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Features of mir-466-3p binding sites in mRNA genes with different functions

Abstract: The importance of miRNA in cellular regulation is gaining momentum. We searched miRNA binding sites using the MirTarget program. We identified several miRNAs that have greater than 300 target genes in humans, with multiple binding sites per gene. We observed that miR-466-3p 1,463 binding sites with high affinity (with $\Delta G/\Delta G_m$ values greater than or equal to 90%).
Key words: binding, sites, genes, functions.

Introduction

Numerous microRNAs (miRNAs) that regulate the expression of several hundred genes by binding to their mRNAs have been identified [1-4]. These miRNAs play key roles in the regulation of many biological processes and changes in their concentrations are observed in various pathologies in humans and other animals [5-7]. Changes in miRNA concentrations can be a cause or a consequence of a disease.

Materials and methods

The human gene mRNAs were taken from the GenBank (http://www.ncbi.nlm.nih.gov) using Lextractor002 script (http://sites.google.com/site/malaheenee/software). Human miR-466-3p was taken from the miRBase site (http://mirbase.org).

The target genes for the tested miRNA were revealed using the MirTarget program, which was developed in our laboratory. This program defines the following features of binding: a) the beginning of an miRNA binding with mRNAs; b) the localization of miRNA binding sites in the 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs) and 3'-untranslated regions (3'UTRs) of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between the miRNAs and the mRNAs. The ratio $\Delta G/\Delta G_m$ (%) was counted for each site, where ΔG_m equaled the free energy of a miRNA binding with its perfect complementary nucleotide sequence. The miRNA binding sites located on the mRNAs had $\Delta G/\Delta G_m$ ratios of 90% and more. We also note the position of the binding sites on the mRNA, beginning from the first nucleotide of the mRNA's 5'UTR. The MirTarget program computes the interactions between the nucleotides of miRNAs and those of target gene mRNAs. It found bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), and G and U, as well as between A and C via a hydrogen bond [8]. The distance between A and C was equal to the G-C, A-U, and G-U distances [9]. The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively. The free binding energies of these nucleotide pairs were accepted as the same values (3:2:1:1).

Results and their discussion

One miRNA that has multiple binding sites is miR-466-3p, which has more than 300 target genes. Their mRNAs include a total of 1,463 binding sites, including from 1 to 18 sites in *RAPGEFL1* and *SYT2*, 25 in *PRKX*, and 38 sites in *DONSON* mRNA. The miRNA binding sites have $\Delta G/\Delta G_m$ ratios of 90% or greater. This indicates a high degree of interaction between miRNAs and mRNAs. Multiple binding sites increase the probability that miRNAs interact with mRNAs, and as a consequence, the translation of such mRNAs is reduced.

The target genes of miR-466-3p serve various functions. Many are transcription factors and kinases, some are involved in the cell cycle, apoptosis, and other processes (Table «a»).

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Table «a» – The characteristics of miR-466-3p binding in the 5'UTR of mRNA target genes

Gene	Start positions of binding sites, nt	ΔG, kJ/mole	Gene	Start positions of binding sites, nt	ΔG, kJ/mole
BHLHE41	89 (1)*	-106.1	*RUNDC3B*	172-160 (10)	-106.1÷-106.2
CASQ2	31 (1)	-106.1	*SPOP*	79 (1)	-106.2
CD97	140-138 (11)	-106.2÷108.3	*STAT6*	102-100 (5)	-106.2÷108.3
CELF4	299 (1)	-108.3	*TSHZ2*	22 (1)	-106.2
CHRNA2	9-23 (3)	-106.2÷108.3	*VWA3A*	17-23 (2)	-108.3÷110.4
GRIA2	52-68 (3)	-106.2	*XPO1*	499-507 (5)	-106.2
KIAA0319	265-291 (14)	-106.2	*ZNF331*	409-415 (4)	-106.2
NOS1AP	2099 (1)	-106.2			
Note. *In brackets are number of binding sites					

One of the objectives of this work was to identify the target genes of miR-466-3p, which may contribute to the development of several diseases. The mRNA of *ADAT2, ADCYAP1R1, BHLHE40, CDK6, FASLG, FGFR3, FLT1, MACC1, MAPKAPK2, MECOM, SPN, STAT6,* and *UGT8* genes, which are involved in the development of lung and breast cancer, are associated with miR-466-3p, indicating an increased probability that miR-466-3p influences these types of cancer. *ADRBK2, BHLHE40, CD36, EGR3, NDUFS2, NFAT5, NOS1, PLA2G7, S1PR2,* and *STAT6* genes are responsible for diseases of the cardiovascular system, and their mRNAs also interact with miR-466-3p. *CDK6* and *MECOM* genes are involved in cell cycle regulation and apoptosis, and their mRNA sequences contain targets for miR-466-3p. We identified the *BHLHE40* gene, which exhibits changes in expression in lung cancer, breast cancer, cardiovascular diseases, and circadian rhythm disorders. *BHLHE40* is a transcription factor that is expressed in various tissue types. It encodes a protein involved in the control of circadian rhythms, cell differentiation, proliferation, cell cycle, apoptosis, and the development of various diseases. Therefore, a change in the expression of this gene after binding with miR-466-3p can cause many diseases.

