IRSTI 34.15.25 https://doi.org/10.26577/eb.2022.v90.i1.06

M.O. Myrzabekova[1,2]*, S.B. Labeit[3], R.Ye. Nyiazova[1]

[1]Al-Farabi Kazakh National University, Kazakhstan, Almaty
[2]Kazakh National Agrarian University, Kazakhstan, Almaty
[3]University of Heidelberg, Germany, Heidelberg
*e-mail: moldir.myrzabek@gmail.com

IN SILICO IDENTIFICATION OF *EQQUS CABALLUS* MIRNAS WITH THE POTENTIAL TO AFFECT HUMAN GENE EXPRESSION

miRNAs exist that are codified by non-human genomes but are still present in circulation.These miRNAs have been termed as xeno-miRNAs. XenomiRNAs in humans have been identified in various exogenous sources previously. The aim of this work is to identify xeno-miRNA from Eqqus caballus (domestic horse or in brief eca) which have analogs can bind to human genes. The MirTarget program was used to predict miRNA binding to human gene mRNAs.The homologs of eca-miRs were identified by using miRviewer online free available bioinformatic tool. It was identified 15 eca-miRNAs interacted with human mRNA genes with high complementarity, ΔG/ΔGm equal to 98-100%. The characteristics of the interaction of all known eca-miRNAs with mRNAs of human genes were identified. The total number of binding sites for 469 miRNAs are 1605, from which 907 are in CDS, 451 in 3'UTR and 247 in 5'UTR. 93 miRNAs each have one-target genes, 63 miRNAs have two target genes, 67 miRNAs have three to four target genes, and 72 miRNAs have five and more target genes. The free energy of the interaction of the considered miRNAs with the mRNAs of human genes is high and varied from -110 kj/mole to -117 kj/mole. The homology analyses revealed 140 miRNAs candidates shown to be total identical to human miRNAs sequences.

Key words: miRNA, mRNA, milk, xeno-miRNA, Eqqus caballus.

М.О. Мырзабекова[1,2]*, З. Лабейт[3], Р.Е. Ниязова[1]

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
[2]Қазақ Ұлттық Аграрлық Зерттеу Университеті, Қазақстан, Алматы қ.
[3]Хайделберг Университеті, Германия, Хайделберг қ.
*e-mail: moldir.myrzabek@gmail.com

Адам гендерінің экспрессиясына әлеуетті әсері бар *Equus caballus* miRNA-н in silico идентификациялау

Адам геномымен кодталмайтын miRNA-лер, бірақ адам ағзасында табылған miRNA-лер бар, олар xeno-miRNA-лар деп аталады. Xeno-miRNA-лар адам ағзасында әр-түрлі экзогенді көздерден анықталынды. Бұл жұмыстың мақсаты аналогтары адам гендерімен байланысатын Eqqus caballus (үй жылқысы немесе қысқаша eca) xeno-miRNA-ларды идентификациялау болып табылады. MiRNA мен mRNA байланысуы MirTarget бағдарламасымен болжам жасалынды, eca-miRNA гомологтары желідегі қолжетімді онлайн miRviewer биоинформатикалық бағдарлама көмегімен идентификацияланды. ΔG/ΔGm 98-100% көрсеткіштегі адам mRNA гендерімен жоғары комплементарлықпен байланысатын 15 eca-miRNA белгілі болды. Белгілі 17508 адам mRNA гендерімен байланысатын барлық eca-miRNA мен mRNA сипаттамалары анықталды. Eqqus caballus-тың белгілі 469 miRNA-мен байланысқан жалпы байланыс сайттар саны 1605, оның ішінде 907-сі CDS-те, 451-3'UTR-де, 247-5'UTR-де орналасқан. 93 miRNA бір нысана генмен, 63 miRNA екі нысана генмен, 67 miRNA үштен төрт нысана генге дейін, ал 72 miRNA бестен көп генмен байланысты. Қарастырылған miRNA-мен mRNA бос байланыс энергия әсерлесуі жоғары -110 кДж/моль – -117 кДж/моль аралығын құрайды. Гомологиялық анализ адам miRNA-мен толықтай ұқсас 140 кандидат eca-miRNA-ді идентификациялап берді.

Түйін сөздер: miRNA, mRNA, сүт, xeno-miRNA, Eqqus caballus.

М.О. Мырзабекова[1,2]*, З. Лабейт[3], Р.Е. Ниязова[1]
[1]Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы
[2]Казахский Национальный Аграрный Исследовательский Университет, Казахстан, г. Алматы
[3]Хайделберг Университет, Германия, г. Хайделберг
*e-mail: moldir.myrzabek@gmail.com

In silico идентификация miRNA *Eqqus caballus* с потенциальным воздействием на экспрессию генов человека

Существуют miRNA, которые не кодируются геномом человека, но обнаруживаются в организме. Их называют xeno-miRNA. xeno-miRNA у людей были обнаружены из различных экзогенных источников. Целью данной работы является идентификация xeno-miRNA из Eqqus caballus (домашняя лошадь или вкратце eca), аналоги которой могут связываться с генами человека. Связывание miRNA с mRNA генов человека предсказывали с помощью программы MirTarget. Гомологи eca-miRNA были идентифицированы с помощью свободно доступного онлайн-биоинформатического метода miRviewer. Было идентифицировано 15 eca-miRNA, взаимодействующих с генами mRNA человека с высокой комплементарностью, с ΔG/ΔGm равной 98-100%. Выявлены характеристики взаимодействия всех известных eca-miRNA с mRNA генов человека. Общее количество сайтов связывания для 469 miRNA составляет 1605, из которых 907 находятся в CDS, 451 – в 3'UTR и 247 – в 5'UTR. 93 miRNA имеют по одному гену-мишени, 63 miRNA имеют по два гена-мишени, 67 miRNA имеют от трех до четырех генов-мишеней, а 72 miRNA имеют пять и более генов-мишеней. Свободная энергия взаимодействия рассматриваемых miRNA с mRNA генов человека высока и варьирует от -110 кДж / моль до -117 кДж / моль. Анализ гомологии выявил 140 кандидатных eca-miRNA, которые оказались полностью идентичными последовательностям miRNA человека.

Ключевые слова: miRNA, mRNA, молоко, xeno-miRNA, Eqqus caballus.

Abbreviations

miRNA – microRNA;
xeno-miRNA – xenomiRs;
NF – kB transcription factor,
IL2 – interleukin 2,
Hsa – Homo sapiens.

Introduction

MicroRNAs are defined as short, typically approximately 22 nucleotide-long noncoding RNAs. Mechanistically, they are generated by the action of ribonuclease (RNAse) III Dicer activity on precursor transcripts [1, 2]. The Dicer complex's miRNAs then play an important part in the posttranscriptional control of gene expression: complementary messenger RNAs are degraded or have their translational suppression mediated by the so-called Ago-complex (mRNAs), repression can be complete or partial [1, 3].

Plants and mammals both have miRNAs. Overall, they have been predicted to control up to 60% of all human genes [4], thereby modulating virtually every aspect of human physiology and health, including cancer [5], brain development [6], fat storage [7], hematopoiesis, or immunity [8, 9, 10].

Certain presently less characterized miRNA subclasses may resist degradation in the gastro-intestinal tract and then be dietary taken up and further distributed by the body circulation. These miRNAs have been termed as xenomiRs. XenomiRs have been found in a variety of exogenous sources, including both human and animals sources [11]. The suspected main route of entry into human is by the diet when animal products are ingested. Exosomes containing miRNA from bovine milk, for example, have been found to penetrate the circulatory system of humans [12].

Milk is an important biological nutrient, it's the only option diet for babies that ensures their development and health in the short and long term [13, 14].

From an evolutionary perspective it is remarkable that fresh milk has been consumed for thousands of years in Central Asia and neighbouring regions. Herodotus in the 5th century BC described how Scythians used to consume it [15]. Therefore, it is conceivable that since the neolithicum cross-species co-evolutionary adaptions may have occured between eca and human for example.

High levels of miRNAs are detected in secreted body fluids including serum, urine, saliva, seminal fluid, and milk [16, 17]. Milk-derived miRNAs are also secreted by the lactating mammary epithelial cell in exosomes, which are microvesicles of ~30–100 nm that are packaged and secreted into extracellular fluids [18]. Recently, there has been

much interest in milk-derived miRNAs as potential regulators of the neonatal gastrointestinal and immune systems [19]. MiRNAs in milk from a variety of sources animals have been detected in the same way as other bodily fluids [20, 21] milk microRNAs have paved the way for prognostic, diagnostic, and functional investigations [22], because milk is the biological fluid with the largest concentration of miRNAs [23].

