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M.O. Myrzabekova, R.Ye. Niyazova, A.T. Ivashchenko

Al-Farabi Kazakh National University,
Kazakhstan, Almaty, e-mail: moldir.myrzabek@gmail.com

FEATURES OF BINDINGS OF MIRNA WITH GENES OF MYB FAMILY TRANSCRIPTION FACTORS OF *B. TAURUS, E. CABALLUS, O. ARIES*

Abstract. Transcription factors (TFs) are the most important proteins in expression of genes and genomes in general. MYB TFs family in animals is little studied and it is necessary to ascertain their properties. In recent years, effect of miRNA on gene expression has been actively studied, since the degree of miRNA influence on expression turns out to be key and determines differentiation, proliferation, cell cycle, apoptosis and other important biological processes. MirTarget program makes it possible to predict miRNA binding characteristics of mRNA with high efficiency, to obtain highly reliable interaction properties of miRNA with MYB family TFs mRNA. Binding characteristics of known miRNAs and mRNA genes of Bos taurus, Equus caballus, Ovis aries MYB family TFs were established. Only 13 bta-miRNAs had binding sites with more than 87%ΔG/ΔGm value in B.taurus TF genes mRNA. mRNA of DNAJC2, MYB, MIER2, MYBL1, MYBL2 genes had only one binding site for different miRNAs. NCOR1 mRNA had binding sites for three miRNAs, located a few hundred nucleotides in CDS. RCOR1 was targeted by five miRNAs that had 14 binding sites. miR-2885, miR-11976, and miR-11975 binding sites were located with nucleotide sequences overlapping in mRNA cluster from 147 nt to 168 nt. These miRNAs had five binding sites in second cluster from 177 to 216 nt. Third cluster of miR-2305, miR-11976, miR-11975 binding sites is localized from 222 to 256 nt. For E.caballus established binding sites of 15 miRNAs with mRNA of ten genes of MYB family. mRNAs of MIER1, MYBL2, RCOR2, RCOR3, SMARCA5, SMARCC2, TERF2 genes can bind with one miRNA. For O.aries, we established ten miRNA binding sites with mRNA of eight genes of MYB family. mRNA of CDC5L, MIER1, MYSM1, RCOR1, RCOR2, RCOR3, SMARCA1 genes can bind with one miRNA with ΔG/ΔGm ratio from 86% to 89%.

Key words: miRNA, gene, MYB, B. taurus, E. caballus, O. aries.

М.О. Мырзабекова, Р.Е. Ниязова, А.Т. Иващенко

әл-Фараби атындағы Қазақ ұлттық университеті,
Қазақстан, Алматы қ., e-mail: moldir.myrzabek@gmail.com

B. taurus, E. caballus, O. aries MYB транскрипциялық факторларының miRNA мен байланысу ерекшеліктері

Аңдатпа. Транскрипция факторлары (ТФ) – жалпы гендік және геномдардың реттелуіндегі ең маңызды белоктар болып табылады. Жануарлардағы MYB транскрипциялық факторлар жанұясы аз зерттелген және олардың қасиеттерін анықтау қажет. Соңғы жылдары miRNA-ның гендік реттелуге әсері белсенді түрде зерттеледі, себебі miRNA-ның экспрессияға әсер ету дәрежесі маңызды болып табылады және дифференцирлеу, пролиферация, жасуша циклі, апоптоз және басқа маңызды биологиялық процестерді айқындайды. MirTarget бағдарламасы mRNA-ның miRNA-мен байланыс сипаттамаларын жоғары тиімділігімен болжауға мүмкіндік береді, соның арқасында MYB жанұя транскрипциялық факторларының mRNA гендеріне байланыстыру арқылы miRNA-ның қасиеттерін алуға болады. Жұмыста қазіргі күні белгілі miRNA мен Bos taurus, Equus caballus, Ovis aries MYB транскрипциялық факторлар mRNA гендерінің байланысу сипаттамалары орнатылған. B. taurus mRNA ТФ гендерінің тек 13 miRNA ΔG/ΔGm мәнімен 87% астам байланысы бар аймақтары бар екені анықталды. mRNA гендері: DNAJC2, MYB, MIER2, MYBL1 және MYBL2 әр-түрлі miRNA үшін тек қана бір байланысқа ие болды. mRNA NCOR1 генінде CDS-те бірнеше жүздеген нуклеотидтерден кейін орналасқан үш miRNA үшін байланысу сайттары болды. RCOR1 гені 14 байланысу сайттары бар бес miRNA-ның межесі болды. miR -2885, miR-11976 және miR-11975 байланысу аймақтары mRNA кластерінде нуклеотидті тізбектердің суперпозициясымен 147 нт-тан 168 нт-ға дейін орналасты. Бұл miRNA-ларда екінші кластерде 177-ден 216-ға дейін болатын бес байланыстыру аймақтары болды. miR-2305, miR-11976 және miR-11975 байланыстыру аймақтарының үшінші кластері 222-ден 256-ға дейін оқшауланған. E. caballus үшін

