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Features of miRNAs binding sites within the C2H2 ZNF family: a *Bos taurus, Eqqus caballus,* and *Ovies aries* comparative approach

Abstract. C2H2 zinc finger genes constitute the largest class of transcription factors in humans and one of the largest gene families in mammals. Using the MirTarget program, we predicted miRNA binding sites (BSs) in CDS, 5'UTR and 3'UTR mRNAs of the ZNF family transcription factors genes of *Bos (B.) taurus, Equus (E.) caballus, Ovis (O.) aries*. We studied interactions of 1025 *B.taurus* miRNAs with 315 mRNAs of zf-C2H2 transcription factors family genes. From established 442 BSs, 196 are located in CDS, 164 in 3'UTR, 82 in 5'UTR. The free binding energy values range from -83 to -127 kJ/mol. mRNAs of several genes have miRNA BSs with overlapping nucleotide sequences (clusters). The cluster of BSs of miR-11975, miR-11976 and miR-2885 were found in 5'UTR, 3'UTR and CDS mRNAs of *FEZF1, SP8, VEZF1, PRDM6, SP3, ZNF366, PRDM13, PRDM12, ZIC4* and *ZFP91* genes. Multiple BSs were predicted for miR-574 in mRNAs of *HIVEP2, KLF7, SNAI2, SP4, ZNF677, ZNF710, ZFP91* genes. We studied binding characteristics between 690 miRNAs and 257 mRNAs of *E.qaballus* zf-C2H2 TFs family genes. The free binding energy ΔG values varied between -87 and -129 kJ/mol. From established 60 BSs, 24 are located in CDS, 21 in 5'UTR, 15 in 3'UTR. The largest ΔG value determined for binding of miR-8996 with *PRDM16* mRNA equals to -129 kJ/mol. The interaction of 152 miRNAs with 223 mRNAs of *O.aries* zf-C2H2 transcription factors genes was identified. The free binding energy values varied in between -85 and -117 kJ/mol. 18 BSs were found in mRNAs of TFs genes, located in CDS and 3'UTR. Therefore, our data suggests that regulation of zf-C2H2 transcription factors by miRNAs may involve their coding regions, thus providing a novel level of complexity when decoding the complex mechanism of miRNA/mRNA interplay and when interpreting conserved motifs within ZF coding sequences.
Key words: miRNA, mRNA, gene, binding site, animal, transcription factor, ZNF.

Introduction

miRNAs are class of ~22-nucleotide "non-messenger" RNAs, generally conserved in evolution, that they have been important regulatory functions. miRNAs play a key role for the control of animal development and physiology [1; 2]. Recent studies demonstrated that animal genomes contain at least 500 genes encoding miRNAs, as well as thousands of genes are targets of miRNA action [3-7]. In animals, miRNAs have been shown suppress mRNA translation and decrease mRNA stability by binding sequences in 3'UTR [8; 9].

The largest family of transcription factors (TF) is zinc-coordinating-group. There are ~20 different types of zinc finger (ZNF) genes domains, the majority is the classical Cys2-His2 (C2H2) [10,11]. The C2H2-type ZNF family has over 700 members, many of which are unique to primates and have appear through gene duplication [12]. The ZNF TFs are known as the most abundant DNA-recognition domain and are stabilized by the coordinated binding of a zinc ion [13]. ZNFs is one of the largest gene families in mammals [14].

Nearly all of the miRNA binding sites which were identified are located in 3′ untranslated region (3′UTR) of target genes in animals. Some recent research has shown that miRNAs are also found in targeted coding sequence (CDS) regions of some species [15-17].

Currently, the effect of miRNAs on the expression of TFs genes in organisms is not sufficiently understood. More systematic and also genome-wide studies on the effects of miRNA on the expression of

transcription factors are currently a topical research theme. For example, the effects of miRNAs on animal gene expression of MYB TFs have been reported in [18]. In this study, we systematically studied miRNA binding motifs in all known C2H2 ZNFs genes, including comparisons of their conservation in three different mammalian species. Surprisingly, our results indicate that mRNA regulation may predominantly involve the ZF coding regions.

Materials and methods

Nucleotide sequences of zf-C2H2 family TFs genes of *B. taurus, E. caballus* and *O. aries* mRNAs were downloaded from Animal Transcription Factor Database (http://www.bioguo.org/Animal TFDB/). Nucleotide sequences of miRNAs were downloaded from miRBase database (http://mirbase.org). The search for binding sites (BSs) of miRNAs in mRNAs of target genes was performed using the MirTarget program [19]. This program defines the following features of binding: a) the start of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in 5'-untranslated regions (5'UTR), CDS and 3'UTR of mRNAs; c) the free energy of interaction miRNA and mRNA (ΔG, kJ/mol); 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; with a miRNA length of 22 nt, ΔG/ΔGm value is higher than 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 nm [20]. G-C, A-U, G-U and A-C interactions form 3, 2, 1 and 1 hydrogen bonds. The miRNA binding sites for mRNA were taken with ΔG/ΔGm ratios equal and more than 85%.