Materials and Methods

The nucleotide sequences of the 17508 mRNAs of targeted genes were downloaded from NCBI GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of the miRNAs were taken from miRBase v.22 (http://www.mirbase.org/). 690 miRNAs encoded by the *Equus caballus* genome are available in the miRBase database. The miRNA BSs in the mRNAs of several genes were predicted using the MirTarget program [24, 25]. This program defines the following features of miRNA binding to mRNA: a) the initiation of the miRNA binding to the mRNAs from the first nucleotide of the mRNAs; b) the localization of the miRNA BSs in the 5′-untranslated region (5′UTR), coding domain sequence (CDS), and 3′-untranslated region (3′UTR) of the mRNAs; c) the schemes of nucleotide interactions between miRNAs and mRNAs d) the free energy of the interaction between miRNA and the mRNA (ΔG, kj/mole); and the ratio ΔG/ΔGm (%) is determined for each site (ΔGm equals the free energy of the miRNA binding with its fully complementary nucleotide sequence). The MirTarget program finds hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The free energy of interactions (ΔG) a pair of G and C is equal to 6.37 kj/mole, a pair of A and U is equal to 4.25 kj/mole, G and U, A and C equal to 2.12 kj/mole [26]. The distances between the bound A and C (1.04 nm) and G and U (1.02 nm) are similar to those between bound G and C, A and U, which are equal to 1.03 nm [27, 28, 29]. The numbers of hydrogen bonds in the G–C, A–U, G–U, and A–C interactions were 3, 2, 1, and 1, respectively. By comparison, MirTarget differs from other programs in terms of finding the BSs of miRNA on the mRNAs of plant genes [30] in that 1) it takes into account the interaction of the miRNA with mRNA over the entire miRNA sequence; 2) it takes into account non-canonical pairs G–U and A–C; and 3) it calculates the free energy of the interaction of the miRNA with mRNA, and when two or more miRNAs are bound with one mRNA or, if the BSs of two different miRNAs coincide in part, the preferred miRNA binding site is considered to be the one for which the free binding energy is greater. The adequacy of the program in terms of finding BSs has been confirmed in several publications [31, 32, 33, 34]. The MirTarget program predicts the BSs of plant and animal miRNAs equally welln [35, 36]. The horse homologs of 140 miRs were identified by using miRviewer online bioinformatic tools which defined the homolog miRNAs by integrating their molecular and structural properties with the results of the BLAST program [37].

Results and Discussion

Characteristics of interactions of eca-miRNAs with human mRNA genes with high complementarity

The table 1 shown the nucleotide sequences of eca-miR-135a, eca-miR-8915, eca-miR-151-5p, eca-miR-1905c, eca-miR-127, eca-miR-136, eca-miR-431, eca-miR-432, eca-miR-433, eca-miR-1282 and eca-miR-9046 BSs that are fully complementary to 10 mRNAs of human genes. The *RTL1* gene with ΔG/ΔGm=100 % with absolute conservative previously shown earlier [34]. Single miRNA eca-miR-1905a has 99% of complementarity; other miRNAs eca-miR-1905b, eca-miR-8910 and eca-miR-196a have 98% of complementarity. Mainly BSs are located in the CDS, four mRNAs have BSs in the 3'UTR and the free energy of interaction of miRNAs with mRNAs of these genes ranges from -110 to -140 kj/mole. Seven miRNAs were identical to human miRNAs.

The interaction of nucleotides of miRNAs and mRNAs of target genes show how effectively these molecules bind. The creation of hydrogen bonds between all of the nucleotides of miRNAs and their binding sites in mRNAs is illustrates in Figure 1.

Characteristics of the interaction of eca-miRNAs with mRNA of human genes

The total number of 690 miRNAs of *Equus caballus* are known in mirBase. Of the 690 known horse miRNAs 469 had BSs with human genes. All miRNAs were searched for 17 508 human target genes. The 907 BSs are located in CDS, 451 BSs are located in 3'UTR and 247 BSs in 5'UTR.

Table 1 – Characteristics of interactions of eca-miRNAs and human mRNA genes with high complementarity

Gene	eca-miRNA	Start of site, nt	Region of miRNA	ΔG, kl/mole	ΔG/ΔGm, %	Length, nt
GLYCTK	eca-miR-135a*	2812	3'UTR	-113	100	23
H2AFX	eca-miR-8915	398	CDS	-136	100	24
H2AFJ	eca-miR-8915	460	CDS	-136	100	24
HIST1H2AJ	eca-miR-8915	325	CDS	-136	100	24
LPPR5	eca-miR-151-5p*	1328	3'UTR	-113	100	21
LYPD3	eca-miR-151-5p*	1608	3'UTR	-113	100	21
MEX3A	eca-miR-1905c	693	CDS	-144	100	25
RTL1	eca-miR-127*	1792	CDS	-121	100	22
RTL1	eca-miR-136	111	CDS	-110	100	22
RTL1	eca-miR-431	3800	CDS	-127	100	23
RTL1	eca-miR-432*	330	CDS	-123	100	23
RTL1	eca-miR-433*	2878	CDS	-119	100	22
SERF2	eca-miR-1282*	1072	CDS	-102	100	20
KBTBD13	eca-miR-9046	1144	CDS	-140	100	25
MEX3B	eca-miR-1905a	942	CDS	-140	99	25
LRFN2	eca-miR-8910	1984	CDS	-134	98	25
MEX3A	eca-miR-1905b	693	CDS	-123	98	22
HOXB8	eca-miR-196a*	1378	3'UTR	-110	98	22
*Identical miRNAs with human						

Gene, miRNA, start of site, region, ΔG, ΔG/ΔGm, nt	Gene, miRNA, start of site, region, ΔG, ΔG/ΔGm, nt
GLYCTK, eca-miR-135a, 2812, 3'UTR, -112, 100, 23 5′ – UCACAUAGGAAUAAAAAGCCAUA – 3′ 3′ – AGUGUAUCCUUAUUUUUCGGUAU – 5′	*RTL1*, eca-miR-127, 1792, CDS, -121, 100, 22 5′ – AGCCAAGCUCAGACGGAUCCGA – 3′ 3′ – UCGGUUCGAGUCUGCCUAGGCU – 5′
H2AFX, eca-miR-8915, 398, CDS, -136, 100, 24 5′ – CUGCCCAACAUCCAGGCCGUGCUG – 3′ 3′ – GACGGGUUGUAGGUCCGGCACGAC – 5′	*RTL1*, eca-miR-136, 111, CDS, -110, 100, 22 5′ – CCAUCAUCAAAACAAAUGGAGU – 3′ 3′ – GGUAGUAGUUUUGUUUACCUCA – 5′
H2AFJ, eca-miR-8915, 460, CDS, -136, 100, 24 5′ – CUGCCCAACAUCCAGGCCGUGCUG – 3′ 3′ – GACGGGUUGUAGGUCCGGCACGAC – 5′	*RTL1*, eca-miR-431, 3800, CDS, -127, 100, 23 5′ – CCUGCAUGACGGCCUGCAAGACA – 3′ 3′ – GGACGUACUGCCGGACGUUCUGU – 5′
HIST1H2AJ, eca-miR-8915, 325, CDS, -136, 100, 24 5′ – CUGCCCAACAUCCAGGCCGUGCUG – 3′ 3′ – GACGGGUUGUAGGUCCGGCACGAC – 5′	*RTL1*, eca-miR-432, 330, CDS, -123, 100, 23 5′ – CCACCCAAUGACCUACUCCAAGA – 3′ 3′ – GGUGGGUUACUGGAUGAGGUUCU – 5′
LPPR5, eca-miR-151-5p, 1328, 3'UTR, -112, 100, 21 5′ – ACUAGACUGUGAGCUCCUCGA – 3′ 3′ – UGAUCUGACACUCGAGGAGCU – 5′	*RTL1*, eca-miR-433, 2878, CDS, -119, 100, 22 5′ – ACACCGAGGAGCCCAUCAUGAU – 3′ 3′ – UGUGGCUCCUCGGGUAGUACUA – 5′
LYPD3, eca-miR-151-5p, 1608, 3'UTR, -112, 100, 21 5′ – ACUAGACUGUGAGCUCCUCGA – 3′ 3′ – UGAUCUGACACUCGAGGAGCU – 5′	*SERF2*, eca-miR-1282, 1072, CDS, -102, 100, 20 5′ – AAGCAGAAAAAGGCAAACGA – 3′ 3′ – UUCGUCUUUUUCCGUUUGCU – 5′
MEX3A, eca-miR-1905c, 693, CDS, -144, 100, 25 5′ – CUACCGCGUGGGUGGGGCUGGUGGUG – 3′ 3′ – GAUGGCGCACCACCCCGACCACCAC – 5′	*KBTBD13*, eca-miR-9046, 1144, CDS, -140, 100, 25 5′ – CUGCCCGGCCAGUUCGUCAACAGCA – 3′ 3′ – GACGGGCCGGUCAAGCAGUUGUCGU – 5′

Figure 1 – Shemes of the interaction of nucleotide sequences of eca-miRNAs and mRNA human genes

A total number of 93 miRNAs with one-target genes were identified, in this group there were several miR-3p/mir-5p pairs: eca-miR-340-3p and eca-miR-340-5p, eca-miR-508-3p and eca-miR-508-5p that originating from the same-pre-miRNA, have binding sites in mRNAs of different genes. We arranged the data into several groups: in the group of 63 miRNAs with two target genes, there were eca-miR-146b-3p and eca-miR-146-5p pairs that originated from same pre-miRNA (Table 2).

