The Binding Sites of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p in the Human mRNAs

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Abstract. miRNAs are important regulators of the translation of protein-coding genes. Their binding sites are located in all parts of mRNAs, but the features of localization remain unclear. The binding of 2,036 human miRNAs with the mRNAs of 12,175 human genes was studied using the miRTarget program, which was developed in our laboratory. It was predicted that miR-619-5p, miR-5095, miR-5096 and miR-5585-3p bind with high affinity to the mRNAs of the 1215, 832, 725 and 655 genes, respectively. miRNAs binding sites are located in the 3'-UTRs, CDSs and 5'-UTRs. The mRNAs of some genes had multiple miR-619-5p, miR-5095, miR-5096 and miR-5585-3p binding sites. The many predicted target genes are participants of cell cycle and apoptosis. The possible functional properties of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p are discussed.

Keywords: miR-619-5p. miR-5095. miR-5096. miR-5585-3p.

1 Introduction

MicroRNAs (miRNAs) participate in the regulation of the expression of protein-coding genes at the post-transcriptional stage [1]. miRNAs, as a part of the RNA-induced silencing complex, bind to mRNAs and interfere with translation or promote mRNA destruction [2]. The study of the properties of miRNAs and their influences on the expression of the genes that participate in all key processes of cells was established in the last 20 years. The actions of miRNAs on the cell cycle [3], apoptosis [4],

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differentiation [5], growth and development in plants [6] and animals [7] have been shown. Connections between miRNA expression and the development of various diseases has been established. miRNA concentrations change in cancer [8]. Metabolic disturbances change miRNA concentrations in cells [9]. It is possible to normalize some processes using miRNAs [10]. The aforementioned roles do not encompass the full list of the biological processes in which miRNAs participate, which proves the importance of their biological functions.

Despite the appreciable successes in the study of miRNA properties, there are obstacles to establishing the target genes of miRNAs. There are miRNAs that bind to several mRNAs, and one mRNA can be the target of some miRNAs. These circumstances significantly complicate the study of the properties of miRNAs and their diagnostic and medical applications. There are more than 2000 miRNAs in the human genome, and they are thought to act on 50% or more of genes. It will be difficult to draw conclusions about the participation of miRNAs in specific biological processes, and until those conclusions can be drawn, the connections between the majority of miRNAs and their target genes will remain unknown.

In present work, we found a set of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p miRNAs that have hundreds of target genes and bind to mRNAs with high affinity. The predicted binding sites of these miRNAs are located in the 3'-untranslated regions (3'-UTRs), coding domain sequences (CDSs) and 5-untranslated regions (5'-UTRs) of mRNAs. We studied some miRNAs that can be bind with the mRNAs of several hundred human genes.

2 Materials and Methods

The human gene mRNAs were taken from GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of human miR-619-5p, miR-5095, miR-5096 and miR-5585-3p were taken from the miRBase site (http://miRbase.org). The putative target genes for the tested 2,036 miRNAs were predicted using the miRTarget program, which was developed in our laboratory. This program calculates the following features of binding miRNAs with mRNAs: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in the 5'-UTRs, the CDSs and the 3'-UTRs of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole). The ΔG value is calculated for total miRNA length. The ΔG/ΔGm ratio (%) was determined for each site. ΔGm equals the free energy of an miRNA binding with its perfect complementary of full nucleotide sequence. The efficiency of miRNA and mRNA interaction was determined according to the ratio ΔG/ΔGm. The sites were selected only with ΔG/ΔGm ratio of 90% or more. We also noted the positions of the binding sites on the mRNA, beginning from the first nucleotide of the mRNA's 5'-UTR. The distance between A and C (1.04 nm) was equal to those between G and C (1.03 nm), A and U (1.03 nm), G and U (1.02 nm) [11]. Therefore, these pairs form hydrogen bonds and their number in the G-C, A-U, G-U and A-C were found to be 3, 2, 1 and 1, respectively. The free binding energies of these nucleotide pairs were taken as the same values (12,55 kJ/mole, 8,37 kJ/mole, 4,18 kJ/mole and 4,18 kJ/mole, respectively).

