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SPECIAL SESSION ON CAMEL GENOMIC

BINDING SITES OF MiRNAs WITH TRANSCRIPTION FACTORS’ GENES OF CAMELUS FERUS

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Abstract
We searched binding sites of miRNAs in mRNAs of 157 transcription factors’ genes of wild camel (Camelus ferus). The mRNAs of 96 genes of zinc finger transcription factors’ family have 16, 210 and 34 binding sites in the 5’UTRs, CDSs and 3’UTRs, respectively. The mRNA of GLI2 gene has binding sites for eight miRNAs. The mRNAs of GLIS1 and ZNF236 genes contain seven binding sites. In the 3’UTR mRNA of ZFP91 gene were revealed 13 miR-574-5p binding sites arranged located through two nucleotides. The ΔG/ΔGm value is equal to 93%. miR-1322 has one binding site in GLI1, HINFP, HIVEP1, MTF1, SALL4, SP1, ZNF335 and ZNF451 genes, two sites in ZNF142, three sites in EGR1 gene. mRNA of VEZF1 gene has eight miR-1322 binding sites located arranged located through three nucleotides. miRNAs with the length of 25 and 26 nucleotides have the highest binding energy. The ΔG value varied from -114,6 kJ/mole to -138,0 kJ/mole. Some miRNAs with a length of 23 and 24 nucleotides also have a high value ΔG varied from -112,5 kJ/mole to -129,5 kJ/mole. The results show a strong interaction between the expression of genes of transcription factors and miRNAs.

Key words: transcription factor, miRNA, mRNA, camel.

miRNA FAMILIES CAMELUS FERUS TRANSCRIPTION FACTORS’ GENES BINDING SITES

Біз жабайы түр Camelus ferus отбасы транскрипционалық факторларының 157 гендерінің міР-нақ - міР-ның байланысы саїттарын іңдестірдік. Цинк фингер тұқымдаларының 96 транскрипционалық факторлар міР-ның 5'UTR-де 16 саїт, CDS-те 210 саїт және 3'UTR-де 34 саїтта орналасқан. GLI2 ғенінің міР-мың сегіз міР-ның байланысы саїттар бар. GLIS1 ғені ZNF236 міР-нақ міР-ның 16 жеті байланысы саїттары бар. ZFP91 ғена міР-ның 3'UTR міR-574-5p байланыстырының 13 саїтар бар, олар 2 нуклеотидтен кейін орналасқан. ΔG/ΔGm дарек сүйініс 93% төң болды. miR-1322-н ғені ZL11, HINFP, HIVEP1, MTF1, SALL4, SP1, ZNF335 жана ZNF451 ның гендерінің міР-нақ бар, ZNF142-да екі, EGR1 ғенінің уші байланысы саїттар бар. VEZF1 ғена міР-нақ міР-1322 байланыстырының ретімен 3 нуклеотидтен кейін орналасқан сегіз байланысы саїттар бар. ΔG мөлшері -114,6 кДж/моль-дан -138,0 кДж/моль дейін өзгеріп отырды. Қейіріз 23 және 24 нуклеотидтерден тұратын ΔG мөлшері -114,6 кДж/моль-дан -129,5 кДж/моль дейін өзгеріп отырды. Альнан Camelus ferus Zinc finger тұқымдастық транскрипционалық факторларының гендерінің міР-мың, міР-ның байланысы саїттарының сипаттамасы өзгердік, қелешіліктін биосинтезі міР және етісші міР-ның байланысы өзгерді – жетілдірді.

