UDC 577.29

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miRNA binding sites in mRNAs of human tumor suppressor genes

The search of binding sites of 18 miRNAs in 230 mRNAs of human tumor suppressor genes using the MirTarget program has been completed. It was predicted that miR-566, miR-619-5p, miR-1268a, miR-1268b, miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, miR-1273g-3p, miR-1273h-5p, miR-1285-3p, miR-1285-5p, miR-1972, miR-5095, miR-5096, miR-5585-3p и miR-5585-5p have from 2 to 85 target genes. 510 miRNA binding sites with the hybridization free energy of the bonds equaled to or greater than 90% of the maximum were predicted in all mRNA parts. The arranged groups of some miRNA binding sites were revealed in 2D-structure of mRNAs of tumor suppressor genes. The role of miRNAs with arranged binding sites in the regulation of expression of tumor suppressor genes are discussed.

Key words: tumor suppressors, miRNA, mRNA, binding site.

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miRNA-ның онкосупрессорлық гендерінің mRNA-сындағы байланысу сайттары

MirTarget бағдарламасын пайдалана отырып 18 miRNA-ның адамның 230 mRNA онкосупрессор-гендерімен байланысу сайттарын іздестіру жұмыстары жүргізілді. miR-566, miR-619-5p, miR-1268a, miR-1268b, miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, miR-1273g-3p, miR-1273h-5p, miR-1285-3p, miR-1285-5p, miR-1972, miR-5095, miR-5096, miR-5585-3p және miR-5585-5p-ның 2-ден 85-ке дейін нысана-гендері бар болатындығы болжалды. mRNA-ның барлық бөлімдерінде гибридизацияның бос энергиясы 90% және одан жоғары болатын 510 байланысу сайттары іріктеліп алынды. miRNA байланысу сайттарының кезектесіп орналасу орындары mRNA-ның екінші реттік құрылымында анықталды. miRNA-мен байланысу сайттарының кезектесіп орналасу реттілігінің онкосупрессорлық гендердің экспрессиясын реттеудегі маңызы әлі талқылану үстінде.

Түйін сөздер: онкосупрессор, mRNA, miRNA, байланысу сайттары.

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Сайты связывания miRNA в mRNA онкосупрессорных генов

Провели поиск сайтов связывания 18 miRNA в 230 mRNA генов-онкосупрессоров человека, используя программу MirTarget. Нами предсказано, что miR-566, miR-619-5p, miR-1268a, miR-1268b, miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, in-miR-1273g-3p, in-miR-1273h-5p, miR-1285-3p, in-miR-1285-5p, in-miR-1972, in-miR-5095, miR-5096, miR-5585-3p и miR-5585-5p имеют от 2 до 85 генов-мишеней. Отобрано 510 сайтов связывания со свободной энергией гибридизации равной 90% и более во всех участках mRNA. Упорядоченные группы некоторых сайтов связывания с miRNA выявлены во вторичной структуре mRNA. Роль упорядоченно расположенных сайтов связывания в регуляции экспрессии онкосупрессорных генов обсуждается.

Ключевые слова: онкосупрессор**,** mRNA, miRNA, сайты связывания.

Tumor suppressor genes are genes protecting cell from cancer. Dysregulation of these genes cause the loss of their function and promote oncogenesis [1]. One of important expression regulators are microRNAs (miRNAs). miRNAs are a class of short, non-coding RNAs that regulate the translation or degradation of messenger RNAs (mRNAs) [2]. It was shown that multiple changes in expression of some microRNAs had identified in cancer [3]. The miRNA level was lower in cell lines of 13 non-small-cell lung carcinomas but was 11-fold higher in another cell line [4]. 130 miRNAs showed significant differential expression in breast tumors compared to the normal adjacent tissue [5]. The expression levels of these 38 miRNAs were changed more than two-fold in cells of colon carcinoma [6]. Change fold of miRNA concentration at cancer was shown in many investigations, but their target genes did not have studied enough. Therefore, it is important to define miRNAs which are bound to mRNAs of tumour suppressor genes. Development of methods for prediction and identification of microRNA binding sites helps to understand their functions. An individual mRNA may be simultaneously targeted by multiple miRNAs. Currently, about 2500 human miRNAs were discovered, but part of them and their target-genes need to be well known.

The tumor suppressor genes were selected. It is possible to find miRNA binding sites with high level of reliability using the MirTarget program written in our laboratory. The localization of miRNA binding sites in secondary structure of mRNAs was not studied. According to these unresolved questions the following

aims of our work were set: (a) to find miRNAs binding sites with mRNAs of tumor suppressor genes, (b) to identify different arranged groups of miRNA binding sites, (c) to reveal features miRNA interactions with different mRNAs of tumor suppressor genes in two-dimensional structures (2D-structures).