3 Results

3.1 Features of miR-619-5p, miR-5096, miR-5585-3p and miR-5095

The binding powers between the 2,036 tested hsa-miRNAs and the mRNAs of 12,175 human genes were calculated. Some of these miRNAs had greater numbers of target genes than others. For example, miR-619-5p, miR-5095, miR-5096, and miR-5585-3p were found to be capable of binding to more than 600 genes each. Additionally, the binding sites for these miRNAs were located in an orderly manner in a region of the mRNAs with a length of 200 nt. miR-619-5p, miR-5095, miR-5096, and miR-5585-3p had different miRNA binding site origins, lengths, quantities, and miRNA binding site properties, among other features. Some of the characteristics of these miRNAs are outlined below.

With a length of 22 nt, miR-619-5p is coded in an intron of the slingshot protein phosphatase 1 gene (*SSH1*), which is located on chromosome 12. We found that miR-619-5p has 1811 predicted binding sites on 1215 target mRNAs. Of these, 1772 miR-619-5p binding sites are located in 3'-UTRs, 26 sites are in located in 5'-UTRs, and 13 sites are located in CDSs. The mRNAs of 197 genes were found to have completely complementary binding sites for miR-619-5p. The mRNAs of 27 genes had four binding sites. Seven genes had five binding sites, and the mRNAs of the *CATAD1, ICA1L, GK5, POLH,* and *PRR11* genes had six miR-619-5p binding sites. The mRNAs of the *OPA3* and *CYP20A1* genes had eight and ten binding sites, respectively. All of these sites are located in the 3'-UTRs.

With a length of 21 nt, miR-5096 is coded in an intron of the BMP2 inducible kinase gene (*BMP2K*), which is located on chromosome 4. We found that miR-5096 has 997 predicted binding sites on 832 target mRNAs. Of these, 984 miR-5096 binding sites are located in 3'-UTRs, nine sites are located in 5'-UTRs, and four sites are located in CDSs. The mRNAs of 42 genes have completely complementary binding sites for miR-5096. The mRNAs of the *IP09* gene were found to have four binding sites, and the *PRR11* gene has five binding sites. The mRNAs of the *OPA3* and *CYP20A1* genes have six and 11 miR-5096 binding sites, respectively. All of these sites are located in 3'-UTRs.

With a length of 22 nt, miR-5585-3p is coded in an intron of the transmembrane protein 39b gene (*TMEM39B*), which is located on chromosome 4. We found that 725 target gene mRNAs have 844 predicted binding sites for miR-5585-3p. Nine of these binding sites are located in 5'-UTRs, two sites are located in CDSs, and 833 sites are located in 3'-UTRs. The mRNAs of the *CYP20A1* and *GPR155* genes each have four binding sites.

With a length of 21 nt, miR-5095 is coded in an intron of the sterol carrier protein 2 gene (*SCP2*), which is located on chromosome 1. We found that 655 target gene mRNAs have 734 predicted binding sites. Fourteen of these binding sites are located in 5'UTRs, eight sites are located in CDSs, and 712 sites are located in 3'-UTRs. The mRNAs of two genes have completely complementary binding sites for miR-5095. The mRNAs of the *OPA3* and *SPN* genes each have four binding sites.

3.2 miRNA Binding Sites in 5'-UTRs, CDSs and 3'-UTRs

The miR-619-5p, miR-5095, miR-5096, and miR-5585-3p binding sites in the 5'UTRs, CDSs, and 3'-UTRs of several genes were predicted using the miRTarget program. Multiple miRNA binding sites were revealed to be in the 5'-UTRs of several genes. For example, miR-619-5p has two binding sites in each of the 5'-UTRs of the *ANAPC16, CYB5D2*, and *PRR5* mRNAs and three binding sites in the *DNASE1* mRNA (Figure 1).