MiRNAs with three and four genes have a total of 126 target genes. A total of 67 miRNAs with three to four target genes were identified. There are 229 target genes for 67 miRNAs in total. The miRNAs number with five and more genes is 72, miRNAs with the most number of target genes are eca-miR-8989 (65 genes), eca-miR-9159 (48 genes), eca-miR-1892 (36 genes), and eca-miR-9164 (24 genes), eca-miR-324-3p (23 genes), eca-miR-326 (20 genes). The total number of target genes for 72 miRNAs is 815. Eca-miR-345-3p and eca-miR-345-5p origin same pre-miRNA. As a result, these miRNAs could drastically alter the metabolism of recipient human cells at high concentrations.

Table 2 shows the characteristics of the binding of some eca-miRNAs to mRNAs of human genes. Each of the 15 miRNAs bind to mRNAs of one target gene, eca-miR-124 has one target gene, eca-miR-1193 has two target genes, and eca-miR-107a has three target genes with a value ΔG/ΔGm of 89-91 %. Eca-miR-145 has four, eca-miR-296 five target genes and eca-miR-346-5p six target genes with a value ΔG/ΔGm of 89-91%. The free energy of miRNA interactions with these genes' mRNAs ranged from -110 kj/mole to -117 kj/mole. 20 target genes are associated with eca-miR-326 with value ΔG/ΔGm equal to 89-94%. The miRNAs eca-miR-146b-3p and eca-miR-146b-5p bind in CDS, 3'UTR, 5'UTR and related NF-kB signaling in innate immune responses [38].

Table 2 – Characteristics of interaction eca-miRNAs with mRNAs of human genes

Gene	eca-miRNA	Start of site, nt	Region of miRNA	ΔG, kl/mole	ΔG/ΔGm %	Length, nt
ADAMTS7	eca-miR-107a	1460	CDS	-110	91	23
APOLD1	eca-miR-107a	656	CDS	-108	89	23
IGHMBP2	eca-miR-107a	365	CDS	-108	89	23
MGRN1	eca-miR-124	4003	3'UTR	-102	92	20
SNX24	eca-miR-145	951	3'UTR	-110	90	23
ARNTL	eca-miR-145	1867	CDS	-110	90	23
PRICKLE4	eca-miR-145	1024	CDS	-113	91	23
WWOX	eca-miR-145	1154	CDS	-110	90	23
CLDND1	eca-miR-146b-3p	345	5'UTR	-110	91	22
MIS12	eca-miR-146b-3p	606	5'UTR	-110	91	22
NCKAP5	eca-miR-146b-5p	2404	CDS	-102	91	22
MOB3C	eca-miR-146b-5p	1002	3'UTR	-102	91	22
ESAM	eca-miR-296	1553	3'UTR	-110	90	22
TMEM198	eca-miR-296	1644	3'UTR	-110	90	22
RNF214	eca-miR-296	483	CDS	-110	90	22
ZNF250	eca-miR-296	983	CDS	-110	90	22
ZNF598	eca-miR-296	1806	CDS	-110	90	22
POLR3A	eca-miR-340-3p	2716	CDS	-106	89	23
NCOA7	eca-miR-340-5p	6034	3'UTR	-100	94	22
HBZ	eca-miR-345-3p	490	3'UTR	-110	90	22
SCRT1	eca-miR-345-3p	2699	3'UTR	-110	90	22
TCL1B	eca-miR-345-3p	817	3'UTR	-110	90	22

Table continuation

Gene	eca-miRNA	Start of site, nt	Region of miRNA	ΔG, kl/mole	ΔG/ΔGm %	Length, nt
C9orf131	eca-miR-345-3p	2291	CDS	-113	91	22
HRC	eca-miR-345-3p	1491	CDS	-110	90	22
ITGA7	eca-miR-345-3p	2007	CDS	-110	90	22
NOS1	eca-miR-345-3p	1246	CDS	-110	90	22
PRKCG	eca-miR-345-3p	2247	CDS	-110	90	22
PRRT3	eca-miR-345-3p	1339	CDS	-110	90	22
PTCHD2	eca-miR-345-3p	292	CDS	-110	90	22
ELFN2	eca-miR-346-5p	6433	3'UTR	-115	89	23
DNAJC5	eca-miR-346-5p	194	5'UTR	-115	89	23
NFIA	eca-miR-346-5p	127	5'UTR	-115	89	23
CAMSAP2	eca-miR-346-5p	97	5'UTR	-117	90	23
TENM4	eca-miR-346-5p	35	5'UTR	-117	90	23
BAI2	eca-miR-346-5p	2168	CDS	-117	90	23
RYBP	eca-miR-508-3p	823	CDS	-104	89	23
RNASEL	eca-miR-508-5p	1550	CDS	-108	89	23
EML4	eca-miR-1193	143	5'UTR	-100	90	21
SOWAHB	eca-miR-1193	1911	CDS	-100	90	21
APEH	eca-miR-326	725	CDS	-110	91	21
ATG7	eca-miR-326	2928	3'UTR	-113	93	21
ATXN1	eca-miR-326	3622	3'UTR	-110	91	21
AZI1	eca-miR-326	3287	CDS	-110	91	21
C2orf54	eca-miR-326	2250	3'UTR	-113	93	21
EHD3	eca-miR-326	637	CDS	-110	91	21
FGF23	eca-miR-326	852	CDS	-113	93	21
FZD10	eca-miR-326	2236	3'UTR	-113	93	21
GIGYF1	eca-miR-326	4902	3'UTR	-110	91	21
HEMK1	eca-miR-326	703	CDS	-115	95	21
KIFC2	eca-miR-326	1707	CDS	-110	91	21
KRBA1	eca-miR-326	950	CDS	-113	93	21
LOXHD1	eca-miR-326	2727	CDS	-110	91	21
LRRN4CL	eca-miR-326	977	CDS	-113	93	21
MAP1A	eca-miR-326	8687	CDS	-110	91	21
MYL9	eca-miR-326	876	3'UTR	-110	91	21
RASSF1	eca-miR-326	1280	3'UTR	-110	91	21
SLC22A17	eca-miR-326	1570	CDS	-110	91	21
STYK1	eca-miR-326	1100	CDS	-110	91	21
TNS1	eca-miR-326	8712	3'UTR	-110	91	21
CLDND1	eca-miR-146b-3p	345	5'UTR	-110	91	22
MIS12	eca-miR-146b-3p	606	5'UTR	-110	91	22
MOB3C	eca-miR-146b-5p	1002	3'UTR	-102	91	22
NCKAP5	eca-miR-146b-5p	2404	CDS	-102	91	22

Characteristics of the interaction of Human homologs eca miRNAs with mRNA of human genes

In our bioinformatics analyses, we identified human homolog miRNA candidates in *Equus cabalus* miRNAs. The miRviewer and miRBase program searched homologs of this miRNAs. Our homology analyses revealed 140 miRNAs candidates shown to be 100% similar to human miRNA sequence. The list of 140 miRNAs in this study given in table 3.

