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Characteristics of interaction of miRNA with mRNA of *E2F* transcription factors family genes

Abstract: Search of binding sites of 6266 miRNA with mRNA of genes of *E2F* transcription factors family was implemented. The mRNA of *E2F1* gene is associated with 13 miRNAs in 5′UTR, CDS and 3′UTR. The mRNA of *E2F2* gene has binding sites for 10 miRNAs. The binding sites of miR-1-875-3p and miR-760-3p are conservative in mRNA of *E2F2* gene of 18 mammalian species. The mRNA of *E2F3* gene contains binding sites for one miRNA in the 5'UTR, for two miRNAs in the 3'UTR and for other seven miRNAs in the CDS. The miR-7-19239-3p, miR-19-42772-5p, miR-3-9461-3p, miR-17-39416-3p can interact with mRNA of *E2F3* gene with energy more than -120 kJ/mole. The mRNA of *E2F4* gene has binding sites for six miRNAs in CDS, 5'UTR and 3'UTR. The mRNA of *E2F5* gene contains binding sites for seven miRNA located only in the CDS. The mRNA of *E2F6* gene has one binding site for miRNA in the 5'UTR, three in the 3'UTR. The mRNA of *E2F7* gene binds miR-14-34881-3p in the CDS. The predicted miRNA binding sites with mRNA of *E2F* gene family help to find associations of miRNAs with their target genes for the development of diagnostic methods of tumourigenesis. The following pairs can be used as associations of miRNA with target genes: miR-6511b-3p, miR-1-1714-3p and miR-6786-5p with mRNA of *E2F1* gene; miR-7-19239-3p, miR-19-42772-5p, miR-3-9461-3p and miR-17-39416-3p with mRNA of *E2F3* gene; miR-5-15026-5p and miR-20-44817-5p with mRNA of *E2F4* gene.
Key words: miRNA, mRNA, transcription factor, *E2F1-8* genes, tumourigenesis.

Introduction

The E2F family of transcription factors includes E2F1-E2F8 proteins, which play an important role in the animal cell cycle [1]. The E2F family of proteins divides by functions into transcriptional activators and repressors. Activators E2F1, E2F2, E2F3a accelerate the cell cycle, and E2F3b, E2F4, E2F5, E2F6, E2F7, E2F8 inhibiting cell cycle. Members of family form heterodimers that increases their stability. The balance between activators and repressors regulates the progress of the cell cycle [2]. Proteins of E2F family regulate transcription of cell cycle genes, negative regulators, checkpoints, proteins of apoptosis, nucleotide synthesis, DNA replication and DNA reparation [3]. A wide range of E2F participation in key processes of cell functioning dictates the need to explore the biological role of members of the E2F family. Some studies found E2F proteins involved in tumourigenesis.

In recent years it was studied the effect of miRNA expression on E2F family of genes in tumorigenesis [4-10]. It was shown the involvement of miRNA in the regulation of the expression of genes of *E2F* family in the cancer of stomach [15], esophagus [11], ovaries [17], colon [12], endometrium [13], lung [14], liver [16] and other; the change in the concentration of various miRNAs and the expression of *E2F* family genes with breast cancer [6-12]. Changes in miRNA concentration and expression of *E2F* family of genes have been studied for *E2F1* [18], *E2F2* [19], and *E2F3* [20, 21] genes. There are several publications for *E2F5* and *E2F7* genes [22-23]. The effect of miRNA on the expression of *E2F4* and *E2F6* has not been studied. In the works cited above, the direct effect of miRNA on mRNA genes of *E2F* family with the establishment of binding sites have not been studied. In connection with this it is needed to identify the influence of currently known 6266 miRNA on the expression of *E2F* family genes.

Materials and methods

Nucleotide sequences of mRNAs of E2F family genes were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). We used the following abbreviations of species names: *Ailuropoda melanoleuca* –