The 5'-UTRs and 3'-UTRs of the *ATAD3C* and *CYB5RL* genes have miR-619-5p binding sites. The CDSs and 3'-UTRs of the *C8orf44, ISY1*, and *ZNF714* genes have miR-619-5p binding sites. The mRNAs of some genes have binding sites for miR-619-5p, miR-5095, miR-5096, and miR-5585-3p within their 5'-UTRs and 3'-UTRs or within their CDSs and 3'-UTRs. The 5'-UTR and 3'-UTR of the *ANAPC16* gene have miR-5095, miR-5096, and miR-5585-3p binding sites. The 5'-UTR and 3'-UTR of the *ATAD3C* gene have miR-5095 and miR-619-5p binding sites. The 5'-UTRs and 3'-UTRs of the *C14orf182* and *CYB5RL* genes have miR-5096 and miR-619-5p binding sites, respectively. miR-5095 and miR-619-5p binding sites were found in the CDS and 3'-UTR of the *ISY1* gene. The CDS and 3'-UTR of the *ZNF714* gene have binding sites for miR-5096 and miR-619-5p, and the *C8orf44* mRNA has only a miR-619-5p binding site. The presence of miR-619-5p binding sites in the CDSs of the *C8orf44, ISY1*, and *ZNF714* genes, which have different functions, and the evolutionary conservation of these sites demonstrate the importance of the role of miRNAs in the regulation of the expression of these genes.

3.3 Multiple miRNA Binding Sites in the mRNAs of Target Genes

The mRNAs of some genes have multiple predicted miRNA binding sites. The nucleotide sequences with lengths of 95 nt that contained miR-619-5p, miR-5096, miR-5095, and miR-5585-3p binding sites are given in Figure 2. These results testify to the high degree of homology between the miRNA binding sites in the mRNAs of different genes. In addition to these binding sites, many other nucleotide sequences found in mRNAs were also found to be homologous. It is possible that the nucleotide sequences adjacent to the binding sites are binding sites for other miRNAs.

3.4 Target Genes of Cell Cycle and Apoptosis having miRNA Binding Sites

miR-619-5p, miR-5095, miR-5096, and miR-5585-3p have many general predicted target genes associated with the cell cycle and apoptosis (Table 1). Therefore, the expression of these genes is strong controlled by the miRNAs. For example, all of the revealed target genes associated with the cell cycle and apoptosis of miR-5585-3p are target genes of miR-619-5p. The quantity of the target genes for miR-619-5p and miR-5096 that are associated with apoptosis is two-fold higher than the number of target genes associated with the cell cycle. It is possible that the expression of apoptosis-related genes is exposed to more influence of miRNA inhibition than the expression of target genes associated with the cell cycle. The *ATM* and *VHL* genes are general participants in cell cycle regulation and apoptosis and have miR-619-5p, miR-

5095, miR-5096, and miR-5585-3p binding sites. Therefore, these genes can define a ratio of speeds for the cell cycle and apoptosis processes.

4 Discussion

If the concentration of a miRNA is lower than the concentration of its target mRNAs, the miRNA will poorly suppress the cell cycle and apoptosis. If there is hyperexpression of a miRNA in comparison with the mRNAs of its target genes, there will be a higher inhibition of apoptosis than of the cell cycle, which will lead to an increase in cell proliferation. This phenomenon is observed in tumour cells [12, 13]. Unfortunately, there is insufficient experimental proof that miR-619-5p, miR-5095, miR-5096, and miR-5585-3p participate in tumourigenesis. However, all of the predicted target genes of these miRNAs participate in the development of breast cancer and lung cancer [14-19].