Material and methods

The human gene mRNAs were obtained from GenBank (http://www.ncbi.nlm.nih.gov) using Lextractor002 script (http://sites.google.com/site/malaheenee/software), which was written in our laboratory. Human miRNA sequences and information regarding their origin was obtained from the miRBase database (http://mirbase.org).

The search for target genes of miRNAs was achieved using the MirTarget program, which was written in our laboratory. This program defines the following features of binding: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in the 5'-untranslated regions (5'UTRs), the coding domain sequences (CDSs) and the 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 mRNAs. The ΔG/ΔGm ratio (%) was determined for each site (ΔGm equals the free energy of a miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on the mRNAs had ΔG/ΔGm ratios 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 MirTarget program calculated the interactions between the nucleotides of the miRNAs and those of the mRNA target gene. This program identified hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C [7]. The distance between A-C was same as that as between G-C, A-U, and G-U [8] nucleotides. The number of hydrogen bonds between G-C, A-U, G-U and A-C and the value of their free energy of binding is equal to 3, 2, 1 and 1, respectively.

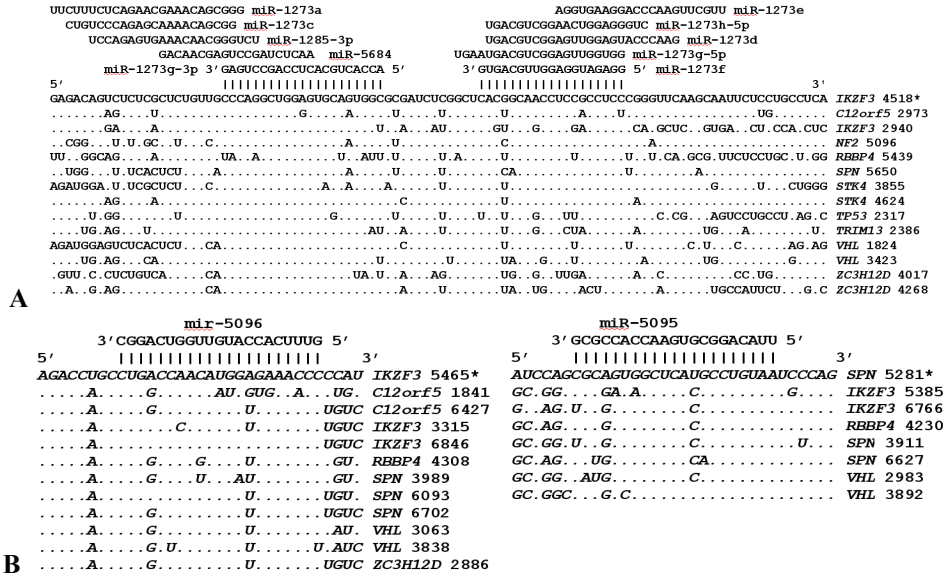
The UNAFold 3.7 program (http://unafold.software.informer.com/) was used for creation 2D-structure of mRNAs.

Results and discussion

Previously binding sites of 2563 miRNAs in 13000 human genes with $\Delta G/\Delta G_m$ ratio of 90% were predicted in our laboratory (unpublished data). These genes were selected as genes participating in different kinds of carcinogenesis, including lung cancer and breast cancer. The most of these miRNAs have intronic origin (in-miRNAs) and some are intergenic (ig-miRNAs). The arranged binding sites of these miRNAs were located in the 5'UTRs, CDSs and 3'UTRs of all studied target genes. 12 miRNAs (miR-619-5p, miR-1273a, miR-1273c, miR-1273d, miR-1273e, miR-1273f, miR-1273g-3p, miR-1273h-5p, miR-1285-3p, miR-5095, miR-5096 and miR-5585-3p) have high quantity of binding sites and more than 400 target genes for each of them and arranged binding site localization in corresponding group. The arranged binding sites are binding sites of different miRNAs, which have overlapping nucleotide sequences or are located within the same distance in different mRNAs of genes. miR-619-5p, miR-5095, miR-5096 and miR-5585-3p create one group with arranged binding sites [9] and miR-1273 family with miR-1285-3p is another group [10, 11]. In the present work, the target genes of apoptosis have arranged groups of miRNA binding sites that are presented at Figure 1A, B.