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Ame, Bos taurus – Bta, Canis familiaris – Cfa, *Capra hircus – Chi, Chlorocebus sabaeus – Csa, Coturnix japonica – Cja*, *Cricetulus griseus – Cgr, Felis catus – Fca, Gorilla gorilla – Ggo, Heterocephalus glaber – Hgl, Loxodonta africana – Laf*, *Lipotes vexillifer – Lve, Macaca fascicularis – Mfa, Macaca mulatta – Mml, Macaca nemestrina – Mne, Monodelphis domestica – Mdo, Mus musculus – Mmu, Nannospalax galili – Nga*, *Nomascus leucogenys – Nle*, *Ovis aries – Oar, Pan paniscus – Ppa, Pan troglodytes – Ptr, Papio Anubis – Pan, Pongo abelii – Pab, Pteropus alecto – Pal*, *Rattus norvegicus – Rno, Rhinopithecus bieti – Rbi, Rhinopithecus roxellana – Rro*, *Saimiri boliviensis – Sbo, Sus scrofa – Ssc, Tupaia chinensis – Tch, Ursus maritimus – Uma.*

2565 miRNA were downloaded from miRBase (http://mirbase.org). Where as 3701 miRNA were taken from the publication of Londin E. et al. [24]. Nucleotide sequences of miRNAs were listed with our notation: miR-1-875-3p(cggcucugggucuguggggagc); miR-1-1714-3p(cggcggcggcggagcgcggg); miR-1-2558-3p(ccuucuucucccccaccccagc); miR-2-3313-3p(ggcggcggcggcggcggcggccccg); miR-2-4804-5p(ugagcaacacagugagacuccuuu); miR-3-9461-3p(cccggcagcgguggcagcgguag); miR-5-14523-3p(ucgcgcucgcugccuucucccc); miR-5-15026-5p(agccagggcaggccgggccuccg); miR-5-16438-3p(gcggcggcagcuggggcgggaa); miR-5-16871-5p(uaaaauuaauugcaguuuuugu); miR-6-17487-3p(cgcacacacacacacagacaccu); miR-7-19239-3p(gguggcggcggcggcuccgggcu); miR-8-24124-3p(gcuccugccuugccggagucug); miR-9-26166-3p(cuguccugccggcggccguggc); miR-10-5299-5p(cggcggcggccgccgcggg); miR-10-25954-5p(agggagccucuguuggggcuggaa); miR-11-18690-5p(cuggggggaggaggaagaggaga); miR-11-23098-5p(cugaggggcaggaggugggag); miR-14-34881-3p(cugggcuuguggggaccccugg); miR-16-36797-3p(ugaugcccucgcccucccuagu); miR-16-37595-3p(cgaccucggcccugcccuugca); miR-17-34996-5p(ugaacccgagaggaagagauugc); miR-17-36033-3p(ugccccgaccugaccccggcccucg); miR-17-38391-3p(ccuucuccuccucucccucccua); miR-17-39416-3p(gccucgccgccgccucugcugc); miR-17-42540-3p(cggcggcggcgcgccggguc); miR-18-39953-5p(ccgccccgccgcucccggcgcccc); miR-19-42593-3p(ccucccccuuuccaccccaguga); miR-19-42772-5p(gggccggcggcggcggcgccucu); miR-19-43065-3p(ucuguccaccuugcuucuucagg); miR-19-43662-5p(uggcuggaggagcuggggguguca); miR-20-44817-5p(ggcuagagcccgacgggggcccgg); miR-21-40861-3p(gucccccucucucccuuccccaa); miR-22-44137-3p(cucccugcagcggucagaggauc).

Binding sites for tested miRNAs were revealed using the MirTarget program [25]. This program defines the following features of binding: a) beginning of miRNA binding with mRNAs; b) localization of miRNA binding sites in the 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs) and 3'UTRs of mRNAs; c) free energy of hybridization (ΔG, kJ/mole); d) schemes of nucleotide interactions between miRNAs and mRNAs. The ratio ΔG/ΔGm (%) was counted for each site, where ΔGm is free energy of miRNA binding with its perfect complementary nucleotide sequence. The miRNA binding sites located on the mRNAs have ΔG/ΔGm ratios of 90% and more. We also note the position of the binding sites on the mRNA, beginning from the first nucleotide of the 5'UTR of mRNA. 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 one hydrogen bond.

In the interaction of miRNA with mRNA, the program allows one unpaired nucleotide only in mRNA, but not in miRNA, since it is bound to the RISK complex. Contrary to the hypothesis that miRNA binds with mRNA only in the 3'UTR, and interacts with mRNA only due to the "seed" of the site, the program takes into account the interaction of miRNA with mRNA over the entire length in 5'UTR, CDS and 3'UTR at the basis of physico-chemical properties of these molecules.