It was predicted that miR-619-5p, miR-5095, miR-5096, and miR-5585 can bind to the mRNAs of 1215, 832, 725, and 655 genes, respectively. The nucleotide sequences of these miRNAs form hydrogen bonds with their target mRNAs, and the free energies of these bonds are equal to or greater than 90% of the maximum possible free energy. The miR-619-5p, miR-5095, miR-5096, and miR-5585-3p binding sites are located in the 3'-UTRs of target genes. Obviously, the maintenance of the nucleotide sequences for the binding site of one miRNA in the CDSs of such a high number of genes is complicated. The miRNA binding sites are located in the 5'-UTRs of some genes, but the number of such genes was small. The mRNAs of some genes had multiple miR-619-5p, miR-5095, miR-5096, and miR-5585-3p binding sites. It is possible that the identification of a large number miRNA binding sites in the mRNAs of some genes will be necessary for the reliable control of their gene expression.

The prediction of a large number of miRNA binding sites in the mRNAs of the genes investigated in this study presumably indicates new functional opportunities. It is possible that these miRNAs are coordinators of gene expression that participate in many major biological processes. The influences of miRNAs on the expression of genes that encode proteins participating in the cellular cycle [3, 20, 21, 22] and apoptosis [3, 12, 23, 24, 25] has been shown previously. Proteins that define the limiting stages of multistage processes need to be controlled to manage the multistage processes. One or several miRNAs that regulate the expression of several hundreds of genes will create a system of interconnected processes in cells and organisms. This role for these miRNAs is quite possible because these miRNAs circulate in the blood and can access nearly all cells of an organism [26, 27].

The present results are the basis for the study of the systemic roles of typical miRNAs in the regulation of gene expression in human cells based on new ideas of miRNA properties.

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Table 1. Target genes of cell cycle and apoptosis having miRNA binding sites.