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MYB жанұясының он mRNA генінің 15 miRNA үшін байланыстыру орындары орнатылды. mRNA гендері MIER1, MYBL2, RCOR2, RCOR3, SMARCA5, SMARCC2 және TERF2 бір miRNA-дан әрекет етеді. O. aries үшін MYB жанұясының сегіз mRNA генінің он miRNA үшін байланыстыру сайттары орнатылды. mRNA CDC5L, MIER1, MYSM1, RCOR1, RCOR2, RCOR3 және SMARCA1 гендері ΔG/ΔGm қатынасы 86%-дан 89%-ға дейін бір miRNA арқылы байланысады.

Түйін сөздер: miRNA, ген, MYB, B. taurus, E. caballus, O. aries.

М.О. Мырзабекова, Р.Е. Ниязова, А.Т. Иващенко
Казахский национальный университет имени аль-Фараби,
Казахстан, г. Алматы, e-mail: moldir.myrzabek@gmail.com

Особенности связывания miRNA с генами транскрипционных факторов семейства MYB *B. taurus, E. caballus, O. aries*

Аннотация. Транскрипционные факторы (ТФ) являются важнейшими белками в регуляции экспрессии генов и в целом геномов. Семейство транскрипционных факторов семейства MYB у животных мало изучено и требуется выяснение их свойств. В последние годы активно изучается влияние miRNA на экспрессию генов, поскольку степень влияния miRNA на экспрессию оказывается ключевой и определяет дифференцировку, пролиферацию, клеточный цикл, апоптоз и другие важнейшие биологические процессы. Используемая программа MirTarget позволяет с высокой эффективностью предсказывать характеристики связывания miRNA с mRNA и благодаря ей можно получить высоко достоверные свойства miRNA при связывании с mRNA генов транскрипционных факторов семейства MYB. В работе установлены характеристики связывания известных в настоящее время miRNA с mRNA генов транскрипционных факторов семейства MYB Bos taurus, Equus caballus, Ovis aries. Было выявлено, что только 13 miRNA имели сайты связывания с величиной ΔG/ΔGm более 87% в mRNA генов ТФ B. taurus. mRNA генов DNAJC2, MYB, MIER2, MYBL1 и MYBL2 имели только по одному сайту связывания для разных miRNA. mRNA гена NCOR1 имела сайты связывания для трех miRNA которые расположены через несколько сотен нуклеотидов в CDS. Ген RCOR1 был мишенью пяти miRNA которые имели 14 сайтов связывания. Сайты связывания miR-2885, miR-11976 и miR-11975 располагались с наложением нуклеотидных последовательностей в кластере mRNA с 147 нт по 168 нт. Эти же miRNA имели пять сайтов связывания во втором кластере с 177 нт по 216 нт. Третий кластер сайтов связывания miR-2305, miR-11976 и miR-11975 локализован с 222 нт по 256 нт. Для E. caballus нами установлены сайты связывания 15 miRNA с mRNA десяти генов семейства MYB. На mRNA генов MIER1, MYBL2, RCOR2, RCOR3, SMARCA5, SMARCC2 и TERF2 действуют по одной miRNA. Для O. aries нами установлены сайты связывания десяти miRNA с mRNA восьми генов семейства MYB. С mRNA генов CDC5L, MIER1, MYSM1, RCOR1, RCOR2, RCOR3 и SMARCA1 связывается по одной miRNA с отношением ΔG/ΔGm от 86% до 89%.

Ключевые слова: miRNA, ген, MYB, B. taurus, E. caballus, O. aries.