Results and discussion

Characteristics of miRNAs binding to mRNAs of Bos taurus zf-C2H2 transcription factors genes. We studied interactions of 1025 *B. taurus* miRNAs with 315 mRNAs of zf-C2H2 transcription factors family genes. We established 442 binding sites: 196 are located in CDS, 164 in 3'UTR, 82 in 5'UTR. The free binding energy (ΔG) values ranged from -83 to – 127 kJ/mol. mRNAs of several genes have miRNA binding sites with overlapping nucleotide sequences (clusters) located in 5'UTR, CDS, or 3'UTR.

Table 1 presents results of the prediction of characteristics of miRNAs binding with mRNAs of *BCL11B*, *PRDM2, RREB1, SP4, ZNF628, ZNF710, ZNF142, ZNF236, ZNF687, ZNF652, ZNF467, ZFP91* genes.

Table 1 – Characteristics of miRNAs binding to mRNAs of *B. taurus* zf-C2H2 transcription factors genes

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
BCL11B	bta-miR-7865	465	5'UTR	-102	91	19
	bta-miR-1281	865		-93	96	17
	bta-miR-2885	2240	CDS	-108	91	19
	bta-miR-2388-3p	2467		-104	91	21
	bta-miR-2309	4516	3'UTR	-115	87	23
	bta-miR-1777b	4519		-113	90	20
	bta-miR-1777a	4520		-106	86	20
	bta-miR-11971	7367		-102	94	20
	bta-miR-1281	8096		-91	93	17

Table 1 continued

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
PRDM2	bta-miR-2450a	1235	CDS	-102	89	21
	bta-miR-2450b	1235		-108	88	23
	bta-miR-324	1606		-110	87	23
	bta-miR-6528	2997		-100	90	20
	bta-miR-3141	3002		-100	94	18
	bta-miR-6528	3285		-100	90	20
	bta-miR-7865	5719	3'UTR	-104	92	19
RREB1	bta-miR-2361	310	5'UTR	-89	93	20
	bta-miR-2359	312		-87	91	20
	bta-miR-1281	5424	CDS	-91	93	17
	bta-miR-2328-3p	6522	3'UTR	-113	91	21
	bta-miR-11972	7387		-115	89	21
	bta-miR-3141	8270		-98	92	18
SP4	bta-miR-2285ah-5p	122	5'UTR	-110	90	22
	bta-miR-1777a	123		-110	90	20
	bta-miR-2374	123		-110	90	21
	bta-miR-1281	214	CDS	-91	93	17
	bta-miR-376d	2949	3'UTR	-93	90	21
ZNF 628	bta-miR-2881	2322	CDS	-104	92	18
	bta-miR-2305	2331		-113	91	20
	bta-miR-1777a	2333		-110	90	20
	bta-miR-3957	2504		-102	91	20
	bta-miR-11981	2565		-119	87	23
	bta-miR-11976	2575		-119	89	21
	bta-miR-4444	3625		-93	94	18
	bta-miR-7865	3712		-104	92	19
	bta-miR-2899	4345		-98	92	18
	bta-miR-12030	4493		-110	93	19
ZNF710	bta-miR-128	322	5'UTR	-100	90	21
	bta-miR-7865	623		-102	91	19
	bta-miR-1777b	5760	3'UTR	-113	90	20
	bta-miR-1777a	5761		-110	90	20
	bta-miR-1296	6906		-110	90	22
	bta-miR-574	7086-7096		-113-115	87-93	19-24
	bta-miR-2304	7097		-96	90	20
ZNF142	bta-miR-2324	1772	CDS	-113	87	23
	bta-miR-8548	2412		-83	93	17
	bta-miR-12022	5731	3'UTR	-98	90	21
	bta-miR-10161-5p	5735		-106	88	23
	bta-miR-12032	5796		-106	89	21
	bta-miR-12054	6956		-96	94	18

Table 1 continued

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
ZNF236	bta-miR-2892	72	5'UTR	-119	89	22
	bta-miR-11975	77		-115	90	20
	bta-miR-411c-3p	2454	CDS	-100	89	22
	bta-miR-149-3p	3994		-117	90	22
	bta-miR-1224	4556		-106	89	21
	bta-miR-23b-3p	5810		-106	88	23
ZNF687	bta-miR-2305	1	5'UTR	-115	93	20
	bta-miR-3141	1		-100	94	18
	bta-miR-2882	438	CDS	-104	91	19
	bta-miR-2475	2269		-108	88	23
	bta-miR-2285ah-5p	2541		-110	90	22
ZNF652	bta-miR-2485	1582	CDS	-96	90	21
	bta-miR-149-3p	2026		-115	89	22
	bta-miR-1777b	2153		-117	93	20
	bta-miR-1777a	2154		-115	93	20
	bta-miR-11989	8441	3'UTR	-110	87	23
ZNF467	bta-miR-7865	16	5'UTR	-104	92	19
	bta-miR-6528	152		-102	92	20
	bta-miR-2305	158		-113	91	20
	bta-miR-1777b	1605	CDS	-117	93	20
	bta-miR-1777a	1606		-110	90	20
ZFP91	bta-miR-11976	177-189	CDS	-121-127	90-95	21
	bta-miR-11975	178-190		-115-121	90-95	20
	bta-miR-2885	180		-110	93	19
		189		-110	93	19
		192		-110	93	19
	bta-miR-574	1849-1870	3'UTR	-110-117	87-92	24
	bta-miR-11988	1895		-113	95	22