Results and their discussion

It was found that 13 miRNAs contacted with mRNA of *E2F1* gene. One miRNA of them is associated in the 5'UTR, five miRNA – in the 3'UTR and seven miRNA – in the CDS, which shows a clear preference for the binding of miRNA in the beginning of mRNA (table 1). The mRNA of *E2F1* gene has one binding site for miR-1913 arranged in the 5'UTR. Binding of miRNA in the 5'UTR has a biological significance, because it allows to miRNA stopping protein synthesis earlier, and do not waste energy on synthesis of abortive protein in the case of miRNA binding in the 3'UTR. The free energy of interaction of miR-6511b-3p, miR-1-1714-3p and miR-6786-5p with mRNA of *E2F1* gene is more than -115 kJ/mole, indicating a strong binding of these miRNAs and more effective suppression of E2F1 protein synthesis.

E2F2 gene consists of a smaller number of nucleotides and possibly therefore its mRNA has binding

sites only for ten miRNAs (table 1). There are no multiple binding sites for miRNAs. The mRNA of *E2F2* gene contains seven binding sites in the 3'UTR, three sites in the CDS and there are no sites in the 5'UTR. Among the mRNA areas, that having a miRNA binding sites, we prefer protein coding regions, which largely reflect the connection of a miRNA with gene function. Therefore, we chose miR-760-3p to determine how conservative this binding in the evolution of *E2F2* gene.

Table 1 – Characteristics of miRNAs binding with mRNA *E2F1, E2F2* genes

Gene *E2F1* miR-1913, 29*, -115, 90, 22; miR-6786-5p, 267, -115, 90, 21; miR-10-5299-5p, 290, -115, 95, 19; miR-X-44865-3p, 292, -115, 92, 20; miR-1-1714-3p, 381, -119, 95, 20; miR-17-36033-3p, 446, -129, 87, 25; miR-22-44137-3p, 764, -115, 89, 23; miR-16-36797-3p, 1382, -115, 93, 22; miR-6-17487-3p, 1642**, -113, 90, 23; miR-21-40861-3p, 2178**, -110, 90, 22; miR-4749-3p, 2322**, -108, 91, 20; miR-6511b-3p, 2326**, -121, 93, 23; miR-6813-3p, 2537**, -108, 91, 21
Gene *E2F2* miR-1-875-3p, 623, -115, 90, 22; miR-760, 624, -106, 93, 20; miR-4539, 1406, -113, 90, 22; miR-548m, 2091**, -93, 90, 21; miR-2-4804-5p, 4107**, -113, 90, 24; miR-5684, 4121**, -98, 92, 20; miR-1273g-3p, 4127**, -113, 96, 21; miR-1273f , 4160**, -96, 92, 19; miR-17-34996-5p, 4165**, -110, 90, 23; miR-10-25954-5p, 4482**, -119, 89, 24
Gene *E2F3* miR-7-19239-3p, 24*, -125, 89, 23; miR-19-42593-3p, 371, -115, 89, 23; miR-5-16438-3p, 457, -115, 87, 22; miR-17-42540-3p, 449, -115, 92, 20; miR-3-9461-3p, 461, -121, 89, 23; miR-11-18690-5p, 530, -110, 90, 22; miR-19-42772-5p, 553, -127, 90, 23; miR-17-39416-3p, 692, -123, 94, 22; miR-1-2558-3p, 3172**, -113, 90, 22; miR-5-16871-5p, 4430**, -93, 92, 22
Gene *E2F4* miR-5-15026-5p, 11*, -125, 91, 23; miR-5-14523-3p, 42*, -117, 92, 22; miR-6791-3p, 159, -108, 91, 21; miR-20-44817-5p, 534, -123, 89, 23; miR-11-23098-5p, 1745**, -110, 91, 21; miR-19-43662-5p, 1756**, -115, 89, 23
Gene *E2F5* miR-9-26166-3p, 66, -113, 90, 22; miR-8-24124-3p, 71, -115, 92, 22; miR-16-37595-3p, 84, -115, 90, 22; miR-6068, 103, -110, 90, 21; miR-7-19239-3p, 130, -125, 89, 23; miR-18-39953-5p, 163, -129, 90, 23; miR-6791-3p, 232, -108, 91, 21
Gene *E2F6* miR-19-43065-3p, 228*, -115, 92, 22; miR-151a-5p, 2212**, -104, 92, 21; miR-151b-5p, 2215**, -93, 96, 18; miR-17-38391-3p, 3023**, -115, 90, 23
Gene *E2F7* miR-14-34881-3p, 71*, -119, 93, 22
Note. miRNA (the number of binding sites); the beginning of binding site; the miRNA region: * – 5'UTR, ** – 3'UTR; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt)