Table 3 – *Equus caballus* homologs miRNAs with human miRNAs

Horse miRNAs
eca-let-7d, eca-let-7e, eca-let-7g, eca-miR-103, eca-miR-105, eca-miR-106b, eca-miR-107b, eca-miR-1185, eca-miR-122, eca-miR-125a-3p, eca-miR-125a-5p, eca-miR-125b-5p, eca-miR-1264, eca-miR-127, eca-miR-128, eca-miR-1289, eca-miR-1291a, eca-miR-1296, eca-miR-1298, eca-miR-129a-5p, eca-miR-129b-5p, eca-miR-130a, eca-miR-130b, eca-miR-132, eca-miR-133a, eca-miR-133b, eca-miR-135a, eca-miR-135b, **eca-miR-141**, eca-miR-145, **eca-miR-146a, eca-miR-148a**, eca-miR-148b-3p, eca-miR-149, eca-miR-151-5p, eca-miR-15b, eca-miR-183, eca-miR-186, eca-miR-187, eca-miR-188-3p, eca-miR-188-5p, eca-miR-18a, **eca-miR-191a**, eca-miR-192, eca-miR-193b, eca-miR-194, eca-miR-196a, eca-miR-196b, eca-miR-197, eca-miR-199a-5p, eca-miR-199b-5p, eca-miR-199b-3p, eca-miR-19a, eca-miR-19b, eca-miR-200b, eca-miR-205, eca-miR-20a, eca-miR-20b, **eca-miR-21**, eca-miR-212, eca-miR-214, eca-miR-216a, eca-miR-216b, eca-miR-217, eca-miR-221, eca-miR-222**, eca-miR-223**, eca-miR-23a, eca-miR-27a, **eca-miR-27b**, eca-miR-28-3p, eca-miR-28-5p, **eca-miR-29a**, **eca-miR-29b**, eca-miR-301a, eca-miR-301b-3p, eca-miR-302b, **eca-miR-30c, eca-miR-30d**, eca-miR-30e, eca-miR-31, eca-miR-323-3p, eca-miR-328, eca-miR-330, eca-miR-331, eca-miR-335, eca-miR-33b, eca-miR-346, eca-miR-34c, eca-miR-361-5p, eca-miR-370, eca-miR-376c, eca-miR-379, eca-miR-381, eca-miR-383, eca-miR-423-3p, eca-miR-423-5p, eca-miR-424, eca-miR-432, eca-miR-433, eca-miR-448, eca-miR-450a, eca-miR-454, eca-miR-485-3p, eca-miR-485-5p, eca-miR-486-5p, eca-miR-487b, eca-miR-490-5p, eca-miR-491-3p, eca-miR-491-5p, eca-miR-492, eca-miR-495, eca-miR-496, eca-miR-497, eca-miR-499-3p, eca-miR-499-5p, eca-miR-500, eca-miR-502-3p, eca-miR-502-5p, eca-miR-505, eca-miR-509-5p, eca-miR-551a, eca-miR-551b, eca-miR-582-5p, eca-miR-598, eca-miR-628a, eca-miR-652, eca-miR-671-3p, eca-miR-671-5p, eca-miR-708, eca-miR-711, eca-miR-761, eca-miR-873, eca-miR-874, eca-miR-876-5p, eca-miR-92a, eca-miR-92b, eca-miR-93, eca-miR-99b, eca-miR-99a
Human miRNAs
hsa-let-7d-5p, hsa-let-7e-5p, hsa-let-7g-5p, hsa-miR-103a-3p, hsa-miR-105-5p, hsa-miR-106b-5p, hsa-miR-107, hsa-miR-1185-5p, hsa-miR-122-5p, hsa-miR-125a-3p, hsa-miR-125a-5p, hsa-miR-125b-5p, hsa-miR-1264, hsa-miR-127-3p, hsa-miR-128-3p, hsa-miR-1289, hsa-miR-1291, hsa-miR-1296-5p, hsa-miR-1298-5p, hsa-miR-129-5p, hsa-miR-129-1-3p, hsa-miR-130a-3p, hsa-miR-130b-3p, hsa-miR-132-3p, hsa-miR-133a-3p, hsa-miR-133b, hsa-miR-135a-5p, hsa-miR-135b-5p, **hsa-miR-141-3p**, hsa-miR-145-5p, **hsa-miR-146a-5p**, **hsa-miR-148a-3p**, hsa-miR-148b-3p, hsa-miR-149-5p, hsa-miR-151a-5p, hsa-miR-15b-5p, hsa-miR-183-5p, hsa-miR-186-5p, hsa-miR-187-3p, hsa-miR-188-3p, hsa-miR-188-5p, hsa-miR-18a-5p, **hsa-miR-191-5p**, hsa-miR-192-5p, hsa-miR-193b-3p, hsa-miR-194-5p, hsa-miR-196a-5p, hsa-miR-196b-5p, hsa-miR-197-3p, hsa-miR-199a-5p, hsa-miR-199b-5p, hsa-miR-199a-3p, hsa-miR-19a-3p, hsa-miR-19b-3p, hsa-miR-200b-3p, hsa-miR-205-5p, hsa-miR-20a-5p, hsa-miR-20b-5p, **hsa-miR-21-5p**, hsa-miR-212-3p, hsa-miR-214-3p, hsa-miR-216a-5p, hsa-miR-216b-5p, hsa-miR-217-5p, hsa-miR-221-3p, hsa-miR-222-3p, **hsa-miR-223-3p**, hsa-miR-23a-3p, hsa-miR-27a-3p, **hsa-miR-27b-3p**, hsa-miR-28-3p, hsa-miR-28-5p, **hsa-miR-29a-3p, hsa-miR-29b-3p**, hsa-miR-301a-3p, hsa-miR-301b-3p, hsa-miR-302b-3p, **hsa-miR-30c-5p**, **hsa-miR-30d-5p**, hsa-miR-30e-5p, hsa-miR-31-5p, hsa-miR-323a-3p, hsa-miR-328-3p, hsa-miR-330-5p, hsa-miR-331-3p, hsa-miR-335-5p, hsa-miR-33b-5p, hsa-miR-346, hsa-miR-34c-5p, hsa-miR-361-5p, hsa-miR-370-3p, hsa-miR-376c-3p, hsa-miR-379-5p, hsa-miR-381-3p, hsa-miR-383-5p, hsa-miR-423-3p, hsa-miR-423-5p, hsa-miR-424-5p, hsa-miR-432-5p, hsa-miR-433-3p, hsa-miR-448, hsa-miR-450a-5p, hsa-miR-454-3p, hsa-miR-485-3p, hsa-miR-485-5p, hsa-miR-486-5p, hsa-miR-487b-3p, hsa-miR-490-5p, hsa-miR-491-3p, hsa-miR-491-5p, hsa-miR-492, hsa-miR-495-3p, hsa-miR-496, hsa-miR-497-5p, hsa-miR-499a-3p, hsa-miR-499a-5p, hsa-mir-500a, hsa-miR-502-3p, hsa-miR-502-5p, hsa-miR-505-3p, hsa-miR-509-5p, hsa-miR-551a, hsa-miR-551b-3p, hsa-miR-582-5p, hsa-miR-598-3p, hsa-miR-628-5p, hsa-miR-652-3p, hsa-miR-671-3p, hsa-miR-671-5p, hsa-miR-708-5p, hsa-miR-711, hsa-miR-761, hsa-miR-873-5p, hsa-miR-874-3p, hsa-miR-876-5p, hsa-miR-92a-3p, hsa-miR-92b-3p, hsa-miR-93-3p, hsa-miR-99b-5p, hsa-miR-99a-5p.
hsa-let-7d-5p, hsa-let-7e-5p, hsa-let-7g-5p, hsa-miR-141-3p, **hsa-miR-146a-5p**, **hsa-miR-148a-3p**, **hsa-miR-191-5p, hsa-miR-21-5p, hsa-miR-223-3p, hsa-miR-27b-3p, hsa-miR-29a-3p, hsa-miR-29b-3p, hsa-miR-30c-5p, hsa-miR-30d-5p –** associated with pathological and immune responses

The number of target genes for homolog miRNAs *Equus caballus* is 342. Total of 369 BSs mainly of them (239) are located in the CDS, 98 BSs in the 3'UTR, 32 BSs in the 5'UTR. MiRNAs bind one to 39 target genes. The miRNAs with the largest number of target genes are eca-miR-671-5p (39 genes), eca-miR-151-5p (11 genes).

Eca-miR-125a-3p and eca-miR-125a-5p; eca-miR-188-3p and eca-miR-188-5p; eca-miR-199b-3p and eca-miR-199b-5p; eca-miR-28-3p and eca-miR-28-5p; eca-miR-423-3p and eca-miR-423-5p; eca-miR-502-3p and eca-miR-502-5p; eca-miR-671-3p and eca-miR-671-5p, originating from the same pre-miRNA, have binding sites in the mRNAs of several genes.