The mRNA of *BCL11B* gene has binding sites with miR-7865, miR-1281, miR-2885, miR-2388-3p, miR-2309, miR-1777b, miR-1777a, miR-11971. BSs of miR-7865, miR-2885 are localized in 5'UTR, of miR-2388-3p and miR-2309 are located in CDS, of others in 3'UTR. Cluster of mir-2309, mir-1777a and mir-1777b BSs is localized in 3'UTR with a total length of 24 nt. The largest ΔG value is determined for miR-2309 BS equal to -115 kJ/mol.

The mRNA of *PRDM2* gene has binding sites with miR-2450a, miR-2450b, miR-324, miR-6528, miR-3141, miR-7865. BSs of miRNAs are located in CDS and for miR-7865 in 3'UTR. Two clusters of binding sites are found in CDS, first cluster for miR-2450a and miR-2450b BSs with a total length of 23 nt and second cluster for miR-6528 and miR-3141 BSs also with a length of 23 nt. miR-6528 has two binding sites.

The mRNA of *RREB1* gene has binding sites with miR-2361, miR-2359 miR-1281, miR-2328-3p, miR-11972, miR-3141 located in 5'UTR, CDS, 3'UTR. We identified miRNA binding sites located with overlapping of nucleotide sequences. Cluster of miR-2361 and miR-2359 BSs with a length of 22 nt is localized in 5'UTR. Binding sites of miR-2361 and miR-2359 are located across two nucleotides in 5'UTR.

The mRNA of *SP4* gene has binding sites with miR-2285ah-5p, miR-1777a, miR-2374, miR-1281, miR-376d located in 5'UTR, CDS, 3'UTR. miR-

1777a and miR-2374 BSs are located with overlay in 5'UTR.

The mRNA of *ZNF628* gene has the largest number of binding sites with ten miRNAs: miR-2881, miR-2305, miR-1777a, miR-3957, miR-11981, miR-11976, miR-4444, miR-7865, miR-2899, miR-12030. All BSs are located in CDS. miR-2881, miR-2305 and miR-1777a have overlapping binding sites which form a cluster. Binding sites of miR-11981 and miR-11976 also form a cluster. In this case it is important the location of binding sites mainly in protein coding part of mRNA. The greater free binding energy is determined for miR-11976 equal to-127 kJ/mol.

The mRNA of *ZNF710* gene has binding sites with miR-128, miR-7865, miR-1777b, miR-1777a, miR-1296, miR-574, miR-2304 located in 3'UTR and 5'UTR. Cluster of BSs is found for miR-1777b and 1777a in the 3'UTR. miRNAs BSs are located with overlapping of 19 nt. miR-574 has polysites in 3'UTR, located through two nucleotides. Binding sites of miR-574 and miR-2304 form a cluster, located from 7094 to 7118 nt.

The mRNA of *ZNF142* gene has binding sites with miR-2324, miR-8548, miR-12022, miR-10161-5p, miR-12032, miR-12054 located in CDS and 3'UTR. Binding sites of miR-12022 and miR-10161-5p form a cluster in 3'UTR, located from 5731 to 5758 nt.

The mRNA of *ZNF236* gene has binding sites with miR-2892, miR-11975, miR-411c-3p, miR-149-3p, miR-1224, miR-23b-3p located in 5'UTR and 3'UTR. In 5'UTR binding sites of miR-2892 and miR-11975 are located with overlapping of five nucleotides.

The mRNA of *ZNF687* gene has binding sites of five miRNAs: miR-2305, miR-3141, miR-2882, miR-2475, miR-2285ah-5p located in 5'UTR and 3'UTR. miR-2305 and miR-3141 BSs are located with an overlay in 5'UTR.

mRNAs of *ZNF652, ZNF467* genes have binding sites with miR-2485, miR-149-3p, miR-1777b, miR-1777a, miR-11989, miR-7865, miR-6528, miR-2305 located in CDS, 3'UTR, 5'UTR. Binding sites of miR-1777b and miR-1777a in CDS mRNA of *ZNF652, ZNF467* genes form a cluster with overlapping of 19 nucleotides.

miR-11976, miR-11975, miR-2885, miR-574, miR-11988 bind in mRNA of *ZFP91* gene. Polysites of miR-11976, miR-11975 and miR-2885 form a cluster with a total length of 34 nt, located across three nucleotides. miR-11976, miR-11975 and miR-2885 bind with ΔG values equal to -127 kJ/mol, -115 kJ/mol and -110 kJ/mol. Described miRNAs also form a cluster with mRNA of *MYB* transcription factors [18]. Also found polysites for miR-574 in 3'UTR mRNA of *ZFP91* gene, located through two nucleotides. The great free binding energy is equal to -127 kJ/mol.