Туйын сөздер: транскрипционалық факторлар, міР-нақ, mRNA, түйе

САЙТЫ СВЯЗЫВАНИЯ MIrNA С ГЕНОМAMI ТРАНСКРИПЦИОНАЛЬНЫХ ФАКТОРОВ CAMELUS FERUS

Проведен поиск сайтов связывания miР-нақ в mRNA 157 геном транскрипционных факторов дикого верблюда (Camelus ferus). В mRNA 96 геном транскрипционных факторов семейства цинк фингер найдены 16, 210 и 34 сайтов связывания в 5'UTR, CDS и 3'UTR соответственно. mRNA генов GLI2 имела сайты связывания для восьми miР-нақ. mRNA генов GLIS1 и ZNF236 содержали по семь сайтов связывания міР. В 3'UTR mRNA гена ZFP91 выявлено 13 сайтов связывания с міР-574-5p, которые упорядоченно располагались через 2 нуклеотиды. Величина ΔG/ΔGm равнялась 93%. miR-1322 связывалось с одним сайтом в генах GLI1, HINFP, HIVEP1, MTF1, SALL4, SP1, ZNF335 и ZNF451, два сайта в ZNF142, три сайта в EGR1. mRNA гена VEZF1 имела восьмь последовательно расположенных через три нуклеотида сайтов связывания miR-1322. Наибольшую энергию связывания имели miRNAs с длиной 25 и 26 нуклеотидов. Величина ΔG изменялась от -114,6 kJ/mole до -138,0 kJ/mole. Некоторые miRNAs 3-4 нуклеотиды тоже имели высокое значение ΔG, которое изменялось от -112,5 kJ/mole до -129,5 kJ/mole. Полученные данные показывают сильную зависимость экспрессии генов транскрипционных факторов от miRNAs.

Ключевые слова: транскрипционные факторы, miР-нақ, mRNA, верблюď

Introduction
The expression of most eukaryotic genes is dependent on TFs. They play an important role in the regulation of key processes in cells. Furthermore, the expression of many genes is regulated by the binding of miRNAs to mRNAs. MiRNAs comprise a class of non-coding RNAs, which play a key role in the regulation of gene expression. MiRNAs are involved in many biological processes, including cell cycle, apoptosis, differentiation, development of skeletal muscle, immune reactions, responses to stress, and others. Since the synthesis of TFs may also be regulated by miRNAs, it is important to establish miRNAs, which can inhibit the synthesis of TFs. We identified the characteristics of binding miRNAs with miRNAs
of ZNF family genes, the most numerous family of TFs. Our objects of study were the ZNF family genes of wild camel, because these animals are different from other mammals, amazingly adapted to abnormal environmental conditions and many stress factors (Kaczensky et al., 2014).

Materials and methods

The nucleotide sequences of mRNAs of 157 TF genes within the ZNF family of Camelus ferus were taken from GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of 2563 Homo sapiens miRNAs were taken from the miRBase database (http://mirbase.org). We used human miRNAs because camel miRNAs have not yet been identified. The search of miRNAs binding sites in the target genes mRNAs was performed using MiRTarget (Ivashchenko et al., 2014). This program defines: the beginning of binding sites of miRNAs with mRNAs; the location of sites in the 5’-untranslated region (5’UTR), a protein-coding portion (CDS) and the 3’-untranslated region (3’UTR) of mRNA; the free energy of hybridization (ΔG, kJ/mole) and patterns of interaction miRNAs nucleotides with mRNAs. We expected value of ΔG/ΔGm (%), where ΔGm is the free energy of miRNA binding with fully complementary nucleotide sequence. Binding sites of miRNAs with mRNAs selected with the value of ΔG/ΔGm equal or more than 90%. The position of the binding sites was shown from the first nucleotide (n.) of mRNA.

Results and discussions

We searched the binding sites of 2563 Homo sapiens miRNAs with the mRNA of 157 Camelus ferus ZNF family TF genes. In this paper, we present data on miRNAs binding to mRNAs of 32 Camelus ferus ZNF orthologous human ZNF. We predicted two binding sites in the 5’UTRs, 38 sites in CDSs and one site in the 3’UTR of 32 mRNAs of Camelus ferus TF genes. The value of ΔG/ΔGm, characterizing the degree of affinity of miRNA to mRNA, varied from 90 to 96%. The mRNA of GLIS1 Glis Family Zinc Finger 1) gene has binding sites for miR-3142, miR-4417 and miR-7515. The mRNA of ZNF423 gene has binding sites for miR-143-3r, miR-3155b and miR-4481. Thus, the expression of genes GLIS1 and ZNF423 is under strong control of miRNAs (Table 1). The protein GLIS1 contains transactivation and repressor functions. The ZNF423 gene may play multiple roles in signal transduction during development. Two miRNAs bind with mRNAs of GLI2, HIVEP2, MTF1, TRERF1 and WIZ genes. The TRERF1 gene encodes a transcriptional regulating protein which regulates the CYP11A1 gene (Gizard et al, 2005). The WIZ gene is conserved in chimpanzee, dog, cow, mouse, rat and chicken. This gene contains a nuclear localization signal from the critical region for velo-cardio-facial syndrome (Nomura et al., 1991).