Data given in Table 2 show that heptapeptide TPHGPEG, encoded by the binding site of miR-1-875-3p and miR-760-3p, conservative in 18 animal species, which indicate the stability of miR-1-875-3p and miR-760-3p connection with the expression of *E2F2* gene in the process of long evolution. Binding sites of miR-760-3p and miR-1-875-3p were determined in the area of 15% from the beginning in CDS. These miRNAs binding sites were determined in mRNA of *E2F2* gene of rat, mice and, therefore, these animals can be used in an experiment of studying the action of miR-760-3p and miR-1-875-3p.

The mRNA of *E2F3* gene contains binding sites for 10 miRNAs, which have one binding site (table 1). The miR-7-19239-3p is bind in the 5'UTR, miR-1-2558-3p and miR-5-16871-5p in the 3'UTR and other miRNAs are bind in the CDS mRNA of *E2F3* gene.

The miR-7-19239-3p, miR-19-42772-5p, miR-3-9461-3p, miR-17-39416-3p can interact with energy more than -120 kJ/mole, which indicates a strong binding to mRNA of *E2F3* gene. However, not all miRNAs can be synthesized at one time in each cell. In order to suppress protein synthesis the miRNA concentration should be comparable with the concentration of mRNA, to reduce the number of free mRNA and cause inhibition of translation. Should be awared that about half of all miRNAs are derived

from introns, and are synthesized together with the host gene, which can not be expressed in a given cell at a given time.

The miR-19-42593-3p is linked in region of mRNA from 371 nt to 420 nt. This binding site of miR-19-42593-3p encoded oligopeptide AAV-VAAAAAA (table 3).

E2F4 gene contains binding sites for six miR-NAs. Two miRNAs bind in the CDS and two miR-NAs in 5'UTR and 3'UTR (Table 1). The free energy of interaction (ΔG) of miR-5-15026-5p and miR-20-44817-5p with mRNA of *E2F4* gene is equaled to or more than -120 kJ/mole, indicating a strong binding of these miRNA and more effective suppression of E2F4 protein synthesis.

The mRNA of *E2F5* gene contains binding sites for seven miRNAs located in the CDS. Binding sites of the following miRNAs encode oligopeptides indicated in brackets: miR-6068 (PPPQPPQ), miR-9-26166-3p (GQQAPAG), miR-8-24124-3p (QAPAGQG), miR-16-37595-3p (GQGQGQR), miR-7-19239-3p (APQPPPPP), miR-18-39953-5p (GGAGGGSS), miR-6791-3p (LLQEAKD). Tables 4-7 show the conservatism of these oligopeptides in the E2F5 protein.

Table 2 – Conservatism of TPHGPEG heptapeptide encoded by miR-760-3p and miR-1-875-3p binding site in mRNA *E2F2* gene

Region of E2F2	Object
APGTCLDA**TPHGPEG**QVVRCLPA	*Hsa, Ppa, Mml, Nle, Chi, Fca, Ptr, Oar, Pab, Rro, Cja, Ggo, Lve*
ASGTCLDA**TPHGPEG**QVVRCLPA	*Mfa*
APGTCLDA**TPHGPEG**QAVRCVPA	*Ame*
AAGTCLDA**TPHGPEG**QAVRCVPA	*Cfa*
ALGTCLDA**TPHGPEG**QIVRCVPA	*Rno, Mmu*
Note. In bold, a conservative heptapeptide encoded by miR-760-3p and miR-1-875-3p binding site	

Table 3 – Conservatism of decapeptide AAVVAAAAAA encoded by miR-19-42593-3p binding site in mRNA *E2F3* gene

Region of E2F3	Object
VTAGGGEG**AAVVAAAAAA.**SMDKRALL	*Hsa, Lve, Ptr, Mne, Pab, Nle, Bta, Csa, Oar, Cgr, Mmu, Pal*
VTAGGGEG**AAVVAAAAAAA**SMDKRALL	*Nga*
VTAGGGEG**AAAAAAAAAA.**SMDKRALL	*Mdo*
VTAGGGEG**AAVVAAAAAA.**SMDTAGSLL	*Ggo*
Note. In bold, a conservative decapeptide encoded by miR-19-42593-3p binding site	