Introduction

Transcriptional regulation is a crucial step in gene expression regulation because the genetic information is directly read from DNA by sequence-specific transcription factors (TFs) [1]. Regulation of gene expression controls the spatial and temporal expression pattern and influences all biological processes in organisms. In this regulation, transcriptional regulatory system plays a key role and involves diverse proteins, including RNA polymerase, basal and sequence specific DNA-binding transcription factors (TFs), transcription cofactors and chromatin remodeling proteins [2]. An important characteristic of this family is the presence of a highly conserved MYB domain at their N end. Previously biochemical and molecular characteristics of MYB were studied [3]. These transcription factors are involved in many physiological and biochemical processes. The features of their structure, classification, multifunctionality, mechanisms of combinatorial control, evolution and functional redundancy at the present time were described [3]. The MYB transcription factor contains a MYB domain that is highly conserved across all eukaryotes and is located at the N terminus, whereas the C terminus is variable, acting as a transacting domain, involves in the regulation of a wide range of functions of MYB protein [4]. MYB genes are part of a large gene family of transcription factors found in animals and plants. In humans, it includes Myb-related protein B and Myb proto-oncogene like1 [5]. miRNAs are small, endogenous, single-stranded, noncoding RNA molecules ranging in length from 18–25 nt that are found in eukaryotic cells. They regulate

approximately 60% of the mammalian protein coding genes, primarily through the interaction with mRNAs. This effect is exerted by binding to complementary regions of messenger transcripts to repress their translation or less frequently inducing their degradation [6]. miRNAs are short RNAs that post-transcriptionally regulate the expression of target genes by binding to the target mRNAs. Although a large number of animal miRNAs has been defined, only a few targets are known [7]. In contrast to plant miRNAs, which usually bind nearly perfectly to their targets, animal miRNAs bind less tightly, with a few nucleotides being unbound, thus producing more complex secondary structures of miRNA target duplexes [8]. TFs are most fascinating owing to their complex regulation function. Here we use the transcription factor family of MYB. As the object of study, selected farm animals from them TFs MYB are cows, horses and sheep. The use of functional genomics approaches in animal husbandry is limited and requires additional research. At present, the effect of miRNA gene expression in human and animal TF has not been studied enough and therefore we have begun a systematic study of the effect of miRNA on the expression of transcription factors, including the MYB family.

Materials and methods

The nucleotide sequences mRNA of transcriptional factors of *Bos taurus, Equus caballus* and *Ovis aries MYB* family were downloaded from Animal TFDB (http://www.bioguo.org/Animal TFDB/). Nucleotide sequences of miRNAs were downloaded from database miRBase (http://mirbase.org). The search for binding sites of miRNA in mRNA of target genes was performed using the program MirTarget [9]. This program defines the following features of binding: a) start of the initiation of miRNA binding to mRNAs; b) localization of miRNA binding sites in 5'-untranslated regions (5'UTR), coding domain sequences (CDS) and 3'-untranslated regions (3'UTR) of mRNAs; c) free energy of interaction miRNA and mRNA (ΔG, kJ/mole); and d) schemes of nucleotide interactions between miRNAs and mRNAs. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its perfect complementary nucleotide sequence). ΔG/ΔGm ratios were taken on the assumption that the members of miRNA family generally differ by no more than 1-2 nt, that with a miRNA length of 22 nt, ΔG/ΔGm value is more 90%. With a larger difference in the number of mismatched nucleotides, the probability of two or more miRNAs to bind in one site increases. With a larger difference in the number of mismatched nucleotides, the probability of two or more miRNAs to bind in one site increases, which excludes the natural property of miRNA to interact selectively with mRNA of target gene. The MirTarget program identifies the positions of BSs on mRNA, beginning from the first nucleotide of mRNA's 5'UTR. The MirTarget program finds hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, A and C. The distances between A and C are equal to those between G and C, A and U, G and U and equal to 1.02 nanometer [10]. The numbers of hydrogen bonds in G-C, A-U, G-U and A-C interactions are found to be 3, 2, 1 and 1, respectively. MirTarget program does not work directly with miRBase and NCBI databases. The search for target genes from 17,494 genes in a special format from NCBI for the known miRNAs from miRBase will be available on request at mirtarget8@gmail.com. The miRNA binding sites for mRNA are taken with a ΔG/ΔGm ratio of more than 85%.