In addition to mir-466, we identified several miRNAs that have greater than 300 target genes in humans, with multiple binding sites per gene. We observed that miR-3960 has 2,563 mRNA binding sites with high affinity for 375 human genes. For example, miR-3960 has 565 binding sites in 5' UTRs and 515 sites in mRNA coding sequences (CDS). The mRNA regions that contain several miR-3960 binding sites have starting sites located through one, two, or three nucleotides. The nucleotide sequences of the binding sites located in CDSs encode polyalanine or polyproline. We determined that most of the target genes for the miRNAs examined encode transcription factors. Specifically, miR-3960, miR-3620-5p, and miR-8072 bind with genes involved in cell cycle regulation and apoptosis. Hsa-miR-1322 has more than 2,000 binding sites in the mRNAs of 1,058 genes. This includes 1,889 binding sites in CDSs, 215 binding sites in 5' UTRs, and 160 binding sites in 3' UTRs. Between 2 and 28 binding sites were arranged sequentially with start positions overlapping with three nucleotides of the adjacent binding site. The nucleotide sequences of these sites in CDSs encode oligopeptides with the same and/or different amino acid sequences. We found that 33% of the target genes encoded transcription factors. The miR-1322 binding sites has arranged binding sites were arranged in the CDSs of the orthologous genes *MAMLD1*, *MAML2,* and *MAML3* genes. These sites encode a polyglutamine oligopeptide ranging from six 6 to 47 amino acids in length [1, 10].

We identified 266 target human genes for miR-574-5p and six target genes for miR-574-3p. The miR-574-5p binding sites were mainly located mainly in the 3' UTRs, and theirthere number is equal towere 1,429 in total. The miR-574-5p binding sites were located in the 3' UTRs of the mRNA sequences of 244 genes mRNAs, in the 5' UTRs of 20 genes mRNAs and in the CDSs of two genes mRNAs. The miR-574-5p binding sites in the CDS of *FGFRL1* and *REM2* genes mRNAs encode the oligopeptides HTHTHTHS and DTDMDTDT in the relevant proteins. The beginning start sites of multiple miR-574-5p binding sites arranged located through two nucleotides and the number of binding sites in one region

ranged from 1 to 37. The target genes of miR-574-5p have been implicated in the development of breast and, lung cancer and other diseases. A significant substantial part portion of the target genes of miR-574-5p are transcription factors and kinases, which are involved in apoptosis and the cell cycle. The synthesis of miR-574-5p and miR-574-3p depends on the expression of the master's gene *FAM114A1* expression, which is the target for of 15 miRNAs. Changes in the expression of miR-574-5p and miR-574-3p are correlated with changes in the expression of their target genes, at which are associated with the development of many pathologies, including cardiovascular diseases and cancer [11].

Table «a» shows the characteristics of miR-466-3p binding sites in the 5' UTRs of 15 target genes mRNAs with the value of ΔG/ΔGm values that were equal or greater than 90%. The number of miR-466-3p binding sites with mRNAs offor these genes varied from one 1 to 14 (Table «b», «c»), indicating the variation in the probability of an interaction between miR-466-3p interaction and target mRNAs, since the greater the length of the section of longer binding sites, is associated with a higher the probability of interaction. It is possible that the mRNA of the *KIAA0319* gene may contacts with two RISC complexes containing miR-466-3p, since the length of the site from nucleotides 265 to 314 nt, which contains multiple binding sites, is 50 nt.