The role of some studied miRNAs is given in Table 2.

Table 2 – Information on role of bta-miRNAs

miRNA	Role of miRNA	A source of information
miR-122	HCV pathogenesis	PMID:29769341
miR-1281	apoptosis	PMID:31884421
miR-149	muscle protein synthesis (MPS) and anabolism	PMID:28341051
mir-1777a	mastitis resistance	PMID:22084936
miR-2304	I. MODULATION OF HOST IMMUNE RESPONSE	PMID:23504566
miR-2361	BHV-1 pathogenesis	PMID:31176405
miR-2881	myotube MPS and anabolism	PMID:28341051
miR-2885	liver diseases	PMID:24428929
miR-2899	energy metabolism; mastitis resistance	PMID:31208329; PMID:31096910
miR-34a	II. ENERGY METABOLISM IN SKELETAL MUSCLE; MAMMARY METABOLISM	PMID:31208329; PMID:30639019

miR-574 has multiple sites in mRNAs of *HIVEP2, KLF7, SNAI2, SP4, ZNF677, ZNF710, ZFP91* genes (Table 3). In all mRNAs of genes miR-574 has poly-sites, located mainly in 3'UTR through two nucleotides. In mRNA of *ZFP91* gene miR-574 has eleven, in *KLF7* ten, in mRNA of *HIVEP2, SNAI2* genes six binding sites. miR-574 has five binding sites in mRNA of *ZNF710* gene, three – in *ZNF677* gene, one binding site in *SP4* gene. The largest ΔG value is determined for BSs in mRNA of *KLF7* gene equal to -121 kJ/mol.

Table 3 – Characteristics of miR-574 binding with 3'UTR mRNA of *B. taurus* zf-C2H2 transcription factor genes

Gene	Start of site, nt	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %
HIVEP2	9694-9704	-113-119	87-94
KLF7	1324-1342	-113-121	87-95
	1352	-110	87
SNAI2	1059-1069	-113	87-93
SP4	1849	-117	92
ZNF677	2419-2423	-113-115	88-90
ZNF710	7086-7096	-113-115	87-93
ZFP91	1849-1870	-110-117	87-92

A previous study demonstrated that in the case of human miR-574-5p, interaction with it target mRNAs involves many binding sites located across two nucleotides [21]. miR-574 is of particular interest because the change in its expression detected in various pathologies. Thus, miR-574-3p is a potential therapeutic and prognostic biomarker in human colorectal cancer cells, it's up-regulation had prohibited the cell proliferation of human colorectal cancer cells *in vitro* and increased the apoptosis level [22]. miR-574-3p expression levels were decreased in spinal chordoma patients [23]. miR-574 has been found to be upregulated in several types of cancers, including human osteosarcoma, lung cancer, bladder cancer and prostate cancer [24-27].

In mRNAs of *REPIN1, ZNF592, ZNF771* genes the cluster of miR-1777b and 1777a binding sites were found (Table 4). In all genes binding sites are located through one nucleotide in 3'UTR mRNA of *REPIN1*, *ZNF592* genes, in 5'UTR mRNA of *ZNF771* gene.

In mRNA of *ZNF699* gene binding sites of miR-3432 and miR-3432a are located with overlay, BSs of miR-3432b across one nucleotide.

Table 4 – Characteristics of miRNAs binding with mRNAs of *REPIN1, ZNF592, ZNF771 B. taurus* zf-C2H2 transcription factor genes

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
REPIN1	bta-miR-1777b	3209	3'UTR	-113	90	20
	bta-miR-1777a	3210		-113	91	20
ZNF592	bta-miR-1777b	4903	3'UTR	-117	93	20
	bta-miR-1777a	4904		-110	90	20
ZNF771	bta-miR-1777b	14	5'UTR	-115	92	20
	bta-miR-1777a	15		-110	90	20
	bta-miR-34a	1011	CDS	-104	89	22
ZNF699	bta-miR-3432	4180	3'UTR	-106	91	22
	bta-miR-3432a	4180		-106	91	22
	bta-miR-3432b	4181		-98	90	21

The cluster of binding sites of miR-11975, miR-11976 and miR-2885 were found in 5'UTR, 3'UTR and CDS of mRNAs of *FEZF1, SP8, VEZF1, PRDM6, SP3, ZNF366, PRDM13, PRDM12, ZIC4* and *ZFP91* genes (Table 5).

In mRNAs of *SP8* and *VEZF1* genes miRNAs have polysites located across three nucleotides. In mRNA *of SP8* gene was predicted seven binding sites of miR-11975, six BSs of miR-11976 and three BSs of miR-2885. In 5'UTR mRNA of *VEZF1* gene predicted six binding sites of miR-11976, four BSs of miR-11975 and two BSs of miR-2885 in 5'UTR. The starts of miR-11976, mir-11975 binding sites in mRNAs of *PRDM6, SP3, SP4, ZNF366* genes are located across one nucleotide. In mRNAs of *PRDM12, PRDM13, ZIC4* genes mir-11976 and mir-2885 BSs are located with overlay. The largest free binding energy were characterized for miR-11976 and varied between -127-(-119) kJ/mol, for miR-11975 – (-121-(-115)) kJ/mol and for miR-2885 – (-110-(108)) kJ/mol.