miRNA	Cell cycle genes	Apoptosis genes
miR-619-5p	**ATM,* 9793, 9 8; *AURKA,* 426, 98; *BRCA1,* 6412, 98; *BRCA2,* 10746, 9 6; *CLSPN*, 7342, 93; *IL2RA,* 2083, 91 ; *IRF1,* 2659, 98; *IRF1,* 2235, 95; *IRF1,* 2523, 91; *KLK10,* 2139, 95; *PDCD4,* 3221, 1 00; *PDCD4,* 3355, 91; *RBBP4*, 4019, 97; *RBBP4*, 42 36, 9 5; *RBBP4,*4747, 91; *RBL1*, 3669, 97; *RBL1,* 3535, 93; *TBRG1*, 3312, 9 8; *TBRG1,* 3436, 91 ; *VHL,* 2989, 98; *VHL,* 3764, 100; *VHL,* 3898, 100; *VHL,* 3125, 93; *VHL,* 2686, 91.	*APAF1,* 6737, 95; *ATM,* 9793, 98; *CASP10, 3*247, 93; *CASP14,* 2606, 95; *CASP6,* 1163, 95; *CASP8,* 2488, 93; *CFLAR,* 1932, 95; *CFLAR, 5*910, 9 5; *CTSB*, 3193, 98; *CTSB,* 3326, 96; *CTSB,* 3503, 96; *CTSB,* 3638, 96; *DFFA*, 1795, 98; *DFFA,* 2745, 98; *DFFA,* 3125, 95; *DNASE1*, 602, 98; *DNASE1,* 501, 91; *DNASE1,* 735, 9 1; *FOXO3,* 6098, 9 6; *IKBIP,* 2324, 91, *IL10,* 1216, 98; *IL1R1*, 2165, 95; *IL1R1,* 2030, 95; *IL2RA,* 2083, 91; *IRAK1,* 2701, 98; *NAIP*, 5923, 95; *NAIP,* 6058, 95; *SCAF11,* 5459, 98; *SPN*, 3917, 95; *SPN*, 5287, 100; *SPN*, 601 8, 9 5; *SPN*, 6633, 95; *TNFRSF10A,* 1621, 10 0; *TNFRSF10D,* 1532, 1 00; *TNFSF10*, 1583, 95; *TNFSF10,* 1450, 91; *VHL,* 2989, 98; *VHL,* 3764, 10 0; *VHL,* 3898, 100; *VHL,* 3125, 93; *VHL,* 2686, 91.
miR-5096	*ATM,* 9882, 92; *BRCA1,* 6486, 98; *CLSPN*, 7416, 91 ; *IRF1,* 2597, 98; *IRF1,* 2731, 87; *RBBP4*, 430 8, 92; *RBL1*, 3609, 96; *VHL*, 3063 , 94 ; *VHL*, 3838, 94	*ATM*, 9882, 92; *CASP6*, 10 97, 94; *CFLAR*, 2 006, 92; *CFLAR*, 4705, 91; *CTSB*, 3265, 92; *DFFA*,1595, 98; *DNASE1*, 674, 91; *DNASE2*, 1613, 98; *FOXO3*, 6038, 96; *IL10*, 1290, 94; *NAIP*, 5997, 96; *SCAF11*, 5532, 94; *SPN*, 3989, 91; *SPN*, 6093, 100; *SPN*, 6 702, 9 8; *TNFRSF10A*, 1695, 91; *VHL*, 3063, 94; *VHL*, 3838, 94.
miR-5095	*ATM*, 9787, 93; *AURKA*, 420, 93; *BIRC5*, 3 52, 91; *BRCA1*, 6406, 91; *IRF1*, 2229, 95; *IRF1*, 2653, 95; *KLK10*, 2133, 91; *PDCD4*, 3215, 91; *RBBP4*, 4230, 1 00; *RBL1*, 3529,93; *TBRG1*, 3306, 95 ; *VHL*, 3892, 93; *VHL*, 2 983, 91.	*ATM*, 9787, 93; *BIRC5*, 352, 91; *CFLAR*, 5904, 91, *DFFA*, 1789, 9 8; *DFFA*, 2 739, 95; *DNASE1*, 59 6, 91; *IKBIP*, 2318, 91; *IL10*, 1210, 98; *IRAK1*, 2695, 95; *NAIP*, 5917, 91; *SPN*, 3911, 95; *SPN*, 5281, 91; *SPN*, 6012 , 91; *SPN*, 662 7,91; *VHL*, 389 2, 9 3; *VHL*, 2983, 91.
miR-5585-3p	*ATM*, 9950, 95; *BRCA1*, 6554, 95; *CLSPN*, 7487, 91; *GTSE1*, 2657, 91; *IL2RA*, 2223, 91; *IRF1*, 2800, 95; *RBBP4*, 4376, 95; *TBRG1*, 3 443, 9 5; *VHL*, 4041, 97.	*ATM*, 9950, 95; *CASP10*, 3389, 93; *CASP14*, 2613, 96; *CTSB*, 3645, 91; *DFFA*, 1940, 98; *DFFA*, 3265, 95; *DNASE1*, 7 42, 91; *FOXO3*, 6 105, 9 1; *IKBIP*, 2465, 96; *IL2RA*, 2223, 91; *SCAF11*, 56 00, 91; *TNFSF10*, 1590, 93; *VHL*, 4041, 97.

Note. * - gene, binding site position in mRNA, nt; $\Delta G/\Delta G_m$ ratio, %.