Table 4 – Variability of decapeptide encoded by miR-6068 binding site in mRNA *E2F5* gene

Region of E2F5	Object
QGQGQR**PPPQPPQ**AQAPQP	*Hsa Ggo*
QGQGQR**PPPHPPQ**AQAPQP	*Nle*
QGQGQR**PQPQPPQ**AQAPQP	*Ptr Mml Csa*
QGQDQR**PQPQPPQ**AQAPQP	*Cja*
QGQGQR**PQPQPSQ**AQPPQQ	*Ame*
QGQGQR**PQPQQSQ**AQPPPP	*Lve*
QGQGQR**PQPPQPQ**PPQQPP	*Laf*
QGQGQR**PQPPPSQ**AQPPPP	*Ssc*
QGQGQR**PQ**A**Q**S**PQ**AQAPQP	*Rro*

Table 5 – Conservatism of decapeptide APQPPPPP encoded by miR-7-19239-3p binding site in mRNA *E2F5* gene

Region of E2F5	Object
QPPQAQ**APQPPPP**PQLGGA	*Hsa, Ptr, Mml, Csa*
QPPQAQ**APQPPPP**QQLGGA	*Ggo*
HPPQAQ**APQPPPP**PQLGGA	*Nle*
QSPQAQ**APQPPP.**LQLGGA	*Rro*

Table 6 – Conservatism of decapeptides encoded by miR-9-26166-3p, miR-8-24124-3p, miR-16-37595-3p binding site in mRNA E2F5 gene

Regions of E2F5			Object
miR-9-26166-3p	miR-8-24124-3p	miR-16-37595-3p	
AEPASS**GQQAPAG**QGQGQR	PASSGQ**QAPAGQG**QGQRPP	GQQAPA**GQGQGQR**PPPQPP	*Hsa*
AEPASS**GQQAPAG**QGQGQR	PASSGQ**QAPAGQG**QGQRPQ	GQQAPA**GQGQGQR**PQPQPP	*Ptr*
AEPASS**GQQAPAG**QGQGQR	PASSGQ**QAPAGQG**QGQRPP	GQQAPA**GQGQGQR**PPPHPP	*Nle*
AEPASS**GQQAPPG**QGQGQR	PASSGQ**QAPPGQG**QGQRPQ	GQQAPP**GQGQGQR**PQPQPP	*Mml*
AEPASS**GQQAPPG**QGQGQR	PASSGQ**QAPPGQG**QGQRPP	GQQAPP**GQGQGQR**PPPQPP	*Ggo*
AEPASS**GQQAPPG**QGQDQR	PASSGQ**QAPPGQG**QDQRPQ	GQQAPP**GQGQDQR**PQPQPP	*Cja*
AEPASS**GQQAPPG**QGQGQR	PASSGQ**QAPPGQG**QGQRPQ	GQQAPP**GQGQGQR**PQPQPP	*Csa*
AEPASS**GQQAPPG**QGQGQR	PASSGQ**QAPPGQG**QGQRPQ	GQQAPP**GQGQGQR**PQAQSP	*Rro*
AEPASS**GQQAPQG**QGQGQR	PASSGQ**QAPQGQG**QGQRPQ	GQQAPQ**GQGQGQR**PQPPQP	*Laf*
VLALRA**GQQAPQG**QGQGQR	ALRAGQ**QAPQGQG**QGQRPQ	GQQAPQ**GQGQGQR**PQPQPS	*Ame*
AEPGGS**GQPAPQG**QGQGQR	PGGSGQ**PAPQGQG**QGQRPQ	GQPAPQ**GQGQGQR**PQPQQS	*Lve*
AEPASS**GQPAPEG**QGQGQR	PASSGQ**PAPEGQG**QGQRPQ	GQPAPE**GQGQGQR**PQPPPS	*Ssc*

Table 7 – Conservatism of decapeptide LLQEAKD encoded by miR-6791-3p binding site in mRNA *E2F5* gene