Table «b» – The characteristics of miR-466-3p binding in the 3'UTR of mRNA target genes having 1-4 sites

Gene	Start positions of binding sites, nt	ΔG, kJ/mole	Gene	Start positions of binding sites, nt	ΔG, kJ/mole
ADAMTS4	3382 (1)	-110.4	*LSAMP*	2285 (1)	-106.2
ADRB3	2451 (1)	-110.4	*MAPKAPK2*	2587-2589 (2)	-106.2÷-108.3
AKIRIN1	1057 (1)	-106.2	*MECOM*	5099 (1)	-110.4
AQP3	1254-1477 (2)	-106.2	*MED24*	3606-3612 (4)	-106.2÷-108.3
ARHGAP26	8270 (1)	-106.2	*METTL16*	3581-3585 (4)	-106.2÷-108.3
ARNT	3104 (1)	-106.2	*MVB12B*	3440-3438 (2)	-106.2÷-108.3
ATAD1	1830 (1)	-106.2	*MYO16*	6426 (1)	-106.2
ATG10	1928 (1)	-108.3	*NACC2*	2484-5875 (2)	-106.1÷-108.3
ATP6V0D1	1201-1211 (3)	-106.1÷110.4	*NAV1*	9412-9418 (4)	-106.2
ATXN7L3B	3359 (1)	-108.3	*NCOA3*	5966-5974 (2)	-106.2÷-108.3
BAHCC1	8118 (1)	-108.3	*NET1*	2642 (1)	-106.2
BHLHE40	1687-1693 (4)	-106.2÷-108.3	*NFAT5*	8540-8548 (3)	-106.2÷-108.3
BNC2	3528-3534 (3)	-106.2	*NPAP1*	5206 (1)	-106.2
BSN	15555 (1)	-106.1	*NPTXR*	5706-5712 (3)	-106.2÷-108.3
C16orf52	1410-1416 (2)	-108.3÷-110.4	*NR1D2*	3337 (1)	-110.4
C19orf59	816 (1)	-106.1	*OCLN*	3570-3572 (2)	-106.2÷-108.3
C3orf72	2537-2543 (2)	-108.3	*PARD3B*	4736 (1)	-110.4
C9orf47	1531 (1)	-106.2	*PDE7A*	2666 (1)	-106.2
CACNA1B	8725-8731 (4)	-106.2÷-108.3	*PHKA1*	5211-5213 (4)	-106.2÷-108.3
CADM3	2634-2646 (2)	-106.2	*PIK3R1*	6519-6523 (4)	-106.2÷-108.3
CD19	1861-1863 (2)	-106.2÷-108.3	*PLAG1*	4546 (1)	-106.2
CD3EAP	3086-3092 (4)	-106.2	*PLCXD1*	2548 (1)	-108.3
CEP135	5219-5221 (2)	-106.2÷-108.3	*PPARGC1A*	2806-2822 (2)	-106.2
CEP85L	3355 (1)	-106.2	*PPARGC1B*	9983 (1)	-106.2
CISD2	1146-1144 (4)	-106.2÷-108.3	*PPIC*	781-787 (4)	-106.2
CLEC4D	1001 (1)	-108.3	*PRR5L*	3504 (1)	-106.2
CLN8	2553-2555 (2)	-106.2	*PRSS23*	1454-1458 (3)	-106.2
CLRN1	1303-1309 (4)	-106.2÷-108.3	*PSD3*	4157-4161 (2)	-106.2÷-110.4

Continuation of Table «b»