Table 1. Characteristics of miRNAs binding sites with mRNAs of Camelus ferus ZNF family transcription factors localized in CDSs, 5’UTRs (*) and 3’UTRs (**)  

<table>
<thead>
<tr>
<th>Gene</th>
<th>miRNA</th>
<th>Position</th>
<th>ΔG/ΔGm</th>
<th>Gene</th>
<th>miRNA</th>
<th>Position</th>
<th>ΔG/ΔGm</th>
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<tbody>
<tr>
<td>E4F1</td>
<td>mir-8616-3p</td>
<td>1037</td>
<td>92</td>
<td>SP6</td>
<td>mir-7162-3p</td>
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<tr>
<td>EGR1</td>
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<td>454</td>
<td>933</td>
<td>TRERF1</td>
<td>mir-1273f</td>
<td>1661</td>
<td>90</td>
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<tr>
<td>EGR4</td>
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<td>92</td>
<td>TRERF1</td>
<td>mir-3960</td>
<td>1669</td>
<td>93</td>
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<td>GFI1</td>
<td>mir-4481</td>
<td>43</td>
<td>93</td>
<td>WIZ</td>
<td>mir-1260a</td>
<td>182</td>
<td>94</td>
</tr>
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<td>GLI2</td>
<td>mir-669</td>
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<td>93</td>
<td>WIZ</td>
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<td>92</td>
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<td>GLI2</td>
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<td>ZFPM1</td>
<td>mir-1306-3p</td>
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<td>GLIS1</td>
<td>mir-3142</td>
<td>1405</td>
<td>91</td>
<td>ZKSCAN4</td>
<td>mir-6828-3p</td>
<td>899</td>
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<td>GLIS1</td>
<td>mir-4417</td>
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<td>90</td>
<td>ZNF142</td>
<td>mir-6132</td>
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<td>GLIS1</td>
<td>mir-7515</td>
<td>1922</td>
<td>91</td>
<td>ZNF143</td>
<td>mir-4313</td>
<td>25**</td>
<td>93</td>
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<td>HINFP</td>
<td>mir-877-3p</td>
<td>146</td>
<td>93</td>
<td>ZNF212</td>
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<td>HIVEP2</td>
<td>mir-1260b</td>
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<td>HIVEP2</td>
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<td>KLF15</td>
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<td>KLF4</td>
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<td>ZNF423</td>
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<td>2918</td>
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<td>KLF9</td>
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<td>ZNF423</td>
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<td>5158</td>
<td>93</td>
<td>ZNF536</td>
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</table>

The HIVEP2 gene encoded protein regulates transcription by binding to regulatory regions of various cellular and viral genes that may involve in growth, development and metastasis of HIV-EP2 (Nomura et al., 1995). The protein encoded by MECOM gene is a transcriptional regulator and oncoprotein that may be involved in hematopoiesis, apoptosis, development, and cell differentiation and proliferation (Bard-Chapeau et al., 2013). The KLF9 is a circadian transcription factor in human epitherins that controls proliferation of keratinocytes (Spörli et al., 2012). The overexpression of ZKSCAN4 in different cell lines also inhibits the transcriptional activities of the tumor protein p53 and the cyclin-dependent kinase inhibitor p21 (Li et al., 2007).

References
GENETIC STRUCTURE AND VARIABILITY IN ALGERIAN DROMEDARY CAMELS ASSESSED BY MICROSATELITE MARKERS

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Abstract

Despite dromedary camels represent an important economic resource in Algeria, knowledge on their genetic diversity and structure is still very poor. Here we contributed to fill this gap by characterizing a total of 198 Algerian camels from 7 sampling areas across the country using 20 STR markers. Nineteen loci were polymorphic (excluding the locus CMS17), with an average of 8.7 ± 5.4 alleles. The average observed heterozygosity was 0.60 ± 0.17, the average expected heterozygosity was 0.64 ± 0.19; four loci deviated significantly from Hardy-Weinberg proportions due to excess of homozygous genotypes. Only a mild significant (P≤0.01) “linkage” disequilibrium was observed in the analysed sample. No clear genetic structure was detected using STRUCTURE. On the contrary, a meaningful stratification was observed when using DAPC, with samples from Adrar and Tamanrasset very well differentiated from all the others, samples from Tindouf well differentiated, though less distant, from the remaining two closer and less differentiated samples (Bechar and Centre). The Neighbor-Net Network analysis confirmed the results obtained using DAPC. The observed results seem to reflect the geographical location of the considered sampling areas. In fact, the region including the areas of Centre (Naama and El bayadh), Bechar and Tindouf represents the western extreme border of Algeria (likely more influenced by gene flow from Morocco and Mauritania); on the contrary, the two southern regions of Adrar and Tamanrasset are more likely to have been influenced by gene flow from Mali and Mali/Niger, respectively.

Keywords: Microsatellites, genetic diversity, Camelus dromedarius, Algeria.