Results and discussion

To identify miRNAs and their targets genes of MYB transcription factor family, we searched for binding sites of 1025 miRNAs of *B.taurus* (bta-miRNA) in mRNA of 23 MYB family genes, 690 miRNAs of *E.caballus* (eca-miRNA) in mRNAs of 25 genes, 153 miRNAs of *O.aries* (oar-miRNA) in mRNAs of 24 genes.

The list of genes of the MYB transcription factor family of three animal species is given in Table 1. Nineteen TF genes were in each of the three animals. Seven other TF genes were in *E. caballus,* five other TF genes were in *O. aries*, four other genes were in *B. taurus.* The *SNAPC4* gene was found only in *E.caballus*. The genomes of *B.taurus*, *E.caballus*, *O.aries* are fully sequenced, but not all genes are annotated and, perhaps, therefore, TF orthologs have not been identified in some animals. Apparently, the same reason is due to the difference in the number of miRNAs in these animal species.

It was revealed that only 13 bta-miRNAs had binding sites in mRNA of *B. taurus* TF genes with ΔG/ΔGm value more than 87% (Table 2). The mRNA of *DNAJC2*, *MYB*, *MIER2*, *MYBL1* and *MYBL2* genes had only one binding site for different miRNAs. The *NCOR1* mRNA had binding sites for three miRNAs which are located through few hundred nucleotides in the CDS.

Table 1 – The list of genes of B. *taurus, E. caballus, O. aries* MYB family transcription factors

№	*B. taurus*	*E. caballus*	*O. aries*
1	*CDC5L*	*CDC5L*	*CDC5L*
2	*DMTF1*	*DMTF1*	*DMTF1*
3	-	*DNAJC1*	*DNAJC1*
4	*DNAJC2*	*DNAJC2*	-
5	*MIER1*	*MIER1*	*MIER1*
6	*MIER2*	-	*MIER2*
7	*MIER3*	*MIER3*	*MIER3*
8	*MYB*	*MYB*	*MYB*
9	*MYBL1*	*MYBL1*	*MYBL1*
10	*MYBL2*	*MYBL2*	*MYBL2*
11	*MYSM1*	*MYSM1*	*MYSM1*
12	*NCOR1*	*NCOR1*	*NCOR1*
13	-	*NCOR2*	*NCOR2*
14	*RCOR1*	*RCOR1*	*RCOR1*
15	*RCOR2*	*RCOR2*	*RCOR2*
16	*RCOR3*	*RCOR3*	*RCOR3*
17	*SMARCA1*	*SMARCA1*	*SMARCA1*
18	*SMARCA5*	*SMARCA5*	*SMARCA5*
19	-	*SMARCC1*	*SMARCC1*
20	*SMARCC2*	*SMARCC2*	*SMARCC2*
21	-	*SNAPC4*	-
22	*TADA2A*	*TADA2A*	*TADA2A*
23	*TERF1*	*TERF1*	*TERF1*
24	*TERF2*	*TERF2*	*TERF2*
25	*TTF1*	*TTF1*	*TTF1*
26	*TERB1*	-	*TERB1*
27	*ZZZ3*	*ZZZ3*	-

Characteristics of the binding of bta-miRNAs to mRNA genes of transcription factors of the MYB family Bos Taurus

The *RCOR1* gene was targeted by five miRNAs that had 14 binding sites. The miR-2885, miR-11976 and miR-11975 binding sites were located with the overlapping of nucleotide sequences in mRNA cluster from 147 nt to 168 nt. These miRNAs had five binding sites in the second cluster from 177 nt to 216 nt. The third cluster of binding sites of miR-2305, miR-11976 and miR-11975 is localized from 222 nt to 256 nt. The advantage of cluster organization of binding sites is compactization of binding sites. The first cluster with a length of 22 nt is 2.7 times smaller than the total length of binding sites of 60 nt. The second cluster, 40 nt long, is 2.5 times less than the length of all binding sites of 100 nt. The third cluster, 35 nt long, is 2.9 times smaller than the sum of the lengths of binding sites of 103 nt. Since it is problematic to support miRNA binding sites in CDS that are not involved in the coding of functionally important amino acids, compaction of these sites is obviously necessary. Figure 1 shows a portion of a protein containing a polypeptide encoded by the nucleotides of three clusters which is located between conserved oligopeptides GKRRGRNNA and SAAAAPNNG of RCOR1 protein.