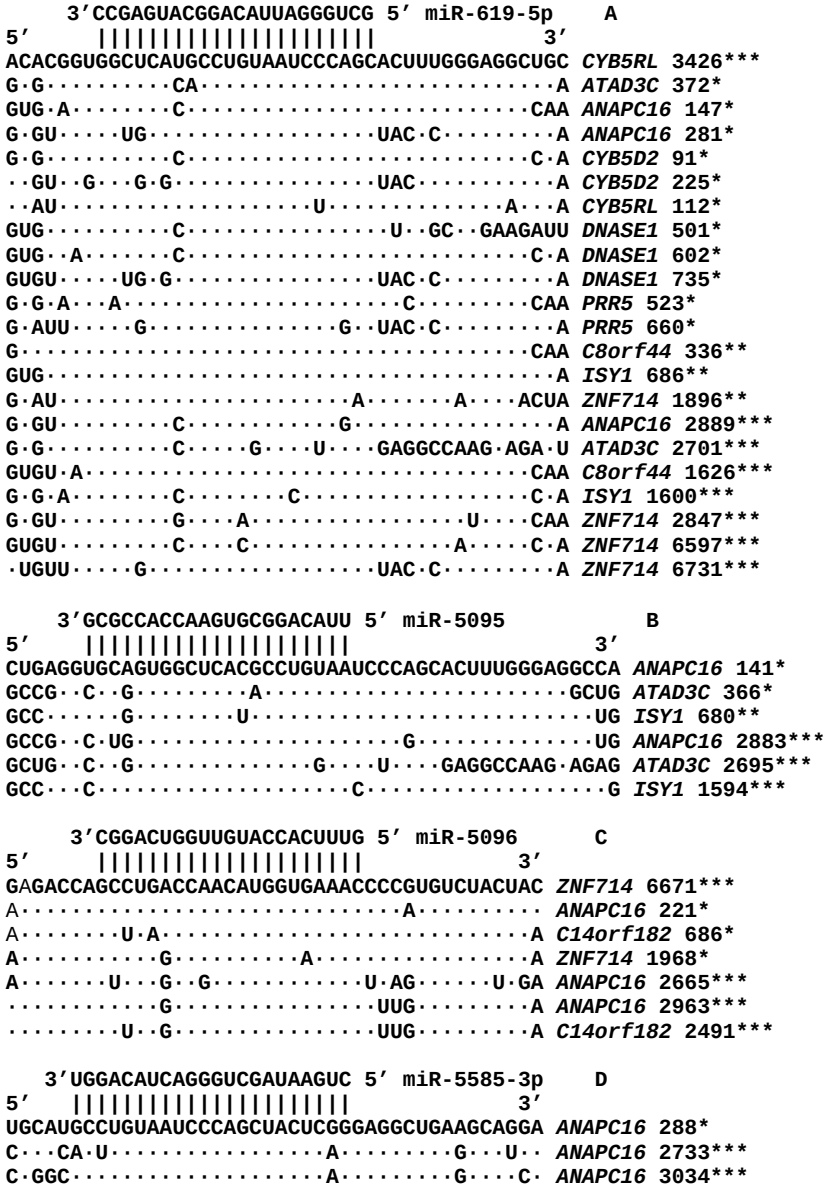