Gene	Start positions of binding sites, nt	ΔG, kJ/mole	Gene	Start positions of binding sites, nt	ΔG, kJ/mole
DDB1	3990-3998 (3)	-106.2÷-108.3	*PTCH1*	6512-6520 (3)	-106.1
DGKG	3890 -3908 (4)	-106.1÷-106.2	*RNF121*	2208-2214 (3)	-106.2
DOK6	1761 (1)	-110.4	*RUNX1*	5455-5459 (2)	-106.2÷-110.4
DPYSL5	4857 (1)	-106.1	*S1PR2*	3189 (1)	-110.4
DTNA	5953 (1)	-106.2	*SBK1*	4530 (1)	-110.4
DYNC1LI1	1796 (2)	-106.2	*SEL1L3*	4370-4374 (3)	-106.2÷-108.3
EGR3	2026 (1)	-108.3	*SGPL1*	3972-3974 (2)	-106.2÷-108.3
EPG5	8002 (1)	-106.2	*SIK1*	2855-2857 (2)	-106.2
EPHB3	4065 (1)	-106.2	*SLC13A2*	2189 (1)	-106.2
F11R	4268 (1)	-106.2	*SLC16A9*	2962 (1)	-106.2
FAM105B	5211 (1)	-106.2	*SLC1A4*	4073 (1)	-108.3
FAM178A	4992-4996 (3)	-106.2÷-108.3	*SLC25A22*	2213 (1)	-106.2
FAM46C	5184 (1)	-106.1	*SLC35E3*	1905 (1)	-106.2
FBXO9	1718-1720 (2)	-106.2÷-112.5	*SLC6A6*	2306-2314 (2)	-106.2÷-110.4
FGFR3	2809 (1)	-110.4	*SLC7A11*	3438-3446 (3)	-106.2÷-108.3
FHOD1	3751-3759 (2)	-106.2	*SOGA3*	8790 (1)	-106.2
FLVCR2	3355 (1)	-106.2	*SPN*	4373-4387 (2)	-106.2÷-108.3
FOXI2	2790 (1)	-106.1	*SPOCK2*	1917 (1)	-108.3
FRMD3	3217	-108.3	*STARD8*	4681 (1)	-110.4
FXYD6	963-1138 (4)	-106.2÷-108.3	*STAT5B*	2717-2721 (3)	-106.2
GADL1	3346 (1)	-106.2	*STRBP*	6071-6073 (2)	-106.2
GOLGA7B	5601 (1)	-106.2	*STX1B*	1032-1180 (4)	-106.2÷-108.3
GPX3	1108 (1)	-106.2	*TFCP2*	3457-3610 (3)	-106.2
GRID1	5455 (1)	-106.2	*TM9SF3*	4448 (1)	-106.2
GRIK4	4357 (1)	-106.1	*TMC7*	4230 (1)	-106.2
GSK3B	4673 (1)	-106.2	*TMOD2*	8207 (1)	-108.3
HIC2	3104-3122 (2)	-106.2÷-108.3	*UGT8*	2500-2516 (3)	-106.2÷-108.3
HIF1AN	2037-2041 (2)	-108.3	*UNC80*	11147 (1)	-110.4
HLF	5348-1554 (3)	-106.2÷-110.4	*USH1G*	2580-2745 (3)	-106.2
ICAM1	2988 (1)	-106.2	*VPS13D*	14819-14821 (2)	-106.2
IGFBP4	1230-1234 (2)	-106.1	*WDR35*	6259 (1)	-106.2
IMPG2	5471 (1)	-114.6	*WDR37*	4026-4030 (3)	-106.2
IRS1	7318-7320 (2)	-106.2÷-108.3	*WDR5B*	2049 (1)	-106.2
ITGAM	3749 (1)	-106.1	*WDR72*	5941	-106.2
KCTD11	2640-2644 (3)	-106.2	*WSCD2*	4274-4276 (4)	-106.2÷-108.3
KCTD16	3932 (1)	-108.3	*ZBTB42*	3075-3077 (3)	-106.2÷-108.3
KIAA0408	5096 (1)	-106.2	*ZDHHC22*	1832-1836 (3)	-106.2
KIAA1804	5327 (1)	-108.3	*ZEB2*	6533 (1)	-106.2
KIAA2022	5693 (1)	-106.2	*ZNF33A*	3169 (1)	-108.3
KIF13A	6965 (1)	-106.2	*ZNF33B*	3103 (1)	-106.2
KIF3C	3122 (1)	-106.1	*ZNF346*	2186-2202 (2)	-108.3
KLHL3	2677-2679 (2)	-106.2	*ZNF428*	1214-1224 (2)	-106.2÷-108.3

Continuation of Table «b»

Gene	Start positions of binding sites, nt	ΔG, kJ/mole	Gene	Start positions of binding sites, nt	ΔG, kJ/mole
KLHL42	3780 (1)	-108.3	*ZNF483*	3636-3638 (3)	-106.2÷-108.3
LFNG	1270 (1)	-108.3	*ZNF562*	3963-3971 (3)	-106.2÷-110.4
LILRB2	2858-2864 (4)	-106.2÷-108.3	*ZNF618*	2835 (1)	-106.2
LONRF1	2685 (1)	-106.1			

Table «c» – The characteristics of miR-466-3p binding in the 3'UTR of mRNA target genes having five and more binding sites