Table 5 – Characteristics of miRNAs binding with mRNAs of *B.taurus FEZF1, SP8, VEZF1 PRDM6, SP3, ZNF366, PRDM13, PRDM12, ZIC4, ZFP91*zf-C2H2 transcription factor genes

Gene	bta-miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
FEZF1	bta-miR-11976	1331	CDS	-119	89	21
	bta-miR-2885	1349		-108	91	19
SP8	bta-miR-11975	511-528	CDS	-115	90	20
	bta-miR-11976	512-527		-127	95	21
	bta-miR-2885	512		-110	93	19
		515		-110	93	19
		524		-108	91	19
VEZF1	bta-miR-11976	11-26	5'UTR	-119	89	21
	bta-miR-11975	18-27		-117	92	20
	bta-miR-2885	11		-108	91	19
		26		-110	93	19
PRDM6	bta-miR-11976	678	CDS	-119	89	21
	bta-miR-11975	679		-117	92	20
SP3	bta-miR-11976	882	CDS	-121	90	21
	bta-miR-11975	883		-121	95	20
ZNF366	bta-miR-11976	969	CDS	-121	90	21
	bta-miR-11975	970		-115	90	20
		1246		-117	92	20
PRDM13	bta-miR-11976	1215	CDS	-121	90	21
	bta-miR-2885	1215		-110	93	19
	bta-miR-11975	1216		-115	90	20
PRDM12	bta-miR-11976	1035	CDS	-119	89	21
	bta-miR-2885	1035		-108	91	19
ZIC4	bta-miR-11976	3946	CDS	-119	89	21
	bta-miR-2885	3946		-108	91	19
ZFP91	bta-miR-11976	177-189	CDS	-121	90	21
	bta-miR-11975	178-190		-115	90	20
	bta-miR-2885	180	CDS	-110	93	19
		189		-110	93	19
		192		-110	93	19

Characteristics of miRNAs binding with mRNAs of Equus qaballus zf-C2H2 transcription factor genes. To detect miRNAs targeted by genes of zf-C2H2 TFs family we studied binding characteristics between 690 miRNAs and 257 mRNAs of *E. qaballus* zf-C2H2 TFs family genes. The free binding energy ΔG values were varied between -87 and -129 kJ/mol. Was established 60 binding sites: 24 located in CDS, 21 in 5'UTR, 15 in 3'UTR. The largest ΔG value determined for binding of miR-8996 with *PRDM16* mRNA equal to -129 kJ/mol. Each of mRNAs of *HIVEP3, PLAGL2, PRDM15, ZNF592* genes have BSs for three miRNAs located in 5'UTR, 3'UTR and CDS (Table 6).

Table 6 – Characteristics of miRNAs binding with mRNAs of *E. caballus* zf-C2H2 transcription factor genes

Gene	miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
EGR3	eca-miR-568	2708	3'UTR	-87	91	20
FEZF2	eca-miR-8953	297	CDS	-98	90	21
GLIS3	eca-miR-9000	75	5'UTR	-119	90	23
HIVEP2	eca-miR-23a	3632	CDS	-102	92	21
HIVEP3	eca-miR-9181	5213	CDS	-108	91	21
	eca-miR-122	7110		-102	89	22
	eca-miR-8984	11837	3'UTR	-98	90	20
IKZF1	eca-miR-8915	4842	3'UTR	-119	87	24
IKZF4	eca-miR-1597	384	5'UTR	-106	89	22
IKZF5	eca-miR-8941	935	CDS	-96	90	20
KLF12	eca-let-7a	4232	3'UTR	-96	87	22
	eca-let-7f	4232		-98	90	22
KLF7	eca-miR-703	5825	3'UTR	-96	90	21
LOC100052677	eca-miR-9140	2136	CDS	-123	87	25
LOC100060110	eca-miR-27b	649	CDS	-100	90	21
LOC100629880	eca-miR-9004	6238	3'UTR	-102	89	22
PLAGL2	eca-miR-432	676	5'UTR	-108	88	23
	eca-miR-7667	727		-113	88	23
	eca-miR-8969	2538	CDS	-104	91	21
PRDM15	eca-miR-135b	2237	CDS	-100	87	23
	eca-miR-135a	2238		-98	87	23
	eca-miR-135b	2238		-102	89	23
PRDM16	eca-miR-7035	5628	3'UTR	-117	86	25
	eca-miR-8996	6349		-129	87	25
WIZ	eca-miR-8931	7515		-113	90	22
ZFP64	eca-miR-769a-5p	1847	CDS	-113	93	22
	eca-miR-769b	1847		-108	91	22
ZFP69B	eca-miR-9167	2294	5'UTR	-119	86	25
ZFX	eca-miR-544b	6514	3'UTR	-96	90	22
ZKSCAN2	eca-miR-7045	301	5'UTR	-121	88	23
ZKSCAN4	eca-miR-141	839	CDS	-102	91	22
ZKSCAN5	eca-miR-197	1451	CDS	-108	89	22