Region of E2F5	Object
TTKFVS**LLQEAKD**GVLDLK	*Hsa, Ggo, Ptr, Mml, Cja, Uma, Fca, Pab, Tch, Ssc, Sbo, Ame, Csa, Rro, Lve, Laf, Mdo, Nga, Mmu*
TAKFVS**LLQEAKD**GVLDLK	*Bta*
TTNFVS**LLQEAKD**GVLDLK	*Nle*

E2F6 gene contains binding sites for one miRNA in the 5'UTR and for three miRNAs in the 3'UTR. Nucleotide sequences of miR-19-43065-3p binding sites in mRNA of *E2F6* gene are shown in Table 9. Binding sites of miR-19-43065-3p are completely homologous in *Homo sapiens, Pan troglodytes, Nomascus leucogenys, Pan paniscus* and there is only one nucleotide replacement in the remaining species. Therefore, for tens of millions of years, miRNA binding sites were remain, which indicates the stability of the process *E2F6* gene expression regulation by miRNA.

mRNA of *E2F7* gene binds to miR-14-34881-3p in the 5'UTR. The nucleotide sequences of binding site of miR-14-34881-3p in mRNA of *E2F7* gene are shown in Table 10. Binding sites of miR-14-34881-3p are completely homologous in *Homo sapiens, Rhinopithecus roxellana, Chlorocebus sabaesus, Papio anubis*. In mRNA of *E2F7* gene of the remaining species this miRNA has only one substitution of nucleotides. That is, for tens of millions of years, the binding sites of miR-14-34881-3p in mRNA of *E2F7* gene persist, indicating that the method of regulating the expression of *E2F7* gene by miRNA is stable.

The miRNA binding sites located in the 5'UTR were found only in monkeys. In other animals, these binding sites were strongly changed, or absent.

Identified binding sites in the protein-coding region of mRNA genes of *E2F* family have several characteristics. Some binding sites are conserved along with flanking sequences of amino acids (Tables 2, 3, 5, 6, 7). Other binding sites are more conservative than flanking sequences. The most common binding sites located between conserved sequences and the length of sites of multiple binding sites vary in mRNA of different animal species (Tables 4, 8). In this connection, the question arises whether the peptides coded by binding sites in the CDS of are any function other than the reflection of binding sites. In the case of a change in the length of binding sites and the corresponding oligopeptides up to their absence, they probably do not play a functional role other than miRNA binding. In the case of high conservatism of encoded oligopeptides and their flanking amino acids, probably these oligopeptides are necessary for the demonstration of function of the complete protein.

Таблица 8 – Variability of oktapeptide GGAGGGSS encoded by miR-18-39953-5p binding site in mRNA of *E2F5* gene

Region of E2F5	Object
PPPPQL**GGAGGGSS**RHEKSL	*Hsa, Ptr, Nle, Csa, Rro*
PPPPPL**GGAGGGSS**RHEKSL	*Ggo*
PPPQQL**GGAGGGSS**RHEKSL	*Mml*
PSQQQL**GGAGGGSS**RHEKSL	*Sbo*
PSQQQL**GGVGGGSS**RHEKSL	*Cja*
TPPPQF**GGVGGGSS**RHEKSL	*Hgl*
PPPPPL**GGGGGGSS**RHEKSL	*Lve*
PPQQPL**GGGGGGSS**RHEKSL	*Ssc*
PPPPQL**GGGGGGG-**RHEKSL	*Ame*
AAPPGN**GGGGSSS-**RHEKSL	*Mdo*
PPQQLA**GGGSS---**RHEKSL	*Tch*
ASCAPP**GAGSS---**RHEKSL	*Bta*
PSAALA**GGSS----**RHEKSL	*Mmu*
QPPRVR**GGSS----**RHEKSL	*Fca*
RSSGRR**GGSS----**RHEKSX	*Pab*

Table 9 – Nucleotide sequences of binding sites of miR-19-43065-3p in 5'UTR of mRNA *E2F6* gene

Nucleotide sequences	Object
GUGCUCGA**GCUGAGCGCGAGAGGGCGGGAGA**GCUCGUGG	*Has, Ptr, Nle, Ppa, Pab*
GUGCUCGA**GCU<u>GAGUGCGAGAGGGCGGGAGA</u>**GCUCGUGG	*Ggo*
GUGAUCGA**GCUGGGCGCGAGAGGGCGGGAGA**GCUCGCAG	*Rro, Rbi*
GUGAUCGA**GCUGGGCGCGAGAGGGCGGGAGA**GCUCGCGG	*Csa*
GUGAUCGA**GCUGCGCGCGAGAGGGCGGGAGA**GCUCGCGG	*Pan, Mml, Mfa*
GCGCUCGA**GC**UA**GGCGCGAGAGGGCGGGAGA**GCUCUCGG	*Cja*
Note. In bold, binding site of miR-19-43065-3p	