Identical eca-miR-127, eca-miR-135a, eca-miR-196a, eca-miR-432, eca-miR-433 are fully complementary to mRNAs of *RTL1, GLYCTK, HOXB8* genes (Table 1). The *RTL1* gene binds with -123 kj/mole.

Table 4 shown similar sequence of some eca-miRNAs, all of 140 eca-miRNAs nucleotide sequence were searched. The free energy of the miRNAs with the mRNAs of these genes chosen higher -100 kj/mole.

We found that the major miRNAs identified in eca largely homolog with those reported in human milk. This suggests a conserved evolutionary a procedure that causes certain milk miRNAs to be released [39, 40, 41, 42, 22, 43].

Animal and human miRNAs have been identified to be homologous. Mir-155, which may be found in milk from both bovine and human sources, was sequenced and found to have a high proportion of sequence similarity. As a result, the existence of such similar miRNAs may result in observable physiological reactions. Similar sequence similarities have also been found in miR-21-5p and miR30a-5p sequences in human and bovine samples [44].

Let-7 family members such as let-7a-5p, let-7b-5p, and let7f, as well as miR-148a, have been demonstrated to decrease the immunological response by influencing the transcription factor NFB (Table 5) were found to be conserved in humans, cows, pigs, and pandas, indicating that it plays an important role in immune modulation [42].

Table 4 – Homolog sequence of eca and hsa miRNAs

miRNA	Sequence
eca-miR-141	UAACACUGUCUGGUAAAGAUGG
hsa-miR-141-3p	UAACACUGUCUGGUAAAGAUGG
eca-miR-146a	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU
eca-miR-148a	UCAGUGCACUACAGAACUUUGU
hsa-miR-148a-3p	UCAGUGCACUACAGAACUUUGU
eca-miR-191a	CAACGGAAUCCCAAAAGCAGCUG
hsa-miR-191-5p	CAACGGAAUCCCAAAAGCAGCUG
eca-miR-223	UGUCAGUUUGUCAAAUACCCCA
hsa-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA
eca-miR-29a	UAGCACCAUCUGAAAUCGGUUA
hsa-miR-29a-3p	UAGCACCAUCUGAAAUCGGUUA
eca-miR-29b	UAGCACCAUUUGAAAUCAGUGUU
hsa-miR-29b-3p	UAGCACCAUUUGAAAUCAGUGUU

Table 5 – Immune response of miRNAs expressed during lactation period

miRNA	Immune response	References
miR-148a-3p	Targets cancer-related (TGIF-2) and drug metabolism-related PXR genes, making it a potential biomarker for milk quality control.	[45], [46], [47]
miR-30d-5p	downstream target DRP1 promote cellular invasion and immunosuppression by direct targeting of GALNT7, increased synthesis of immunosuppressive cytokine IL-10	[48]
miR-30c-2-5p	Involved in oncogenesis and mmunosuppression	[49]
miR-191-5p	Colorectal cancer biomarker, primary effusion lymphoma biomarker, hepatocellular carcinoma biomarker	[50, 51, 52]
miR-21-5p	TLR4 inhibition by targeting the tumor suppressor PDCD4 Modulation of IL-12	[53]
miR-27b-3p	Destabilization of lipopolysaccharide-mediated PPAR mRNA abundance, linked to chronic inflammatory disorders	[54]
miR-146b-5p	Innate immunological responses and NF-kB signaling	[38]

MiR223 has been found to function as a monocyte differentiation factor and to have a role in granulocyte proliferation and activation [55, 56], miR146a is primarily involved in NF-kB-mediated inflammatory responses as well as type 1 interferon generation and signaling [57].

Other miRNAs linked to pathological and immune responses include miR-29a-3p (target interferon, suppresses immune response to intracellular pathogens), miR-141-3p (biomarker for colon cancer), and hsa-miR-223, whose targets tend to be T and granulocyte cell populations, and thus affect the developmental stage of adaptive immune response in infants [58].

The expression of hsa-miR-191-5p and the number of CD4+ T-cells were identified. miR-29b found in bovine milk tends to target the runt-related transcription factor-2 (RUNX2) [58]. Immune-related miRNAs in human breast milk contains exosomal vesicles. In mammals, miRNAs and their potential role in interindividual communication has been suggested after the demonstration of their presence in both cow and human breast milk [44].

A recently published bioinformatics analysis based on homology modelling between animal and human miRNA tried to address this issue by analyzing also the probability that xenomiR might be transported accross cell membranes, thereby augmenting their potential to regulate human mRNAs [59]. In the last years, there has been a steady growth in interest in milk miRNAs, accompanied by a persistent debate about the bioactivity of cross-species or inter-individual microRNA transfer through the diet. Certain miRNAs that are codified by non-human genomes might be ingested and then might be presented to human cells in the blood stream by circulation

Conclusion

The obtained results indicate that *Equus caballus* have a large number of similar miRNAs with human miRNAs. The results also revealed that a single miRNA could bind to one to several mRNA target genes. In addition to the identical with human miRNAs, eca-miRNAs were found to have high complementarity with human mRNA genes. Binding sites miRNAs with high complementarity to mRNA genes were located in the CDS region.

Conflict of interest

The authors have declared no conflict of interest

References

1 Ambros V. The functions of animal microRNAs // Nature. – 2004. – Vol. 431, No. 7006, – P. 350–355.

2 Provost P., Dishart D., Doucet J., Frendewey D., Samuelsson B., Radmark O. Ribonuclease activity and RNA binding of recombinant human dicer //The EMBO Journal. – 2002. – Vol. 21. No. 21. – P. 5864–5874

3 Meister G. Argonaute proteins: Functional insights and emerging roles // Nature Reviews Genetics. 2013.- Vol. 14. No.7. – P. 447–459.

4 Bartel D. P. MicroRNAs: Target recognition and regulatory functions // Cell. – 2009. – Vol. 136. No. 2. – P. 215–233.

5 Feng Y., Li N., Ma H., Bei B., Han Y., Chen G. Undescribed phenylethyl flavones isolated from patrinia villosa show cytoprotective properties via the modulation of the mir-144-3p/nrf2 pathway // Phytochemistry. – 2018. – Vol. 153. – P. 28–35.

6 Meza-Sosa K. F., Pedraza-Alva G., Perez-Martinez L. MicroRNAs: Key triggers of neuronal cell fate // Frontiers in Cellular Neuroscience, – 2014. – Vol. 8. No.175.

7 Mysore R., Zhou Y., Sadevirta S., Savolainen-Peltonen H., Nidhina Haridas P. A., Soronen J., Olkkonen V. M. MicroRNA-192 impairs adipocyte triglyceride storage // Biochimica et Biophysica Acta, – 2016. – Vol. 1861. No. 4. – P. 342–351.

8 Benmoussa A., Lee C.H., Laffont B., Savard P., Laugier J., Boilard E., Provost P. Commercial dairy cow milk microRNAs resist digestion under simulated gastrointestinal tract conditions // Journal of Nutrition, -2016. -Vol. 146, No. 11. -P. 2206–2215.

9 Bissels U., Bosio A., Wagner W. MicroRNAs are shaping the hematopoietic landscape // Haematologica, -2012. – Vol. 97, No. 2. – P. 160–167.

10 O'Connell R.M., Rao D.S., Chaudhuri A.A., Baltimore D. Physiological and pathological roles for microRNAs in the immune system // Nature Reviews Immunology, – 2010. – Vol.10, No.2. – P. 111–122

11 Li Z., Xu R., Li N. MicroRNAs from plants to animals, do they define a new messenger for communication? // Nutr. Metab. – 2018. – Vol.15, No.68. doi: 10.1186/s12986-018-0311-x.

12 Izumi H., Tsuda M., Sato Y., Kosaka N., Ochiya T., Iwamoto H., Takeda Y. Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages // Journal of Dairy Science, – 2015. – Vol. 98, No. 5. – P. 2920–2933.

13 Andreas N.J., Kampmann B., Mehring Le-Doare K. Human breast milk: A review on its composition and bioactivity // Early Human Development, – 2015. – Vol. 91. No.11. – P. 629–635.

14 Le Doare K., Holder B., Bassett A., Pannaraj P.S. Mother's milk: A purposeful contribution to the development of the infant microbiota and immunity // Frontiers in Immunology. – 2018. – Vol. 9. No. 361.

15 West S. Introducing the Scythians: Herodotus on koumiss // Museum Helveticim. – 1999. – Vol.56. – P.76-86

16 Cui J., Zhou B., Ross S.A., Zempleni J. Nutrition, microRNAs, and Human Health // Adv. Nutr. – 2017. – Vol. 8. – P.105–112. doi: 10.3945/an.116.013839.