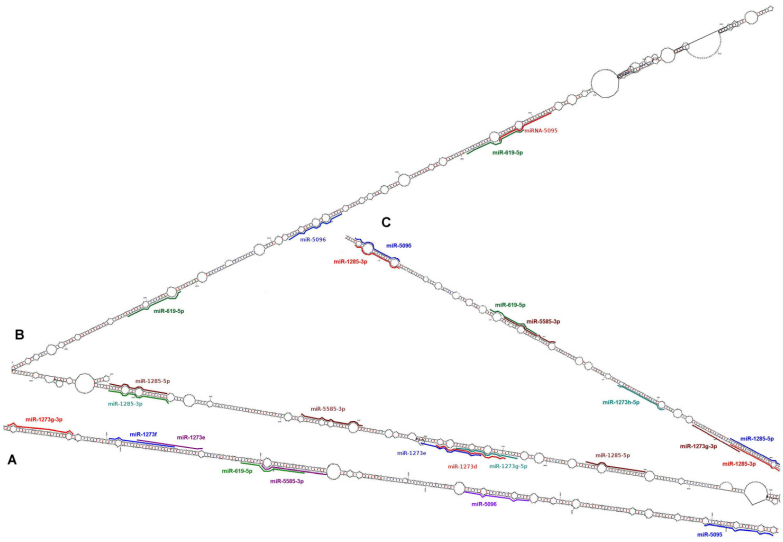
We revealed another six miRNAs (miR-566, ig-miR-1268a, miR-1268b, miR-1285-5p, miR-1972 and miR-5585-5p), their binding sites located near described over arranged binding sites. As a result, 510 miRNA binding sites in 145 mRNAs of human tumor suppressor genes were identified.

Previously it was identified two groups with binding sites with arranged localization: miR-1273 family and miR-619-5p. The binding sites of miR-1273 group have arranged localization in 100 nucleotide parts of some target genes and consist of two subgroup: miR-1273g-3p and miR-1273f. The miR-1273g-3p subgroup contain of miR-1273a, miR-1273c, miR-1285-3p and miR-5684. The distance between the end of miR-1273g-3p binding site and begins of miR-1273f binding site was equaled 12 nucleotide (nt). The miR-1273f subgroup consist of miR-1273d, miR-1273e, miR-1273g-5p and miR-1273h-5p.



The nucleotide sequences of miRNAs corresponding to the binding sites in mRNA regions.
«|» - symbol shows the presence of the hydrogen bond(s) between the miRNA and mRNA nucleotides. The nucleotide sequence the gene with «*» contain arranged binding sites that was selected for comparison with other mRNAs of target genes.
(A): Some binding sites of miR-1273 group. (B): Some binding sites of miR-5096 and miR-5065.

Figure 1 - Schema of the localizations of the aligned nucleotide sequences of some arranged miRNA binding sites in the mRNAs of tumour suppressors.



Lines are shown places of miRNA binding sites on mRNA of *IKZF3* gene.
A - binding sites of miR-1273g-3p, miR-1273f, miR-1273e, miR-5095, miR-5096, miR-5585-3p and miR-619-5p are presented.B - binding sites of miR-5095, miR-619-5p, miR-5096, miR-619-5p, miR-1285-5p, miR-5585-3p, miR-1285-5p, miR-1273g-5p, miR-1273d, miR-1273e and miR-1285-3p are presented.C - binding sites of miR-5096, miR-619-5p, miR-5585-3p, miR-1285-5p, miR-1285-3p, miR-1273g-3p, miR-1273h-5p and miR-1285-3p are presented.

Figure 2 – miRNA binding sites of *IKZF3* gene presented on 2D-structure.

The nucleotide sequences of binding sites in each subgroup are overlapped [10, 11]. The binding sites with this miRNA group were revealed in mRNAs of tumour suppressor genes (Figure 1A). The miR-619-5p group consist of arranged miR-5095, miR-619-5p, miR-5096 and miR-5585-3p binding sites located in 180 nucleotide part of target genes. The homology level of nucleotide sequences is high in binding sites and between them. The binding sites of miR-5095 and miR-619-5p are overlapped. The distance between miR-

5095 and miR-5096 binding sites is equaled 57-59 nt and the distance between miR-5096 and miR-5585-3p is equaled 46-47nt. We revealed that two groups with arranged binding sites are located on the different sides of *IKZF3* mRNA's stem loop in 2-D structure (Figure 2A-C).