Gene	Start positions of binding sites, nt	$\Delta G/\Delta G_m$, kJ/mole	Gene	Start positions of binding sites, nt	$\Delta G/\Delta G_m$, kJ/mole
ABLIM1	4472-4498 (14)	-106.2	*MLLT6*	4051-4075 (6)	-106.2÷-110.4
ADAT2	1950-1964 (8)	-106.2	*MMS22L*	5738-5754 (9)	-106.2
ADCYAP1R1	3841-3853 (7)	-106.2÷-108.3	*MYADM*	1982-1992 (6)	-106.2
ADRBK2	6676- 6696 (11)	-106.2	*MYSM1*	3512-3524 (7)	-106.2
AFF1	6829-6837 (5)	-106.2	*NDRG4*	3108-3116 (5)	-106.2÷-108.3
AKAP11	6559-6579 (11)	-106.2	*NDUFS2*	1917- 1925 (5)	-106.2÷-108.3
ANKLE1	2219-2255 (12)	-106.2	*NKTR*	5828-5838 (6)	-106.2
ARHGAP12	4800-4814 (8)	-106.2	*NOS1*	5560-5568 (5)	-106.2
ATP9A	3509-3523 (8)	-106.2	*OAS3*	6257-6267 (6)	-106.2
BACH1	4266 -4274 (8)	-106.2÷-110.4	*PAK6*	3163-3179 (9)	-106.2
BACH2	5578-5604 (14)	-106.2	*PARN*	2839-2861 (12)	-106.2
BAZ2A	6856-6868 (7)	-106.2	*PCBD2*	1805-1815 (6)	-106.2
BGN	1960-1980 (11)	-106.2	*PCK1*	2388-2414 (9)	-106.2÷-108.3
C11orf75	693-709 (9)	-106.2÷-108.3	*PDE1A*	2793-2805 (7)	-106.2÷-108.3
C21orf91	5113-5121 (5)	-106.2	*PDE3A*	4022-4040 (10)	-106.2
C2orf91	1577-1593 (7)	-106.2	*PEAK1*	7268-7290 (12)	-106.2÷-108.3
CACFD1	2283-2303 (9)	-106.2÷-108.3	*PIGS*	2585-2597 (7)	-106.2
CACNG8	6807-6817 (6)	-106.2	*PKNOX1*	1680-1688 (7)	-106.2÷-108.3
CBX3	1306-1314 (5)	-106.2÷-108.3	*PLA2G7*	1643-1651 (5)	-106.2÷-108.3
CCDC9	1815-1833 (10)	-106.2	*PLEKHA2*	1672-1684 (7)	-106.2
CD2AP	2829-2849 (11)	-106.2÷-108.3	*PRKX*	5366- 5390 (13)	-106.2
CD36	3530-3538 (5)	-106.2÷-108.3	*PTGES*	1664-1688 (13)	-106.2
CDK6	1907-1917 (8)	-106.2÷-108.3	*PTP4A2*	3572-3586 (8)	-106.2÷-108.3
CHRDL1	3452-3474 (12)	-106.2	*PTPN3*	7001-7039 (20)	-106.2
CLASP1	6695-6711 (9)	-106.2	*QSOX2*	3460- 3476 (9)	-106.2÷-108.3
CNOT6	5094-5104 (6)	-106.2÷-108.3	*RABGAP1*	4103-4105 (7)	-106.2÷-108.3
DBT	5929-5965 (19)	-106.2÷-108.3	*RAPGEFL1*	2305-2339 (18)	-106.2
DMXL1	9853-6922 (5)	-106.2÷-108.3	*RDX*	3321-3329 (5)	-106.2
DONSON	2372-2446 (38)	-106.2	*REEP3*	3836-3856 (12)	-106.2÷-108.3
ELOF1	429-455 (14)	-106.2	*RORA*	5293-5307 (8)	-106.2
ETS1	3887-3907 (11)	-106.2	*SAMD4A*	4191-4201 (6)	-106.2
EVI2A	2015-2027 (7)	-106.2	*SEPT3*	1319-1333 (8)	-106.2

Continuation of table «c»