17 Kosaka N., Izumi H., Sekine K., Ochiya T. MicroRNA as a new immune-regulatory agent in breast milk // Silence.- 2010. – Vol.1, No.7, doi: 10.1186/1758-907X-1-7.

18 Alsaweed M. Lai C.T., Hartmann P.E., Geddes D.T., Kakulas F. Human milk cells contain numerous miRNAs that may change with milk removal and regulate multiple physiological processes // Intern. J. Molec. Sci. – 2016. – Vol.17. No. 956. doi: 10.3390/ijms17060956

19 Zempleni J., Aguilar-Lozano A., Sadri M., Sukreet S., Manca S., Wu D., Mutai E. Biological activities of extracellular vesicles and their cargos from bovine and human milk in humans and implications for infants // Journal of Nutrition. – 2017. – Vol.147. No.1. – P. 3–10

20 Melnik B. C. Milk: An epigenetic amplifier of FTO-mediated transcription? Implications for western diseases // Journal of Translational Medicine. – 2015a. – Vol.13. No. 385.

21 Melnik B. C., Schmitz G. MicroRNAs: Milk's epigenetic regulators // Best Practice & Research: Clinical Endocrinology & Metabolism. – 2017a – Vol.31. No. 4. – P. 427–442.

22 Zempleni J., Kusuma R. J., Manca S., Friemel T., Sukreet S., Nguyen C. Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis // American Journal of Physiology-Cell Physiology. – 2016. – Vol.310. No. 10. – P.800–807.

23 Weber J. A., Baxter D. H., Zhang S., Huang D. Y., Huang K. H., Lee M. J., Wang K. The microRNA spectrum in 12 body fluids // Clinical Chemistry. – 2010. – Vol. 56, No. 11. – P. 1733–1741.

24 Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva S. MiR-3960 binding sites with mRNA of human genes // Bioinformation. – 2014. – Vol. 10. – P. 423–427. doi: 10.6026/97320630010423.

25 Ivashchenko A.T., Pyrkova A.Y., Niyazova R.Y., Alybayeva A., Baskakov K. Prediction of miRNA binding sites in mRNA // Bioinformation 12. – 2016. – P. 237–240.

26 Friedman R. A., Honig B. A free energy analysis of nucleic acid base stacking in aqueous solution // Biophys. J. – 1995. – Vol. 69. No. 4. – P. 1528–1535. doi:10.1016/S0006-3495(95)80023-8.

27 Garg A., Heinemann U. A novel form of RNA double helix based on G•U and C•A+ wobble base pairing // RNA. – 2018. – Vol. 24. – P. 209–218. doi: 10.1261/rna.064048.117

28 Kool E.T. Hydrogen bonding, base stacking, and steric effects in DNA replication //Annu. Rev. Biophys. Biomol.Struct. – 2001. – Vol. 30. – P. 1–22. doi: 10.1146/annurev.biophys.30.1.1

29 Leontis N.B., Stombaugh J., Westhof E. The non-Watson-Crick base pairs and their 641 associated isostericity matrices // Nucleic Acids Res. – 2002. – Vol. 30. – P. 3497–3531. doi: 10.1093/nar/gkf481.

30 Dai X., Zhuang Z., Zhao P. Computational analysis of miRNA targets in plants: current status and challenges // Brief. Bioinformatics. – 2011. – Vol.12. – P. 115-121.doi1093/bb/bbq065

31 Davis E., Caiment F., Tordoir X., Cavaillé J., Ferguson-Smith A., Cockett N. RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus // Curr. Biol. – 2005. – Vol. 15. – P. 743–749. doi: 10.1016/j.cub.2005.02.060

32 Wang J., Li Z., Liu B., Chen G., Shao N., Ying X. Systematic study of cis696 antisense miRNAs in animal species reveals miR-3661 to target PPP2CA in human cells // RNA. – 2016a. – Vol. 22. – P. 87–95. doi: 10.1261/rna.052894.115.

33 Wang Y., Li L., Tang Sh., Liu J., Zhang H., Zhi H. Combined small RNA and degradome sequencing to identify miRNAs and their targets in response to drought in foxtail millet // BMC Genomics 17:57. – 2016b. doi: 10.1186/ s12863-016-0364-7

34 Yurikova O.Y., Aisina D.E., Niyazova R.E., Atambayeva S.A., Labeit S., Ivashchenko A.T. The interactions of miRNA-5p and miRNA-3p with the mRNAs of Ortolologous Genes // Mol. Biol. – 2019. – Vol. 53. no. 4. – P. 692-704. doi: 10.1134/S0026898419040189

35 Bari A., Orazova S., Ivashchenko A. miR156- and miR171-binding sites in the protein coding sequences of several plant genes // Biomed Res. Int. – 2013. – Vol. 1. No. 7. doi: 10.1155/2013/307145.

36 Bari A., Sagaidak I., Pinskii I., Orazova S., Ivashchenko A. Binding of miR396 to mRNA of genes encoding growth regulating transcription factors of plants // Russ. J. Plant Physiol. – 2014. – Vol. 61. – P. 807–810. doi:10.1134/S1021443714050033

37 Kiezun A., Artzi S., Modai S., Volk N., Isakov O., Shomron N. miRviewer: a multispecies microRNA homologous viewer // BMC Res Notes.- 2012. – Vol. 5. – P. 92–97.

38 Perri M., Lucente M., Cannataro R., De Luca I.F., Gallelli L., Moro G. Variation in immune- related microRNAs profile in human milk amongst lactating women // Microrna. – 2018. – Vol. 7. – P. 107 – 14. doi: 10.2174/2211536607666180206150503

39 Melnik B.C. Milk disrupts p53 and DNMT1, the guardians of the genome: Implications for acne vulgaris and prostate cancer // Nutrition & Metabolism (London). – 2017. – Vol. 14. No. 55.

40 Melnik B. C., Kakulas F., Geddes D. T., Hartmann P. E., John S. M., Carrera-Bastos P., Schmitz G. Milk miRNAs: Simple nutrients or systemic functional regulators? // Nutrition & Metabolism (London). – 2016. -Vol.13. No. 42.

41 Melnik B. C., Schmitz G. MicroRNAs: Milk's epigenetic regulators // Best Practice & Research: Clinical Endocrinology & Metabolism. – 2017a. – Vol. 31. No. 4. – P. 427–442.

42 Van Herwijnen M.J.C, Driedonks T.A.P., Snoek B.L., Kroon A.M.T., Kleinjan M. Abundantly present miRNAs in milk-derived extracellular vesicles are conserved between mammals // Front Nutr. – 2018. – Vol. 5. No. 81. doi: 10.3389/fnut.2018.00081

43 Zempleni J., Baier S. R., Howard K. M., Cui J. Gene regulation by dietary microRNAs // Canadian Journal of Physiology and Pharmacology. – 2015b. – Vol. 93. No. 12. – P. 1097–1102.

44 Fromm B., Tosar J. P., Lu Y., Halushka M. K., Witwer K.W. Human and cow have identical miR-21–5p and miR-30a-5p sequences, which are likely unsuited to study dietary uptake from cow milk // J. Nutr. – 2018. -Vol. 148. – P. 1506 –7. doi: 10.1093/jn/nxy144.

45 Lujambio A., Calin G.A., Villanueva A., Ropero S., Sanchez-Cespedes M., Blanco D. A microRNA DNA methylation signature for human cancer metastasis // Proc. Natl. Acad. Sci. USA. – 2008. – Vol. 105. No. 13556–61. doi: 10.1073/pnas.0803055105

46 Takagi S., Nakajima M., Mohri T., Yokoi T. Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4 // J. Biol. Chem. – 2008. – Vol. 283. doi: 10.1074/jbc.M709382200

47 Chen X., Gao C., Li H., Huang L., Sun Q., Dong Y. Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products // Cell Res. – 2010. -Vol. 20. – P. 1128–37. doi: 10.1038/cr.2010.80

48 Stittrich A.B., Haftmann C, Sgouroudis E, Kuhl A.A., Hegazy A.N., Panse I. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes // Nat. Immunol. – 2010. -Vol. 11. – P. 1057–62. doi: 10.1038/ni.1945

49 Gaziel-Sovran A., Segura M.F., Di Micco R., Collins M.K., Hanniford D., VegaSaenz De Miera E. miR-30b/30d regulation of GalNAc transferases enhances invasion and immunosuppression during metastasis // Cancer Cell. – 2011. – Vol. 20. – P. 104–18. doi: 10.1016/j.ccr.2011.05.027 43.