mRNA of *IKZF3* gene (IKAROS family zinc finger 3 (Aiolos)) consist of 5'UTR with length 62nt, CDS (1529nt) and 3'UTR (8075nt). 28 miRNA binding sites located in the 3'UTR were predicted. 2D-structure of IKZF3 3'UTR have four long stem loops with many complementary nucleotide pairs, in which was located arranged miRNA binding sites (Figure 2A-C). The binding sites of studied miRNA binding sites located on the both sides of mRNA stem *IKZF3,* which have one or three binding sites located in opposite sides of mRNA in 2D-structure (Figure 1B, C). miR-1285-5p and miR-1285-3p are complementary between themselves as well as miR-5585-3p and miR-5585-5p. 1285-5p and miR-5585-3p belong to miR-619-5p arranged group, and 1285-3p and miR-5585-5p belong to miR-1273 group. Therefore these two arranged group of binding sites have connection of their mRNA part of nucleotide chains for creation such the 2D-structure with four long stem loops.

In order to miRNA as a part of a RNA-induced silencing complex bind to almost complementary sequence of mRNA of a target gene, it needs to overcome the free energy of interaction in two nucleotide chains of mRNA. miRNA binding sites often located in the parts of mRNA, where there are some not complementary nucleotides (1-3 bugles), which destabilize mRNA structure.

As a result, the homology degree of nucleotide sequences in studied gene regions is high, which confirms the important role of these binding sites in the biological function of tumour suppressor genes. The connections between some arranged miRNA binding sites on 2D-structure of mRNA were revealed. Various disruptions in the influence of miRNAs on the expression of target genes containing the arranged groups of binding sites can be the result of strong changes in the metabolism. The change in the regulation of target gene expression via these miRNAs might be the reason underlying many diseases including cancer. For example, the increasing of the miRNA expression can lead to decreasing of tumour suppressor translation and contribute oncogenesis.

References

1 Katoh M., Igarashi M., Fukuda H., Nakagama H., Katoh M. Cancer genetics and genomics of human FOX family genes // Cancer Lett. 2013. – Vol. 328. – No.2. – P.198-206.

2 Lee R.C., Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans* // Science. 2001.-Vol.294. – No.5543. – P. 862-864.

3 Fan M., Krutilina R., Sun J., Sethuraman A., Yang C.H., Wu Z.H., *et al.* Comprehensive Analysis of microRNA (miRNA) targets in breast cancer cells // J. Biol. Chem. 2013. – Vol. 288. – No.38. – P. 27480-27493.

4 Chen S., Wang H., Ng W.L., Curran W.J., Wang Y. Radiosensitizing effects of ectopic miR-101 on non-small-cell lung cancer cells depend on the endogenous miR-101 level // Int. J. Radiat. Oncol. Biol. Phys. 2011. – Vol.81. – No.5. – P. 1524-1529.

5 Romero-Cordoba S., Rodriguez-Cuevas S., Rebollar-Vega R., Quintanar-Jurado V., Maffuz-Aziz A., Jimenez-Sanchez G., *et al.* Identification and pathway analysis of microRNAs with no previous involvement in breast cancer // PLoS One. 2012. – Vol.7. – No.3. – P. e31904.

6 Shin S., Cha H.J., Lee E.M., Jung J.H., Lee S.J., Park I.C., et al. MicroRNAs are significantly influenced by p53 and radiation in HCT116 human colon carcinoma cells // Int J Oncol. 2009. – Vol.34. – No.6. – P.1645-1652.

7 Peng Y., Dai Y., Hitchcock C., Yang X., Kassis E.S., Liu L., *et al*. Insulin growth factor signaling is regulated by microRNA-486, an underexpressed microRNA in lung cancer // Proceedings of the national academy of sciences, 2013. – Vol.110. – No.37. – P.15043

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. – No. 16. – P. 3497-3531.

10 Иващенко А.Т., Берилло О.А., Ниязова Р.Е., Пыркова А.Ю., Атамбаева Ш.А. Особенности сайтов связывания miR-619-5p, miR-5095, miR-5096 и miR-5585-3p с mRNA генов человека // Вестник КазНУ серия биологическая. 2013. – Т. 3(58) – С. 24-29.

11 Берилло О.А., Иващенко А.Т., Ниязова Р.Е., Пыркова А.Ю., Атамбаева Ш.А. особенности сайтов связывания miRNA семейства miR-1273 с mRNA генов человека // Вестник КазНУ серия биологическая. 2013. – Т. 3(58) – С. 30-35.

12 Атамбаева Ш.А., Берилло О.А., Иващенко А.Т., Пыркова А.Ю., Ниязова Р.Е. Особенности сайтов связывания miR-1285-3p, miR-5684 и семейства miR-1273 с mRNA генов человека // Вестник КазНУ серия биологическая. 2013. – Т. 3(58) – С. 36-41.