Gene	Start positions of binding sites, nt	$\Delta G/\Delta G_m$, kJ/mole	Gene	Start positions of binding sites, nt	$\Delta G/\Delta G_m$, kJ/mole
FAM120C	4206-4222 (9)	-106.2	*SERBP1*	5153-5163 (6)	-106.2
FAM126B	3744-3764 (7)	-106.2÷-108.3	*SFN*	1189-1199 (6)	-106.2
FAM180B	928-940 (7)	-106.2	*SH3PXD2A*	8615-8623 (5)	-106.2
FAM212B	1317-3453 (7)	-106.2	*SLC1A5*	2651-2679 (10)	-106.2
FAM216B	1390-1398 (5)	-106.2	*SLC25A44*	1893-1913 (11)	-106.2
FASLG	1603-1613 (6)	-106.2÷-108.3	*SLC30A3*	1865-1905 (9)	-106.2
FGF9	1737-1745 (8)	-106.2÷-108.3	*SLFN5*	3046-3058 (7)	-106.2÷-108.3
FLT1	6910-6922 (8)	-106.2÷-108.3	*SMIM15*	2574-2584 (6)	-106.2
FOXK1	10612- 10628 (9)	-106.2÷-108.3	*SP1*	4146-4158 (7)	-106.2
GGA2	2081- 2103 (12)	-106.2	*ST8SIA1*	4536-4558 (12)	-106.2
GID4	2279 – 2291 (7)	-106.2÷-108.3	*SYNPO2L*	3586-3600 (8)	-106.2
GLCCI1	3955- 3963 (5)	-106.2÷-108.3	*SYT1*	2739-2749 (6)	-106.2
GNAI1	2937-2945 (5)	-106.2	*SYT2*	1863-1907 (15)	-106.2÷-108.3
GPR21	1481- 1503 (12)	-106.2÷-108.3	*SYTL4*	2462-2480 (6)	-106.2÷-110.4
GTPBP1	2471-2485 (8)	-106.2	*TBX4*	1780-1798 (10)	-106.2÷-108.3
HEMGN	1841-1849 (6)	-106.2÷-108.3	*TIPRL*	2688-2702 (8)	-106.2÷-108.3
HPS4	3984-3998 (8)	-106.2	*TMEM132B*	5871-5879 (5)	-106.2
IGF2R	8446-8454 (5)	-106.2	*TMEM2*	6337-6345 (5)	-106.2
JAK2	5183-5199 (9)	-106.2	*TMEM30B*	2800-2812 (7)	-106.2
KCNJ10	3349-3375 (14)	-106.2	*TNFRSF21*	2497-2517 (7)	-106.2
KCNJ12	4656- 4670 (8)	-106.2÷-108.3	*TNIP3*	1604-1620 (9)	-106.2÷-108.3
KCNK10	5523-5547 (13)	-106.2÷-108.3	*UBN2*	10914-10924 (6)	-106.2
KIAA2026	6628-6644 (9)	-106.2	*UMPS*	4221-4241 (10)	-106.2
KIFC1	2488-2512 (13)	-106.2	*UNC5B*	4036-4046 (6)	-106.2
LANCL3	2972-2980 (5)	-106.2÷-108.3	*VAPB*	2523-2537 (8)	-106.2÷-108.3
LSM14A	2048-2070 (10)	-106.2÷-108.3	*WDR3*	3560-3588 (11)	-106.2÷-110.4
MACC1	3950-3966 (10)	-106.2÷-108.3	*WT1*	2704-2714 (6)	-106.2
MARCH5	1863-1879 (9)	-106.2÷-108.3	*ZC3H12C*	6510-6528 (10)	-106.2
MCTS1	1294-1318 (7)	-106.2÷-108.3	*ZDHHC21*	7360-7374 (8)	-106.2
MGAT5	4307-4333 (14)	-106.2÷-108.3	*ZNF670*	1930-1940 (7)	-106.2÷-108.3
MLLT4	7131-7153 (12)	-106.2	*ZXDA*	3942-3952 (6)	-106.2÷-108.3

The binding characteristics of miR-466-3p in the 3'UTR of 147 target genes mRNAs with the value $\Delta G/\Delta Gm$ equal or greater than 90% are shown in Table «b». The number of miR-466-3p binding sites on mRNAs of these genes varied from one to four, and indicates the different probability of miR-466-3p interaction with mRNAs, since the greater the length of the section of longer binding sites, is associated with a higher the probability of interaction. ZNF family transcription factors are among of target genes, and they have less than four binding sites. Only *ZNF670* gene mRNA has seven binding sites (Table «c»). miR-466-3p target genes involved in many biological processes and, therefore, miR-466-3p may participate in the regulation and can cause diseases. Table «d» demonstrates some of these genes.

Table «c» demonstrates characteristics of miR-466-3p binding in the 3'UTR of target genes mRNAs, having five or more binding sites in the mRNA.