50 Xi Y., Formentini A., Chien M., Weir D. B., Russo J.J., Ju J. Prognostic values of microRNAs in colorectal cancer // Biomark Insights. – 2006. – Vol. 2. – P. 113–21. doi: 10.1177/117727190600100009 48.

51 O'hara A.J., Vahrson W., Dittmer D.P. Gene alteration and precursor and mature microRNA transcription changes contribute to the miRNA signature of primary effusion lymphoma // Blood. -2008. – Vol. 111. – P. 2347–53. doi: 10.1182/blood-2007-08-104463 49.

52 Elyakim E., Sitbon E., Faerman A., Tabak S., Montia E., Belanis L. hsamiR-191 is a candidate oncogene target for hepatocellular carcinoma therapy // Cancer Res. -2010. – Vol. 70. – P. 8077–87. doi: 10.1158/0008-5472.CAN-10-1313

53 Sheedy F.J., Palsson-Mcdermott E., Hennessy E.J., Martin C., O'leary J.J., Ruan Q. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21 // Nat. Immunol. – 2010. – Vol.11. – P.141–7. doi: 10.1038/ni.1828

54 Jennewein C., Von Knethen A., Schmid T., Brune B. MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor gamma. (PPARgamma) mRNA destabilization // J. Biol. Chem. -2010. -Vol. 285. -P.11846–53. doi: 10.1074/jbc.M109.066399.

55 Johnnidis J. B., Harris M.H., Wheeler R.T., Stehling-Sun S., Lam M.H., Kirak O. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223 // Nature. – 2008. – Vol. 451: – P. 1125–9. doi: 10.1038/nature06607

56 Haneklaus M., Gerlic M., O'neill L.A., Masters S.L. miR-223: infection, inflammation and cancer // J. Intern. Med. – 2013. 274:215–26. doi: 10.1111/joim.12099

57 Nahid M.A., Pauley K. M., Satoh M., Chan E.K. miR-146a is critical for endotoxininduced tolerance: implication in innate immunity // J. Biol. Chem. – 2009. – Vol. 284. – P. 34590–9. doi: 10.1074/jbc.M109.056317

58 Baier S.R., Nguyen C., Xie F., Wood J.R., Zempleni J. MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK293 kidney cell cultures, and mouse livers // J. Nutr. – 2014. – Vol. 144. – P. 1495–500. doi: 10.3945/jn.114.196436

59 Shu J., Chiang K., Zempleni J., Cui J. Computational characterization of exogenous microRNAs that can be transferred into human circulation // PLoS One. – 2015. – Vol.10

References

1 Andreas N.J., Kampmann B., Mehring Le-Doare K. (2015) Human breast milk: A review on its composition and bioactivity. Early Human Development, vol. 91, no.11, pp. 629–635.

2 Alsaweed M. Lai C.T., Hartmann P.E., Geddes D.T., Kakulas F. (2016) Human milk cells contain numerous miRNAs that may change with milk removal and regulate multiple physiological processes. Intern. J. Molec. Sci., vol.17, no. 956. doi: 10.3390/ijms17060956

3 Ambros V. (2004) The functions of animal microRNAs. Nature, vol. 431, no. 7006, pp. 350–355.

4 Bartel D.P. (2009) MicroRNAs: Target recognition and regulatory functions, Cell, vol. 136, no. 2, pp. 215–233.

5 Benmoussa A., Lee C. H., Laffont B., Savard P., Laugier J., Boilard E., Provost P. (2016) Commercial dairy cow milk microRNAs resist digestion under simulated gastrointestinal tract conditions, Journal of Nutrition, vol. 146, no. 11, pp. 2206–2215.

6 Bissels U., Bosio A., Wagner W. (2012) MicroRNAs are shaping the hematopoietic landscape. Haematologica, vol. 97, no. 2, pp. 160–167.

7 Bari A., Orazova S., Ivashchenko A. (2013) miR156- and miR171-binding sites in the protein coding sequences of several plant genes. Biomed Res. Int., vol. 1, no. 7. doi: 10.1155/2013/307145.

8 Bari A., Sagaidak I., Pinskii I., Orazova S., Ivashchenko A. (2014) Binding of miR396 to mRNA of genes encoding growth regulating transcription factors of plants. Russ. J. Plant Physiol., vol. 61, pp. 807–810. doi:10.1134/S1021443714050033

9 Baier S.R., Nguyen C., Xie F., Wood J.R., Zempleni J. (2014) MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow milk and affect gene expression in peripheral blood mononuclear cells, HEK293 kidney cell cultures, and mouse livers. J. Nutr., vol.144, pp. 1495–500. doi: 10.3945/jn.114.196436

10 Cui J., Zhou B., Ross S.A., Zempleni J. (2017). Nutrition, microRNAs, and Human Health. Adv. Nutr., vol. 8, pp.105–112. doi: 10.3945/an.116.013839.

11 Chen X., Gao C., Li H., Huang L., Sun Q., Dong Y. (2010) Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. Cell Res. vol. 20, pp. 1128–37. doi: 10.1038/cr.2010.80

12 Dai X., Zhuang Z., Zhao P. (2011) Computational analysis of miRNA targets in plants: current status and challenges. Brief. Bioinformatics vol.12, pp.115-121.doi1093/bb/bbq065

13 Davis E., Caiment F., Tordoir X., Cavaillé J., Ferguson-Smith A., Cockett N. (2005). RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. Curr. Biol., vol.15, pp. 743–749. doi: 10.1016/j.cub.2005.02.060

14 Elyakim E., Sitbon E., Faerman A., Tabak S., Montia E., Belanis L. (2010) has-miR-191 is a candidate oncogene target for hepatocellular carcinoma therapy. Cancer Res. vol.70, pp.8077–87. doi: 10.1158/0008-5472.CAN-10-1313

15 Feng Y., Li N., Ma H., Bei B., Han Y., Chen G. (2018). Undescribed phenylethyl flavones isolated from patrinia villosa show cytoprotective properties via the modulation of the mir-144-3p/nrf2 pathway. Phytochemistry, vol. 153, pp. 28–35.

16 Fromm B., Tosar J.P., Lu Y., Halushka M.K., Witwer K.W. (2018) Human and cow have identical miR-21–5p and miR-30a-5p sequences, which are likely unsuited to study dietary uptake from cow milk. J. Nutr. vol. 148, pp. 1506–7. doi: 10.1093/jn/nxy144.

17 Friedman R.A., Honig B. (1995) A free energy analysis of nucleic acid base stacking in aqueous solution. Biophys. J. vol. 69, no.4, pp. 1528–1535. doi:10.1016/S0006-3495(95)80023-8.

18 Garg A., Heinemann U. (2018) A novel form of RNA double helix based on G•U and C•A+ wobble base pairing. RNA. vol. 24, pp.209–218. doi: 10.1261/rna.064048.117

19 Gaziel-Sovran A., Segura M.F., Di Micco R., Collins M.K., Hanniford D., VegaSaenz De Miera E. (2011) miR-30b/30d regulation of GalNAc transferases enhances invasion and immunosuppression during metastasis. Cancer Cell. 20:104–18. doi: 10.1016/j.ccr.2011.05.027 43.

20 Haneklaus M., Gerlic M., O'neill L.A., Masters S.L. miR-223: infection, inflammation and cancer. J. Intern. Med. (2013) 274:215–26. doi: 10.1111/joim.12099

21 Stittrich A.B., Haftmann C, Sgouroudis E, Kuhl A.A., Hegazy A.N., Panse I. The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. (2010) Nat. Immunol. vol.11, pp.1057–62. doi: 10.1038/ni.1945

22 Ivashchenko A., Berillo O., Pyrkova A., Niyazova R., Atambayeva S. (2014) MiR-3960 binding sites with mRNA of human genes. Bioinformation. vol. 10, pp.423–427. doi: 10.6026/97320630010423.

23 Ivashchenko A.T., Pyrkova A.Y., Niyazova R.Y., Alybayeva A., Baskakov K. (2016). Prediction of miRNA binding sites in mRNA. Bioinformation 12, pp.237–240.

24 Izumi H., Tsuda M., Sato Y., Kosaka N., Ochiya T., Iwamoto H., Takeda Y. (2015) Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. Journal of Dairy Science, vol. 98, no. 5, pp. 2920–2933.

25 Jennewein C., Von Knethen A., Schmid T., Brune B. (2010) MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor gamma. (PPARgamma) mRNA destabilization. J. Biol. Chem. vol.285, pp.11846–53. doi: 10.1074/jbc.M109.066399.