Table 6 continued

Gene	miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
ZMAT3	eca-miR-8957	1043	5'UTR	-115	86	25
ZNF142	eca-miR-9121	2971	CDS	-115	86	25
ZNF214	eca-miR-539	936	CDS	-100	89	22
ZNF215	eca-miR-9031	5677	5'UTR	-117	87	25
ZNF324	eca-miR-1379	357	CDS	-123	87	24
	eca-miR-8984	2788	3'UTR	-98	90	20
ZNF333	eca-miR-7045	718	5'UTR	-121	88	23
	eca-miR-493b	1836	CDS	-108	89	22
ZNF34	eca-miR-9005	3807	5'UTR	-117	86	25
ZNF408	eca-miR-9159	4680	3'UTR	-100	89	22
ZNF423	eca-miR-143	2670	CDS	-100	90	21
ZNF445	eca-miR-9115	243	5'UTR	-117	90	24
ZNF45	eca-miR-8945	1888	CDS	-106	88	23
ZNF512	eca-miR-8917	1897	CDS	-108	88	23
ZNF514	eca-miR-8917	218	5'UTR	-110	90	23
ZNF521	eca-miR-9039	399	CDS	-108	88	23
ZNF544	eca-miR-186	1380	5'UTR	-100	89	22
ZNF592	eca-miR-493a	145	5'UTR	-110	91	22
	eca-miR-764-3p	220		-108	91	22
	eca-miR-197	6289	3'UTR	-110	91	22
ZNF614	eca-miR-9163	482	5'UTR	-115	87	24
ZNF615	eca-miR-9000	51	5'UTR	-123	94	23
ZNF618	eca-miR-9164	14187	3'UTR	-102	92	20
ZNF710	eca-miR-9124	2168	5'UTR	-108	88	23
ZNF75D	eca-miR-876-5p	1074	CDS	-100	90	22
ZNF79	eca-mir-9024	1759	5'UTR	-115	86	25
ZSCAN23	eca-miR-211	2324	5'UTR	-102	89	22
ZSCAN29	eca-miR-149	299	5'UTR	-113	88	23

Each of mRNAs of *KLF12, PRDM16, ZFP64, ZNF324*, *ZNF333* genes have BSs for two miRNAs. mRNAs of other genes have BSs with one miRNA. miR-9000 has BSs in 5'UTR mRNA of *GLIS3* and *ZNF615* genes. miR-8984 has binding sites in 3'UTR mRNA of *HIVEP3* and *ZNF324* genes. let-7a and let-7f BSs located with overlay in mRNA of *KLF12* gene in 3'UTR. miR-135b and miR-135a binding sites are located across one nucleotide in CDS mRNA of *PRDM15* gene. miR-769a-5p and miR-769b have by one BS located in CDS mRNA of *ZFP64* gene. miR-7045 has BSs in mRNAs of *ZKSCAN2* and *ZNF333* genes located in 5'UTR. miR-197 also has BS in mRNA of *ZKSCAN5* and *ZNF592* genes located in CDS and 3'UTR. miR-8917 has BSs in mRNA of *ZNF512* and *ZNF514* genes located in CDS and 5'UTR.

Characteristics of miRNAs binding with mRNAs of Ovis aries zf-C2H2 transcription factor genes. The interaction of 152 miRNAs with 223 mRNAs of *O. aries* zf-C2H2 transcription factors genes was identified. The free binding energy ΔG values were varied between -85 and -117 kJ/mol. Was found 18 binding sites: 9 located in CDS and 9 in 3'UTR (Table 7).

Table 7 – Characteristics of miRNAs binding to mRNAs of *O. aries* zf-C2H2 transcription factor genes

Gene	miRNA	Start of site, nt	Region of mRNA	ΔG, kJ/mol	$\Delta G/\Delta G_m$ %	Length, nt
BCL11A	oar-miR-544-3p	1184	CDS	-91	90	21
IKZF1	oar-miR-493-5p	3712	3'UTR	-98	88	22
KLF12	oar-miR-106a	3777	3'UTR	-98	90	21
	oar-miR-23b	969	CDS	-85	91	18
	oar-miR-544-5p	9302	3'UTR	-93	90	21
LOC101110699	oar-miR-1193-3p	752	CDS	-104	89	22
LOC101111419	oar-miR-543-5p	4458	CDS	-91	90	19
LOC101119987	oar-miR-27a	1133	CDS	-100	89	21
MTF1	oar-miR-106a	2630	3'UTR	-96	88	21
PRDM1	oar-let-7d	2784	CDS	-93	90	20
SNAI2	oar-miR-654-5p	196	CDS	-117	87	24
ZFP62	oar-miR-493-5p	4087	3'UTR	-98	88	22
ZIC3	oar-miR-30d	2946	3'UTR	-91	90	19
ZNF236	oar-miR-3959-3p	2362	CDS	-100	89	22
ZNF331	oar-miR-3955-3p	3104	3'UTR	-96	90	21
ZNF541	oar-miR-410-5p	325	CDS	-110	88	23
ZNF606	oar-miR-329a-5p	4099	3'UTR	-102	89	22
	oar-miR-329b-5p	4098	3'UTR	-106	89	23

The largest ΔG value determined for miR-8996 binding with mRNA of *SNAI2* gene and equal to -117 kJ/mol (Table 7). *KLF12* mRNA have binding sites for three miRNAs: oar-miR-106a, oar-miR-23b and oar-miR-544-5p. *ZNF606* mRNA have binding sites for oar-miR-329a-5p and oar-miR-329b-5p. Other genes have BSs for one miRNA with $\Delta G/\Delta G_m$ values more than 85.

