1981-2001 (11)
FAM117B	2078-2104 (14)	*NR2E1*	2953-2981 (15)	*XRCC1*	2035-2051(9)
FAM163A	2108-2122 (8)	*PCDHB16*	198-212 (8)	*ZEB1*	3587-3605 (10)
FAM167A	3152-3178 (9)	*PPARA*	9024-9036 (6)	*ZFP92*	3289-3307 (10)
FAM83C	2511-2523 (7)	*PPP2R1B*	4561-4581 (11)	*ZNF618*	2954-2976 (11)
FBRSL1	4180-4190 (6)	*PRKCB*	7056-7087 (16)	*ZRANB1*	3797-3807 (6)
FOXN3	2420-2446 (13)	*PTCHD1*	2900-2922 (12)	*ZSWIM1*	1627-1645 (9)
Note. In parentheses is shown the number of repeated miR-574-5p binding sites.					

miR-574-5p and miR-574-3p have different properties despite their common origin. miR-574-5p has 13 purines and 10 pyrimidines, while miR-574-3p has 9 purines and 13 pyrimidines. miR-574-5p has 245 target genes despite its longer length, whereas miR-574-3p has only six target genes with $\Delta G/\Delta G_m$ ratios of 90% or more. miR-574-5p and miR-574-3p have binding sites in the mRNAs of 1764 and six target genes, respectively.

The average number of binding sites for miR-574-5p and miR-574-3p in the mRNA of a target gene is 7.2 and 1.0, respectively (table 1). We therefore propose the following hypothesis based on the observed properties of miR-574-5p and miR-574-3p. In addition, studies measuring the concentration of miR-574-3p without miR-574-5p observed that changes in biological liquids correlated with tumour development. Therefore, we hypothesize that it is possible that tumorigenesis is initiated by changes in the concurrent concentration of miR-574-3p and miR-574-5p, since they mature from a common precursor. This hypothesis is supported by the fact that miRNA-5p and miRNA-3p are coded by a single pre-miRNA. The effect of the concentration of miRNA-5p has been investigated without a control comparison with miRNA-3p. Therefore, the results of these studies are incomplete and only a few studies have described the co-expression of miRNA-5p and miRNA-3p. A similar pattern of regulation was observed for miR-574-3p and miR-574-5p, which are specifically downregulated in lymphoblastic cell lines. miR-574-5p binding sites are located in adenine-cytosine repeats (AC-repeats) in mRNA UTRs. A significant positive correlation between the sequence length of 3′ UTRs and the presence of tandem repeat sequences has been reported. Therefore, the majority of miR-574-5p sites are located in 3′ UTRs. Regulation of human *CBR1* gene expression by miR-574-5p depends upon the rs9024 genotype status. It was established that the concentration of serum miR-574-5p was significantly different and was associated with death from sepsis [6]. Moreover, varying miR-574 expression was demonstrated in different diseases and its possible gene targets were investigated [7-12]. miR-574 is overexpressed in myotonic dystrophy, oesophageal squamous cell carcinoma, and early-stage NSCLC. It was shown that TLR9 signaling was elevated due to the expression of miR-574-5p in human lung cancer cells [9]. Furthermore, miR-574-3p plays a significant role in cellular processes and might also be required for mesenchymal stem cell multipotency [10]; it has also been associated with neurodegenerative diseases and gastric cancer [11]. *In*

vitro transfection of mesenchymal stem cells with pre-miR-574-3p resulted in inhibition of chondrogenesis, indicating its role during the commitment of mesenchymal stem cells towards chondrocytes. Studies have shown that nucleotide repeats are connected to miRNA target gene regulation. Therefore, we studied CA-repeats of genes that are targets for miR-574-5p. We determined that 162 miR-574-5p binding sites are located in 5′ UTRs, two in CDSs and 1,606 in 3′ UTRs with a $\Delta G/\Delta G_m$ ratio of 90% or more. The target genes identified play important roles in various cell processes involving 36 transcription factors, 26 kinases (including 7 transcription factors), 31 cell cycle related genes, and 27 apoptotic genes. Some of these target genes participate in carcinogenesis. Specifically, 18 and 11 proteins of identified target genes are involved in breast and lung cancer development, respectively. All 11 target genes participating in the development of lung cancer are included in the group of 18 target genes that regulate breast cancer development. We established that the predicted target genes of miRNAs are transcription factors, kinases, and cell cycle and apoptosis-related genes that participate in the development of various diseases such as breast cancer, lung cancer, and cardiovascular diseases, etc.

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