A region of three clusters with a length of about 100 nt can at the same time binds no more than three miRNAs as part of a RISC complex. That is, between miRNAs, there is competition for binding to mRNA. There is a greater probability of binding miRNA to mRNA, for which the value of free energy of interaction is greater and the miRNA concentration is higher. These two factors will determine which miRNA will more suppress the expression of *RCOR1* gene. For example, miR-11976 and miR-11975 were associated with mRNA of gene with ΔG values -129 kJ/mole and -123 kJ/mole, which are 97% of the maximum ΔG value.

Figure 1 – Logo plot of amino acid variability in the region of RCOR1 protein containing a polypeptide encoded by binding sites of three clusters mRNA RCOR1 gene of B. *taurus, E. caballus, O. aries* and H. *sapiens*

Each of *RCOR2, RCOR3, SMARCC2* and *ZZZ3* genes was the target of one miRNA. A common feature of miRNA associations and target genes is the localization of binding sites predominantly in CDS. Only three sites are located in 5'UTR, one in 3'UTR. The binding of miRNA in 5'UTR has an important biological value, as it allows miRNA to stop protein synthesis earlier and not to waste energy on the synthesis of abortive protein in the case of miRNA binding in 3'UTR.

Table 2 – Characteristics of bta-miRNA binding to mRNA genes of *B.taurus* MYB family transcription factors

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/ mole	$\Delta G/\Delta G_m$ %	Length, nt
bta-DNAJC2	bta-miR-2322-5p	29	5'UTR	-104	87	23
bta-MYB	bta-miR-6528	14	5'UTR	-100	90	20
bta-MIER2	bta-miR-12035	28	CDS	-110	88	23
bta-MYBL1	bta-miR-582	469	CDS	-100	87	23
bta-MYBL2	bta-miR-3154	2087	CDS	-104	89	21
bta-NCOR1	bta-miR-3154	2852	CDS	-104	89	21
	bta-miR-31	5033	CDS	-102	91	21
	bta-miR-2381	5778	CDS	-104	89	21
bta-RCOR1	bta-miR-2885	147	CDS	-113	95	19
	bta-miR-11976	147	CDS	-125	94	21
	bta-miR-11975	148	CDS	-119	93	20
	bta-miR-2885	177	CDS	-113	95	19
	bta-miR-11976	177	CDS	-129	97	21
	bta-miR-11975	178	CDS	-123	97	20
	bta-miR-11976	195	CDS	-123	92	21
	bta-miR-11975	196	CDS	-117	92	20
	bta-miR-11976	222	CDS	-121	90	21
	bta-miR-11975	223-247	CDS	-115	90	20
	bta-miR-2305	227	CDS	-110	90	20
	bta-miR-11976	228	CDS	-121	90	21
	bta-miR-11976	246	CDS	-123	92	21
	bta-miR-1949	2881	3'UTR	-104	87	23
bta-RCOR2	bta-miR-877	1328	CDS	-102	91	20
bta-RCOR3	bta-miR-6528	102	5'UTR	-100	90	20
bta-SMARCC2	bta-miR-2330-5p	3363	CDS	-117	90	23
bta-ZZZ3	bta-miR-1284	651	CDS	-106	89	22

The mRNA of *DNAJC2, MYB, MIER2, MYBL1, MYBL2, RCOR2, RCOR3, SMARCC2* and *ZZZ3* genes have each binding sites for one miRNA with ΔG/ΔGm from 87% to 91.

DNAJC- focused on the relation of function to cell apoptosis and the cell cycle in cancers [11]. *MYB, MYBL1, MYBL2*- the genes encodes a transcription factor that regulates cell proliferation, differentiation, and apoptosis [12]. *MIER1- gene was identified as fibroblast growth factor and* that has been implicated as a tumour suppressor in breast cancer [13,14], predicted to be nuclear proteins [15,16]. *RCOR2 – RCOR3* are the transcriptional co-repressors [17,18].

miR-2381, miR-31 and miR-3154 effect mRNA of *NCOR1* gene in CDS. The ΔG/ΔGm value for these miRNAs change from 89% to 90%. The largest number of miRNA binding sites is found in mRNA of *RCOR1* gene: miR-2885, miR-11976, miR-11975, miR-2305 and miR-1949, binding sites

of all of them are located in the protein-coding region, except miR-1949.