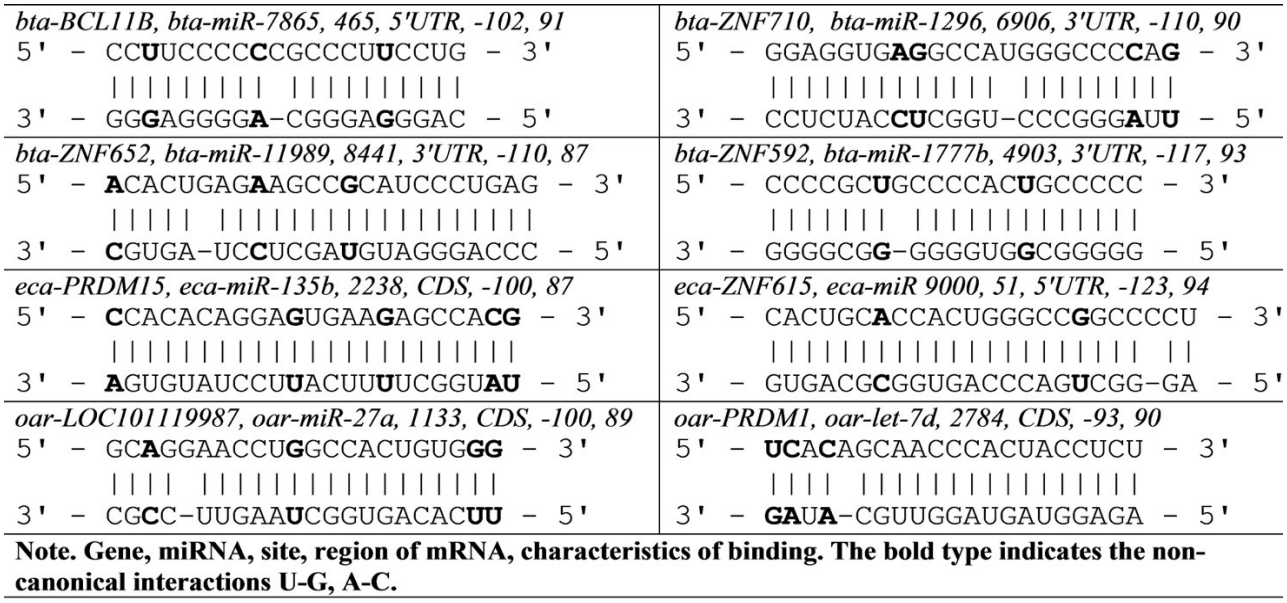
The possible schemes of interaction of miRNA and mRNA nucleotides are shown in Figure 1. The schemes show the following advantages of the MirTarget program, taking into account that: almost of miRNA nucleotides in the interaction with mRNA; the formation of non-canonical pairs G-U and A-C that do not change the double-stranded conformation of the miRNA complex with mRNA, since the distances between G-U and A-C are equal to the distances between G-C and A-U; an important criterion for binding miRNA to mRNA is the free energy of interaction; the localization of the miRNA binding site in 5'UTR, CDS and 3'UTR of mRNA.

The schemes demonstrate a total complementarity between nucleotides. For example, of the 23 nucleotides of eca-miR-135b, all nucleotides formed a double-stranded helical structure with mRNA. We can see three pairs of G-U and two pairs of A-C non-canonical pairs interaction between nucleotides of eca-miR-135b and eca-PRDM15 mRNA.

The level of miRNA interaction with mRNA is given by the size of free energy of their binding. By this indicator several miRNAs were identified. The largest ΔG value -123 kJ/mol is character for eca-miR-9000 binding with *ZNF615* mRNA. Bta-miR-1777b binds to mRNA of *ZNF592* gene with ΔG value equal to -117 kJ/mol, which is 90% of maximum free binding energy, which indicates the strong binding of these miRNAs and more efficient suppression of *ZNF615* and *ZNF592* proteins synthesis.

An amino acid sequence of oligopeptide is encoded by the nucleotides of three clusters which located between conserved oligopeptides PGSSAFSLTSSS and AAAAASSSPFAN of SP8 protein presented in Table 8.

The bta-miR-11975 binding sites in the mRNAs of *SP8* genes are located in CDS and encode the AAAAAAA oligopeptide in the first and third reading frames. Bta-miR-11976 binding sites in *SP8* gene also encode this oligopeptide in the first and third reading frames. The bta-miR-2885 BS in CDS encodes AAAAAAA in the third reading frame.