Table «d» – miR-466-3p target genes involved in many biological processes

Functional group	Genes
Transcription factors	*BACH1*, 4256-4274*, eight sites, -106.2÷-110.4**; *BHLHE40*, 1683-1693, four sites, -106.2÷-108.3; *EGR3*, 2026, one site, -108.3; *FOXI2*, 2790, one site, -106.2; *MECOM*, 5099, one site, -110.4; *RUNX1*, 5455-5459, two sites, -106.2÷-110.4; *ZNF618*, 2835, one site, -106.2.
Kinases	*ADRBK2*, 6676-6696, 11 sites, -106.2; *AKAP11*, 6559-6579, 11 sites, -106.2; *CDK6*, 1895-1919, eight sites, -106.2÷-108.3; *FLT1*, 6910-6936, eight sites, -106.2÷-108.3; *JAK2*, 5183-5199, nine sites, -106.2; *KIAA1804*, 5327, one site, -108.3; *MAPKAPK2*, 2587-2589, two sites, -106.2÷-108.3; *PCK1*, 2388-2414, nine sites, -106.2÷-108.3; *PIK3R1*, 6519-6523, four sites, -106.2÷-108.3; *PRKX*, 1804-5390, 25 sites, -106.2÷-108.3; *SBK1*, 4530, one site, -110.4.
Genes of cell cycle	*BACH1*; *BHLHE40*; *CBX3*, 1306-1314, five sites, -106.2÷-108.3; *CDK6*; *DDB1*, 3990-4004, three sites, -106.2÷-108.3; *MECOM*; *WDR3*, 3560-3588, 11 sites, -106.2÷-110.4; *MACC1*, 2979-3966, 10 sites, -106.2÷-108.3; *MAPKAPK2*; *NDRG4*, 3108-3116, five sites, -106.2÷-108.3; *PIK3R1*.
Genes of apoptosis	*BACH1*; *BHLHE40*; *CD2AP*, 2829-2849, 11 sites, -106.2÷-108.3; *CD36*, 3530-3538, five sites, -106.2÷-108.3; *CDK6*; *CNOT6*, 5094-5104, six sites, -106.2÷-108.3; *DDB1*; *FASLG*, 1603-1613, six sites, -106.2÷-108.3; *FGF9*, 1729-1753, eight sites, -106.2÷-108.3; *FLT1*; *HIF1AN*, 2037-2041, two sites, -108.3; *MACC1*; *MAPKAPK2*; *MECOM*; *OCLN*, 3570-3572, two sites, -106.2÷-108.3; *SLC7A11*, 3438-3446, three sites, -106.2÷-108.3; *SPN*, 4373-4387, two sites, -106.2÷-108.3; *TIPRL*, 2688-2702, eight sites, -106.2÷-108.3; *WDR3*.
Genes of breast cancer	*ADAT2,* 1950-1964, eight sites, -106.2; *ADCYAP1R1*, 3841-3853, seven sites, -106.2÷-108.3; *BHLHE40*; *CBX3; CDK6; CISD2*, 1140-1146, four sites, -106.2÷-108.3; *EGR3;FASLG; FGFR3*, 2809, one site, -110.4; *FLT1; HIF1AN; IRS1*, 7318-7320, two sites, -106.2÷-108.3; *LFNG*, 1270, one site, -108.3; *MACC1; MAPKAPK2; MECOM; MGAT5*, 4307-4333, 14 sites, -106.2÷-108.3; *NFAT5,* 8532-8548, three sites, -106.2÷-108.3; *PEAK1,* 7268-7290, 12 sites, -106.2÷-108.3; *PIK3R1; PTP4A2*, 3562-3586, eight sites, -106.2÷-108.3; *SLC7A11; SPN; STAT6*, 94-102, five sites, -106.2÷108.3; *UGT8,* 2492-2516, three sites, -106.2÷-108.3; *VAPB,* 2523-2537, eight sites, -106.2÷-108.3 27 sites.
Genes of lung cancer	*ADAT2; ADCYAP1R1; BHLHE40; CDK6; DPYSL5*, 4857, one site, -106.2; *FASLG; FGF9; FGFR3; FLT1; HEMGN,* 1841-1849, six sites, -106.2÷-108.3; *MACC1; MAPKAPK2; MECOM; LILRB2,* 2858-2864, four sites, -106.2÷-108.3; *SPN; STAT6; UGT8* 17 sites.
Genes of cardiovas-cular diseases	*ADRBK2; BHLHE40; CD36; EGR3; NDUFS2*, 1917-1925, five sites, -106.2÷-108.3; *NFAT5; NOS1*, 5560-5568, five sites, -106.2; *PLA2G7,* 1643-1651, five sites, -106.2÷-108.3; *S1PR2*, 3189, one site, -110.4; *STAT6*.
Genes of circadian rhythm	*BHLHE40; DBT,* 5929-5965, 19 sites, -106.2÷-108.3; *NR1D2*, 3337, one site, -110.4.
Note: * – start positions of binding sites; ** – energy of hybridization (kJ/mole).	

Typically, the more multiple binding sites available in the mRNA, the more extended is the section. mRNA of *LSM14A* and *PARN* genes having 10 and 12 binding sites, have a length of 45 nucleotides. mRNA of *BACH2, KCNJ10* genes containing 10 and 12 binding sites, have a length of 49 nt. mRNA of *RAPGEFL1, PTPN3* genes containing 18 and 21 binding sites, have a length of 57 nt and 61nt, respectively. mRNA of gene containing 38 binding sites, have a length 97nt. mRNAs of the above genes can bind more than one RISC complex containing miR-466-3p and efficiency of translation of mRNA suppression will be higher. We have developed a program of predicting miRNA binding sites with mRNAs which allows high reliability to establish these sites. At the value of ΔG/ΔGm equal to 90%, the level of p <0.0001. For example, we were able to identify 200 genes mRNAs from 18,000 human genes which have entirely complementary binding sites for unique miR-619.