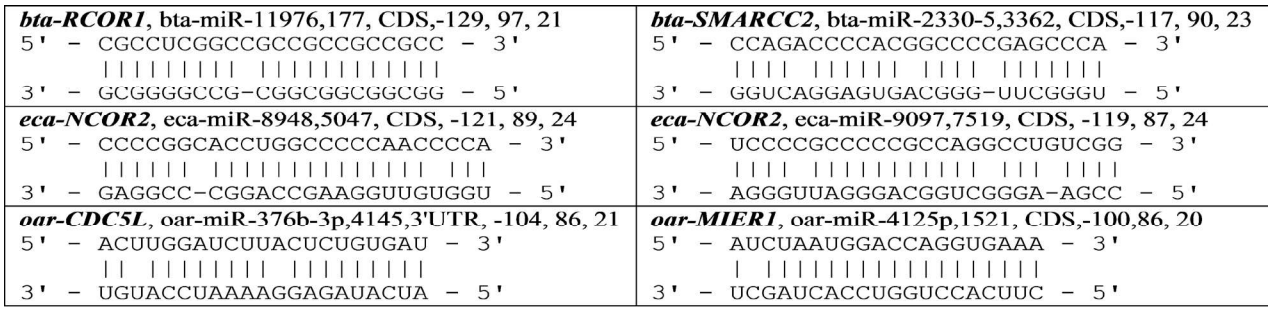
In these work the ΔG/ΔGm value for miRNAs change in the range from 90% to 97%, which indicates the strong influence of individual miRNAs on the expression of MYB genes.

Some mRNAs have several miRNA binding sites. miR-6528 has paired mRNA binding sites for MYB and *RCOR3* genes in 5'UTR. miR-3154 binds to mRNA of *MYBL2* and *NCOR1* genes in CDS.

The degree of miRNA interaction with mRNA is determined by the size of free energy (ΔG) of their binding. According to this indicator, several miRNAs can be identified. Schemes are shown in Figure 1. The largest ΔG value -129 kJ/mole is shown for miR-11976 binding with *RCOR1* mRNA, which is 97% of the maximum free binding energy of these miRNAs. miR-2330-5p binds to mRNA of *SMARCC2* gene with ΔG equal to -117 kJ/mole, which is 90% of maximum free binding energy, which indicates strong binding of these miRNAs and more efficient suppression of RCOR1 and SMARCC2 proteins synthesis.

Characteristics of eca-miRNAs binding to mRNA genes of E. caballus MYB family transcription factors

For *E.caballus*, we established binding sites for 15 miRNAs with mRNA of ten MYB family genes. The results of these studies are shown in table 3. mRNA of *MIER1, MYBL2, RCOR2, RCOR3, SMARCA5, SMARCC2* and *TERF2* genes bind each with one miRNA. The value of ΔG/ΔGm for binding varies from 88% to 93%. Different miRNAs act on mRNA of *NCOR2* gene: miR-8989, miR-9159, miR-8948 and miR-9097 with a ΔG/ΔGm value from 87% to 90%



Picture 1 – Schemes of miRNA interaction with mRNA genes of MYB family B.taurus, E.caballus, O.aries transcription factors

TERF2- is observed in a variety of human cancers, suggesting that *TERF2* plays a key role in tumor initiation and development [19, 20].

The largest number of binding sites has mRNA of *NCOR2* gene in CDS. On each of the mRNA of *SMARCC1* and *TTF1* genes act two miRNAs. The value of ΔG/ΔGm varies from 88% to 90%. The largest ΔG value is determined at miR-8948 interaction on mRNA of *NCOR2* gene, equal to -121 kJ/mole, which is 89% of the maximum free binding energy of these miRNAs. Interaction schemes of mRNA gene *eca-NCOR2* with miRNAs: eca-miR-8948 and eca-miR-9097 are shown in figure 1. 89% and 87% of the nucleotides of these miRNAs bind by hydrogen bonds to mRNA of respective target genes. Of the 15 miRNAs, 14 miRNAs are localized in the CDS and only miR-328 is located in the 5'UTR. This show a stable dependence of the expression of corresponding genes on miRNA, since CDS and 5'UTR are more conservative compared to 3'UTR.