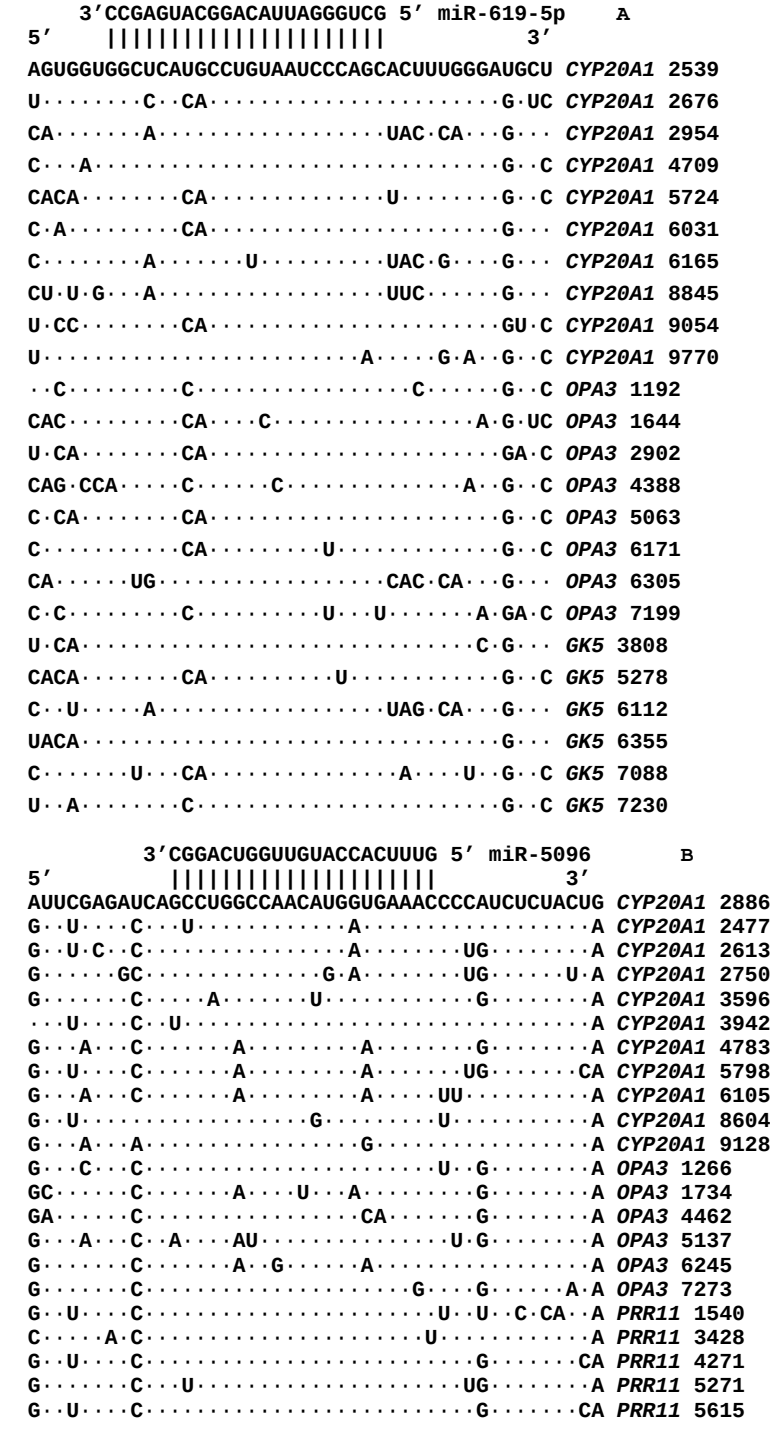