Table 10 – Nucleotide sequences of binding sites of miR-14-34881-3p in 5'UTR of mRNA *E2F7* gene

Nucleotide sequences	Object
UGCCCGGACG**CCGCGGGGU**CCCCGCCAGCCCAGGGCACUCGGC	*Hsa*
UGUCGGGACG**CCGCGGGGU**CCCCGCCAGCCCAGGGCACUCGGC	*Rro, Csa*
UGUUGGGACG**CCGCGGGGU**CCCCGCCAGCCCAGGGCACUCGGC	*Pan*
UGCCCGGACG**CCACGGGGU**CCCCGCCAGCCCAGGGCACUCGGC	*Ggo, Ptr, Ppa*
UACACGGACG**CCACGGGGU**CCCCGCCAGCCCAGGGCACUCGGC	*Nle*
UGUCGGGACG**CCGCGGGGU**CCCCGCCAGUCCAGGGCACUCGGC	*Mml, Mne, Mfa*
UGCCCGGACG**CCGCGGGGU**CCCA**GCCAGCCCAG**GGCACUCGGC	*Cja, Sbo*
Note. In bold, binding site of miR-14-34881-3p	

The nucleotide sequences of binding sites that located in the 5'UTR are flanked by conserved regions without changing in the length of miRNA binding sites (Tables 9, 10). On the basis defined in the present work different miRNA binding sites in mRNA of a gene family can be identified associations miRNA with mRNA which allow their use as diagnostic markers for different oncology diseases.

The mRNA of *E2F1, E2F2, E2F3* genes activate the cell cycle, and the probable cause is that they often demonstrate themselves as oncogenes, because they increase cell proliferation. We have identified an increased number of miRNAs, interacting with mRNA of *E2F1, E2F2, E2F3* genes, probably they work as increased protection against excessive fusion E2F1, E2F2, E2F3 proteins. Less ability of mRNA of *E2F4, E2F5, E2F6, E2F7, E2F8* genes to bind miRNA allows to maintain the required level of apoptosis.

Finding miRNA binding sites raises the question of the level of reliability of found sites. One effective way to establish the reliability of binding sites is finding of binding sites in orthologous genes and identification of orthologous miRNA. Location of binding site in the protein coding region facilitates its conservation in evolution, especially, if the corresponding oligopeptide plays an important role in the function of protein. Similar results were obtained earlier [26, 27].

From cited studies it is known that for all taken miRNAs their concentrations vary with the change in the activity (expression) of *E2F* family genes, none could significantly affect on mRNA, unless the miRNA concentration was much higher than the mRNA concentration. Unfortunately, authors of virtually all publications did not simultaneously control the concentration of miRNA and the concentration of the synthesized protein of the target gene. Therefore, we cannot say with confidence that if the gene expression changes along with the level of miRNA with a positive or negative correlation, then this gene is a target for miRNA. In addition to the direct action of miRNA on the expression of the target gene, miRNA affects on many transcription factors, which in turn can influence on the expression of many genes, including those studied simultaneously with the studied miRNAs.

Conclusion

The obtained results indicate that mRNA of *E2F* genes family bind with miRNA in different degrees. The mRNA of *E2F1, E2F2, E2F3* genes have the largest number of miRNA binding sites which accelerate the cell cycle. Probably therefore the expression of *E2F1, E2F2, E2F3* genes should be largely influenced by miRNA, to prevent an uncontrolled increase in cell proliferation, which is usually observed during tumourigenesis. The predicted miRNA binding sites with mRNA of *E2F* gene family help to find associations of miRNA with their target genes for the development of diagnostic methods of tumourigenesis. The following pairs can be used as associations of miRNA with target genes: miR-6511b-3p, miR-1-1714-3p and miR-6786-5p with mRNA of *E2F1* gene; miR-7-19239-3p, miR-19-42772-5p, miR-3-9461-3p and miR-17-39416-3p with mRNA of *E2F3* gene; miR-5-15026-5p and miR-20-44817-5p with mRNA of *E2F4* gene.

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