Considering the possibility of regulating that the expression of many genes are regulated by miR-466-3p, it should be expected that the concentration of miR-466-3p should notis not expected to change in the norm vary widely; otherwise, excessively high or low levels of miR-466-3p would inevitably lead to the disruption of the expression of genes involved in key biological processes and would result in several pathological conditions. The type of disease will depends on the ratio of the concentrations of miR-466-3p and target mRNAs of target genes in specific cells and tissues of the body [12-18].

References

1. Niyazova R., Berillo O., Atambayeva Sh., Pyrkova A., Alybaeva A., Ivashchenko A. miR-1322 Binding Sites in Paralogous and Orthologous Genes. // Biomed Research International. – 2015. – P. 1-7.

2. Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva Sh. Binding sites of mir-1273 family on the mRNA of target genes. // Biomed research international. – 2014. – P. 1-8.

3. Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva Sh. The properties of binding sites of miR-619-5p, miR-5095, miR-5096 and miR-5585-3p in the mRNAs of human genes. // Biomed research international. – 2014. – P. 2014.

4. Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva Sh. MiR-3960 binding sites with mRNA of human genes. // Bioinformation. – 2014. – Vol. 10. – No. 7. – P. 423-427.

5. Cheng Q., Yi B., Wang A., Jiang X. Exploring and exploiting the fundamental role of microRNAs in tumor pathogenesis. // Onco Targets Ther. – 2013. – Vol. 6. – P. 1675-1684.

6. Hromadnikova I., Kotlabova K., Hympanova L., Krofta L. Cardiovascular and cerebrovascular desease associated microRNAs are dysregulated in placental tissues affected with gestational hypertension, preeclampsia and intrauterine growth restriction. // Plos One. – 2015. – Vol. 10 – No. 9. – e0138383.

7. Faruq O., Vecchione A. microRNA: Diagnostic perspective. // Front Med. – 2015. – 2. – P. 51.

8. Kool E.T. Hydrogen bonding, base stacking, and steric effects in DNA replication. // Annu.Rev. Biophys.Biomol.Struct. – 2001. – Vol. 30. – P. 1–22.

9. Leontis N.B., Stombaugh J., Westhof E. The non-Watson-Crick base pairs and their associated isostericity matrices. // Nucleic Acids Res. – 2002. – Vol. 30. – P. 3497–3531.

10. Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva S. MiR-3960 binding sites with mRNA of human genes. // Bioinformation. – 2014. – Vol. 10. – No. 7. – P. 423-427.

11. Berillo O., Atambayeva Sh., Niyazova R., Akimniyazova A., Ivashchenko A., Pyrkova A. Features of Multiple MiR-574-5p Binding Sites and Functions of Their Target-Genes. // Journal of Bioinformatics and Computational Biology. – submitted for publication.

12. Druz A., Chu C., Majors B., Santuary R., Betenbaugh M., Shiloach J. A novel microRNA mmu-miR-466h affects apoptosis regulation in mammalian cells. // Biotechnol Bioeng. – 2011. – Vol. 108. – P. 1651-1661.

13. Dimmeler S., Zeiher A.M. Endothelial cell apoptosis in angiogenesis and vessel regression. // Circ Res. – 2000. – Vol. 87. – P. 434-439.

14. Folkman J. Angiogenesis and apoptosis. // Semin Cancer Biol. – 2003. – 13. – P. 159-167.

15. Druz A., Betenbaugh M., Shiloach J. Glucose depletion activates mmu-miR-466h-5p expression through oxidative stress and inhibition of histone deacetylation. // Nucleic Acids Res. – 2012. – Vol. 40. – P. 7291-7302.

16. Li Y., Fan X., He X., Sun H., Zou Z., Yuan H., Xu H., Wang C., Shi X. MicroRNA-466 inhibits antiviral innate immune response by targeting interferon-alpha. // Cell Mol Immunol. – 2012. – Vol. 9. – P. 497-502.

17. Luo Y., Liu Y., Liu M., Wei J., Zhang Y., Hou J., Huang W., Wang T., Li X., He Y., Ding F., Yuan L., Cai J., Zheng F., Yang J. Sfmbt2 10th intron-hosted miR-466(a/e)-3p are important epigenetic regulators of Nfat5 signaling, osmoregulation and urine concentration in mice. // Biochim Biophys Acta. – 2014. – Vol. 1839. – P. 97-106.

18. Seo M., Choi J., Rae Rho Ch., Joo Ch., Lee S. Microrna Mir-466 inhibits lymphangiogenesis by targeting Prospero-related homeobox 1 in the alkali burn corneal injury model. // Journal of Biomedical Science. – 2015. – Vol. 22. – P. 3.