Fig. 1. miR-619-5p (A), miR-5095 (B), miR-5096 (C) and miR-5585 (D) Binding Sites in the 5'-UTRs, CDSs and 3'-UTRs of Human Genes. Note: the symbols *, ** and *** indicate the position of the origin of the miR-619-5p binding site from the first nucleotide of the mRNA in the 5'-UTRs, CDSs and 3'-UTRs, respectively.


3’CCGAGUACGGACAUUAGGGUCG 5’ miR-619-5p A
5’ 3’
AGUGGUGGCUCAUGCCUGUAAUCCCAGCACUUUGGGAUGCU CYP20A1 2539
U·····C··CA·····G·UC CYP20A1 2676
CA·····A·····UAC·CA···G··· CYP20A1 2954
C···A·····G··C CYP20A1 4709
CACA·····CA·····U·····G··C CYP20A1 5724
C·A·····CA·····G··· CYP20A1 6031
C·····A·····U·····UAC·G····G··· CYP20A1 6165
CU·U·G···A·····UUC·····G··· CYP20A1 8845
U·CC·····CA·····GU·C CYP20A1 9054
U·····A·····G·A··G··C CYP20A1 9770
··C·····C·····C·····G··C OPA3 1192
CAC·····CA····C·····A·G·UC OPA3 1644
U·CA·····CA·····GA·C OPA3 2902
CAG·CCA·····C·····C·····A··G··C OPA3 4388
C·CA·····CA·····G··C OPA3 5063
C·····CA·····U·····G··C OPA3 6171
CA·····UG·····CAC·CA···G··· OPA3 6305
C·C·····C·····U···U·····A·GA·C OPA3 7199
U·CA·····C·G··· GK5 3808
CACA·····CA·····U·····G··C GK5 5278
C··U·····A·····UAG·CA···G··· GK5 6112
UACA·····G··· GK5 6355
C·····U···CA·····A····U··G··C GK5 7088
U··A·····C·····G··C GK5 7230
3’CGGACUGGUUGUACCACUUUG 5’ miR-5096 B
5’ 3’
AUUCGAGAUCAGCCUGGCCAACAUGGUGAAACCCCAUCUCUACUG CYP20A1 2886
G··U····C···U·····A·····A CYP20A1 2477
G··U·C··C·····A·····UG·····A CYP20A1 2613
G·····GC·····G·A·····UG·····U·A CYP20A1 2750
G·····C·····A·····U·····G·····A CYP20A1 3596
···U····C··U·····A CYP20A1 3942
G···A···C·····A·····A·····G·····A CYP20A1 4783
G··U····C·····A·····A·····UG·····CA CYP20A1 5798
G···A···C·····A·····A·····UU·····A CYP20A1 6105
G··U·····G·····U·····A CYP20A1 8604
G···A···A·····G·····A CYP20A1 9128
G···C···C·····U··G·····A OPA3 1266
GC·····C·····A····U···A·····G·····A OPA3 1734
GA·····C·····CA·····G·····A OPA3 4462
G···A···C··A····AU·····U·G·····A OPA3 5137
G·····C·····A··G·····A·····A OPA3 6245
G·····C·····G····G·····A·A OPA3 7273
G··U····C·····U··U··C·CA··A PRR11 1540
C·····A·C·····U·····A PRR11 3428
G··U····C·····G·····CA PRR11 4271
G·····C···U·····UG·····A PRR11 5271
G··U····C·····G·····CA PRR11 5615

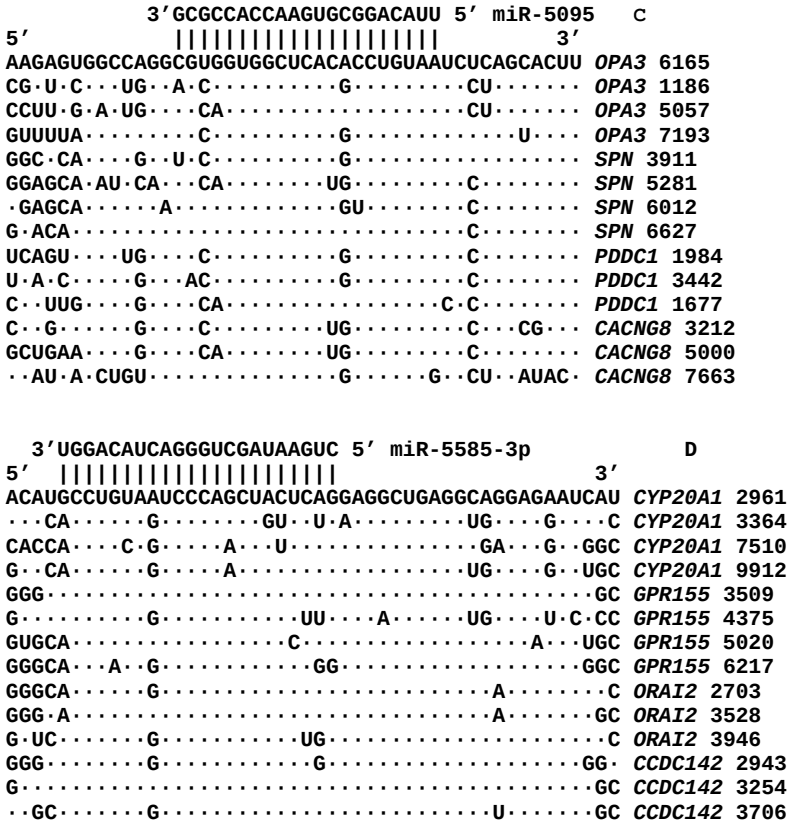


Fig. 2. The Nucleotide Parts of 3'-UTRs having Multiple miR-619-5p (A), miR-5096 (B), miR-5095 (C) and miR-5585-3p (D) Binding Sites.