Characteristics of binding of oar-miRNA to mRNA genes of O. aries MYB family transcription factors

For *O. aries*, we established ten miRNA binding sites in mRNA of eight MYB family genes. The results of these studies are shown in table 4. The mRNAs of *CDC5L, MIER1, MYSM1, RCOR1, RCOR2, RCOR3* and *SMARCA1* genes bind each with one miRNA with a ΔG/ΔGm ratio from 86% to 89%. *CDC5L* gene was shown to be a candidate oncogene in osteosarcoma and cervical tumors [21, 22]. The expression of *MYSM1, SMARCA1* genes may serve as a cancer marker [23-25].

Table 3 – Characteristics of binding of miRNAs to mRNA genes of *E. caballus* MYB family transcription factors

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mole	ΔG/ΔGm %	Length, nt
eca-MIER1	eca-miR-30e	328	CDS	-102	91	22
eca-MYBL2	eca-miR-9036	1228	CDS	-106	88	22
eca-NCOR2	eca-miR-8989	1574	CDS	-100	90	19
	eca-miR-9159	1667	CDS	-100	89	22
	eca-miR-8948	5047	CDS	-121	89	24
	eca-miR-9097	7519	CDS	-119	87	24
eca-RCOR2	eca-miR-139-3p	1440	CDS	-117	93	22
eca-RCOR3	eca-miR-9159	930	CDS	-100	89	22
eca-SMARCA5	eca-miR-7667	313	CDS	-113	88	23
eca-SMARCC1	eca-miR-328	79	5'UTR	-110	88	22
	eca-miR-539	2698	CDS	-100	89	22
eca-SMARCC2	eca-miR-345-3p	3732	CDS	-108	88	22
eca-TERF2	eca-miR-197	277	CDS	-106	88	22
eca-TTF1	eca-miR-129a-5p	2208	CDS	-102	89	21
eca-TTF1	eca-miR-129b-5p	2208	CDS	-102	89	21

Table 4 – Characteristics of oar-miRNAs binding to mRNA *O. aries* MYB family genes of transcription factors

Gene	miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
oar-CDC5L	oar-miR-376b-3p	4145	3'UTR	-104	86	21
oar-MIER1	oar-miR-412-5p	1521	CDS	-100	86	20
oar-MYSM1	oar-miR-26b	6109	3'UTR	-98	86	21
oar-NCOR2	oar-miR-3956-5p	1036	5'UTR	-108	88	23
	oar- miR-125b	2212	5'UTR	-96	86	21
	oar-miR-200b	15582	CDS	-93	86	21
oar-RCOR1	oar-miR-539-3p	4108	3'UTR	-91	87	23
oar-RCOR2	oar-miR-323a-5p	141	5'UTR	-91	87	21
oar-RCOR3	oar-miR-487a-3p	3329	5'UTR	-100	89	22
oar-SMARCA1	oar-miR-544-5p	1223	CDS	-91	88	21

miR-125b, miR-200b and miR-3956-5p bind with mRNA of *NCOR2* gene. The largest ΔG value is determined for miR-376b-3p binding in mRNA of *CDC5L* gene, equal to -104 kJ/mole. miR-412-5p binds to mRNA of *MIER1* gene with ΔG value -100 kJ/mole, which is 86% of the maximum free binding energy. The interaction of these two associations of miRNAs and mRNAs are shown in figure 1. In 5'UTR, miR-125b, miR-323a-5p and miR-487a-3p bind with ΔG/ΔGm from 86% to 87%. In CDS, miR-412-5p, miR-200b, miR-323a-5p and miR-487a-3p bind with ΔG/ΔGm from 86% to 89%. In 3'UTR, miR-26b, miR-376b-3p and miR-539-3p bind with ΔG/ΔGm from 86% to 88%.

Conclusion

The results indicate that mRNA of B. taurus, E. caballus, O. aries MYB family genes can bind with miRNA to varying degrees. The largest number of miRNA binding sites was found in the mRNA of *B. taurus RCOR1* gene. The mRNA of this gene has three clusters of miRNA binding sites, which is a fundamentally new property of miRNAs in animal organisms.

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