26 Johnnidis J.B., Harris M.H., Wheeler R.T., Stehling-Sun S., Lam M.H., Kirak O. (2008) Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature. vol.451:pp. 1125–9. doi: 10.1038/nature06607

27 Kosaka N., Izumi H., Sekine K., Ochiya T. (2010) MicroRNA as a new immune-regulatory agent in breast milk. Silence. vol.1, no.7, doi: 10.1186/1758-907X-1-7.

28 Kool E.T. (2001) Hydrogen bonding, base stacking, and steric effects in DNA replication. Annu. Rev. Biophys. Biomol. Struct. vol.30, pp.1–22. doi: 10.1146/annurev.biophys.30.1.1

29 Kiezun A., Artzi S., Modai S., Volk N., Isakov O., Shomron N. (2012) miRviewer: a multispecies microRNA homologous viewer. BMC Res Notes., vol.5, pp.92–97.

30 Lujambio A., Calin G.A., Villanueva A., Ropero S., Sanchez-Cespedes M., Blanco D. (2008) A microRNA DNA methylation signature for human cancer metastasis. Proc. Natl. Acad. Sci. USA. vol.105, no.13556–61. doi: 10.1073/pnas.0803055105

31 Li Z., Xu R., Li N. (2018) MicroRNAs from plants to animals, do they define a new messenger for communication? Nutr. Metab. vol.15, no.68. doi: 10.1186/s12986-018-0311-x.

32 Leontis N.B., Stombaugh J., Westhof E. (2002) The non-Watson-Crick base pairs and their 641 associated isostericity matrices.Nucleic Acids Res. vol.30, pp.3497–3531. doi: 10.1093/nar/gkf481.

33 Meister G. (2013) Argonaute proteins: Functional insights and emerging roles. Nature Reviews Genetics, vol.14, no.7, pp. 447–459.

34 Meza-Sosa K.F., Pedraza-Alva G., Perez-Martinez L. (2014) MicroRNAs: Key triggers of neuronal cell fate. Frontiers in Cellular Neuroscience, vol. 8, no.175.

35 Melnik B.C. (2015a) Milk: An epigenetic amplifier of FTO-mediated transcription? Implications for western diseases. Journal of Translational Medicine, vol.13, no. 385.

36 Melnik B.C., Kakulas F., Geddes D.T., Hartmann P.E., John S.M., Carrera-Bastos P., Schmitz G. (2016) Milk miRNAs: Simple nutrients or systemic functional regulators? Nutrition & Metabolism (London), vol.13, no. 42

37 Melnik B.C., John S.M., Carrera-Bastos P., Schmitz G. (2016a) Milk: A postnatal imprinting system stabilizing FOXP3 expression and regulatory T cell differentiation. Clinical and Translational Allergy, vol. 6, no.18. https://doi. org/10.1186/s13601-016-0108-9

38 Melnik B.C. (2017) Milk disrupts p53 and DNMT1, the guardians of the genome: Implications for acne vulgaris and prostate cancer. Nutrition & Metabolism (London), vol.14, no. 55.

39 Melnik B.C., Schmitz G. (2017a) MicroRNAs: Milk's epigenetic regulators. Best Practice & Research: Clinical Endocrinology & Metabolism, vol.31, no.4, pp. 427–442.

40 Mysore R., Zhou Y., Sadevirta S., Savolainen-Peltonen H., Nidhina Haridas P. A., Soronen J., Olkkonen V. M. (2016). MicroRNA-192 impairs adipocyte triglyceride storage. Biochimica et Biophysica Acta, vol. 1861, no.4, pp. 342–351

41 Nahid M.A., Pauley K.M., Satoh M., Chan E.K. (2009) miR-146a is critical for endotoxininduced tolerance: implication in innate immunity. J. Biol. Chem. vol. 284, pp. 34590–9. doi: 10.1074/jbc.M109.056317

42 O'Connell R. M., Rao D.S., Chaudhuri A. A., Baltimore D. (2010) Physiological and pathological roles for microRNAs in the immune system. Nature Reviews Immunology, vol.10, no.2, pp. 111–122

43 O'hara A.J., Vahrson W., Dittmer D.P. (2008) Gene alteration and precursor and mature microRNA transcription changes contribute to the miRNA signature of primary effusion lymphoma. Blood. vol. 111, pp. 2347–53. doi: 10.1182/blood-2007-08-104463 49.

44 Provost P., Dishart D., Doucet J., Frendewey D., Samuelsson B., Radmark O. (2002) Ribonuclease activity and RNA binding of recombinant human dicer. The EMBO Journal, vol. 21, no.21, pp. 5864–5874.

45 Perri M., Lucente M., Cannataro R., De Luca I.F., Gallelli L., Moro G. (2018) Variation in immune- related microRNAs profile in human milk amongst lactating women. Microrna. vol.7, pp.107 – 14. doi: 10.2174/2211536607666180206150503

46 Shu J., Chiang K., Zempleni J., Cui J. (2015) Computational characterization of exogenous microRNAs that can be transferred into human circulation. PLoS One. vol.10

47 Stittrich A.B., Haftmann C., Sgouroudis E., Kuhl A. A., Hegazy A. N., Panse I. (2010) The microRNA miR-182 is induced by IL-2 and promotes clonal expansion of activated helper T lymphocytes. Nat. Immunol. vol.11, pp.1057– 62. doi: 10.1038/ni.1945

48 Sheedy F.J., Palsson-Mcdermott E., Hennessy E.J., Martin C., O'leary J.J., Ruan Q. (2010) Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat. Immunol, vol.11, pp.141–7. doi: 10.1038/ni.1828

49 Takagi S., Nakajima M., Mohri T., Yokoi T. (2008) Post-transcriptional regulation of human pregnane X receptor by microRNA affects the expression of cytochrome P450 3A4. J. Biol. Chem. vol. 283, doi: 10.1074/jbc.M709382200

50 Van Herwijnen M.J.C, Driedonks T.A.P., Snoek B.L., Kroon A.M.T., Kleinjan M. (2018) Abundantly present miRNAs in milk-derived extracellular vesicles are conserved between mammals. Front Nutr, vol.5, no.81. doi: 10.3389/fnut.2018.00081

51 Wang J., Li Z., Liu B., Chen G., Shao N., Ying X. (2016a) Systematic study of cis696 antisense miRNAs in animal species reveals miR-3661 to target PPP2CA in human cells. RNA vol.22, pp.87–95. doi: 10.1261/rna.052894.115.

52 Wang Y., Li L., Tang Sh., Liu J., Zhang H., Zhi H. (2016b). Combined small RNA and degradome sequencing to identify miRNAs and their targets in response to drought in foxtail millet.BMC Genomics 17:57. doi: 10.1186/ s12863-016-0364-7

53 Weber J.A., Baxter D.H., Zhang S., Huang D.Y., Huang K.H., Lee M.J., Wang K. (2010) The microRNA spectrum in 12 body fluids. Clinical Chemistry, vol. 56, no. 11, pp. 1733–1741.

54 West S. (1999) Introducing the Scythians: Herodotus on koumiss. Museum Helveticim.vol.56, pp.76-86.

55 Xi Y., Formentini A., Chien M., Weir D. B., Russo J.J., Ju J. (2006) Prognostic values of microRNAs in colorectal cancer. Biomark Insights, vol. 2, pp.113–21. doi: 10.1177/117727190600100009 48.

56 Yurikova O.Y., Aisina D.E., Niyazova R.E., Atambayeva S.A., Labeit S., Ivashchenko A.T. (2019) The interactions of miRNA-5p and miRNA-3p with the mRNAs of Ortolologous Genes Mol. Biol. vol.53, no.4, pp. 692-704. doi: 10.1134/ S0026898419040189

57 Zempleni J., Kusuma R. J., Manca S., Friemel T., Sukreet S., Nguyen C. (2016) Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis. American Journal of Physiology-Cell Physiology, vol.310, no.10, pp.800–807.

58 Zempleni J., Aguilar-Lozano A., Sadri M., Sukreet S., Manca S., Wu D., Mutai E. (2017) Biological activities of extracellular vesicles and their cargos from bovine and human milk in humans and implications for infants. Journal of Nutrition, vol.147, no.1, pp. 3–10.

59 Zempleni J., Baier S.R., Howard K. M., Cui J. (2015b) Gene regulation by dietary microRNAs. Canadian Journal of Physiology and Pharmacology, vol. 93, no.12, pp.1097–1102.