Note. Gene, miRNA, site, region of mRNA, characteristics of binding. The bold type indicates the non-canonical interactions U-G, A-C.

Figure 1 – Schemes of miRNAs interaction with mRNAs of *B. taurus, E. caballus, O. aries* zf-C2H2 family transcription factors genes

Table 8 – The variability of amino acid sequences of ZNF family proteins containing oligopeptide AAAAAAA encoded by the binding sites of miRNAs in CDS mRNAs of genes

Gene	miRNA	Start of site, nt	Region of transcription factor containing oligopeptide AAAAAAA
SP8	bta-miR-11975	511-522	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[1]
		525-528	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[3]
	bta-miR-11976	512-518	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[1]
		521-527	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[3]
	bta-miR-2885	512-515	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[3]
		524	PGSSAFSLTSSS**AAAAAAA**AAAAASSSPFAN[3]
PRDM6	bta-miR-11976	678	SSSTSASSASSC**AAAAAAA**AAAALAGLSALP[1]
	bta-miR-11975	679	SSSTSASSASSC**AAAAAAA**AAAALAGLSALP[1]
ZFP91	bta-miR-11976	177	SRVLRGGRDRGR**AAAAAAA**AAAAVSRRRKAE[1]
		180-189	SRVLRGGRDRGR**AAAAAAA**AAAAVSRRRKAE[2]
	bta-miR-11975	178-190	SRVLRGGRDRGR**AAAAAAA**AAAAVSRRRKAE[3]
	bta-miR-2885	180-192	SRVLRGGRDRGR**AAAAAAA**AAAAVSRRRKAE[2]
Note: Indexes show reading frames – 1, 2, 3.			

In the mRNA of *PRDM6* gene cluster of bta-miR-11976 and bta-miR-11975 BSs encodes AAAAAAA in the first reading frame, which are located between conserved oligopeptides SSSTSASSASSC and AAAALAGLSALP.

In the mRNA of *ZFP91* gene three clusters also encode polyA between oligopeptides SRVLRGGRDRGR and AAAAVSRRRKAE.

Recent studies have revealed that aberrant expression of zinc finger proteins contributes to progression in multiple cancers, including tumorigenicity, metastasis and chemoresistance [28-30]. *PRDM2* gene has a large role in human cancers such as neuroblastoma [31], hepatoma [32], and breast cancer [33]. A significant decrease of *PRDM2* gene expression is observed in high-grade gliomas [34]. *BCL11B* mRNA

is essential for growth of αβ T cells and most of γδ T cells [35]. Oncogenic properties of *ZFP91* is revealed in experiments. Where is found overexpression of *ZFP91* in a screening-type of study in leukemic cells and neoplastic blood cell lines [36]. Overexpression has been connected to cancer pathogenesis in melanoma, pancreatic, breast and lung cancers [37], colon cancer and endometrial cancer cell lines and stomach cancer cell lines [38]. Overexpression of *ZFP91* gene is observed in many types of cancer. Therefore, it is possible to bind the cluster into the protein encoding part has an important role to suppress the expressions of this gene. Also miRNA binds to a complementary sequence in the 3′ untranslated region (3′UTR) of its target mRNA and expression of this mRNA is silenced. The *ZNF628* known to be essential for normal growth and development, found in mammals, conserved, seems to be functionally important [39]. Was identified to have significant association with Alzheimer's disease [40]. This *in silico* research indicates that some miRNAs can regulate gene family by targeting their coding regions, thus providing an important and novel perspective for decoding the complex mechanism of miRNA/mRNA interplay. Overall, miRNA binding sites in coding regions show bigger regulation than 3′UTR and 5′UTR binding.

Conclusion

In this paper, we identified the features of interactions of 1025 *B. taurus* miRNAs with 315 ZNFs genes mRNAs, 690 miRNAs and 257 mRNAs of *E. qaballus* ZNFs genes mRNAs and 152 miRNAs with 223 mRNAs of *O. aries* zf-C2H2 transcription factors genes. Using the MirTarget program, we predicted miRNA BSs in the CDS, 5'UTR and 3'UTR mRNAs of genes. It has been found that some miRNAs may bind to mRNA of more than one target gene. For example, bta-miR-1777b and bta-miR-1777a have BSs in mRNAs of *BCL11B, SP4, ZNF 628, ZNF710, ZNF652, ZNF467, REPIN1, ZNF592* and *ZNF771* genes. miR-574 has polysites in 3'UTR mRNA of *B. taurus* zf-C2H2 transcription factor genes. On the basis of the miRTarget program, the organization of binding sites was established in arranged located sites with overlapping nucleotide sequences. The cluster organization of miRNA BSs, together with the free energy of miRNA interaction with mRNA, causes competition between miRNAs to bind to mRNA. Since hundreds of miRNAs have now been identified in various farm animal species, more systematic and genome-wide studies will be required on the effect of miRNAs. This will address how the expression of transcription factors is regulated during a diverse range of biological processes by the fast growing miRNA signal networks.

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