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Characteristics of miRNA binding sites in mRNAS of human and mouse titin gene

Abstract: We have studied characteristics of miRNA (microRNAs) binding sites in mRNAs (matrix RNAs) of human, primate and mouse titin gene. miRNAs are small non-coding RNAs with the length about 21-22 nucleotides binding with mRNAs of genes and blocking or disturbing their translation. Titin is the largest protein of heart muscle tissue that is a base of myofibril. Defects of titin synthesis lead to malfunction of muscle tissue, for example, to the heart failure which is one of the widest reasons of the death in the world. We have found differences and similarities of characteristics of miRNA binding sites in human and mouse titin gene mRNAs. The differences are the following: different number of binding sites, different values of binding energy and different nucleotide sequences of orthologous human and mouse miRNAs. The similarities are concluded in that all of these sites are located in protein-coding part of mRNA and they all have particular complementarity. But changing some nucleotides can help to get artificial miRNAs with ideal complementarity and maximal effect on expression. We have noticed that characteristics of miRNA binding sites in mRNAs of titin gene between different species of primates are more similar than between human and mouse. It can be explained by different evolutionary distance between these species. So the model of miRNA regulation of mouse titin synthesis is not completely adequate for human titin gene, but weakness of miRNA interaction with mRNA of mouse titin gene can be compensated by increasing of miRNA concentration in relation to mRNA.

Key words: miRNA, mRNA, binding, sites, titin, gene, human, primates, mouse.

Introduction

Titin is a protein of muscle tissue. It is the largest protein in the nature and plays enormous role in providing elasticity and structural integrity of sarcomers [1]. For example, the longest transcript variant of human titin gene encodes 35991 amino acids and includes all 363 exons of this gene. Disturbance of titin synthesis causes the development of serious cardiovascular diseases, such as dilated cardiomyopathy, heart failure, ischemic heart disease, myocardial infarction and etc. Different titin isoforms are synthesized in various types of muscle tissue (heart, smooth and skeletal striated muscle tissue) and are encoded by different combinations of exons [2].

Recently miRNA binding with mRNAs of different genes, participating in the development of cardiovascular diseases, was studied [3-4]. For example, it was proved, that high level of miR-208b expression leads to cardiac hypertrophy in titin-based dilated cardiomyopathy [5]. But characteristics of miRNA interactions with mRNAs of titin gene were not studied. So it was important to establish what kind of miRNAs bind with mRNAs of titin gene? Since miRNA binding sites in mRNAs of orthologous genes can differ it is necessary to study characteristics of miRNA binding with mRNAs of orthologous genes, especially with mRNAs of human and mouse titin gene because mouse is used in experimental research. So it was important to compare characteristics of miRNA binding sites in mRNAs of titin gene. It is possible that differences can be observed in miRNA binding with mRNAs of different titin isoforms.

Materials and methods

Materials of research are titin mRNA nucleotide sequences of *Homo sapiens, Pan troglodytes, Pongo abelii, Macaca fascicularis, Papio anubis, Pan paniscus, Colobus angolensis, Chlorocebus sabaeus, Rhinopithecus roxellana, Callithrix jacchus, Aotus nancymaae*, *Saimiri boliviensis*, *Gorilla gorilla, Nomascus leucogenys* and *Mus musculus*. These sequences were taken from the Genbank (*https://www.*

ncbi.nlm.nih.gov/genbank/). Nucleotide sequences of 2568 miRNAs were taken from the miRBase (*www.mirbase.org/*) and 3707 other miRNAs were discovered in 2015 by Eric Londina and other scientists [6]. Human miRNAs have abbreviation "hsa" (*Homo sapiens*) in the beginning of their names. Mouse miRNAs have abbreviation "mmu" (*Mus musculus*). Free energy of miRNA-mRNA binding (ΔG), ΔG/ΔG_m ratio (%), positions and schemes of potential miRNA binding sites were calculated by the program MirTarget [7-8]. ΔG_m (maximal ΔG) is free energy of miRNA binding with nucleotide sequence that is absolute complementary to this miRNA. ΔG/ΔG_m ratio was used as comparative criterion of miRNA-mRNA interaction. ΔG/ΔG_m ratio should be equal or more than 85% because 15% of ΔG_m correspond to three nucleotides of miRNA sequence which can encode one amino acid. Binding site with value of ΔG/ΔG_m ratio, that is less than 85%, is counted as unreliable because it has low specifity and can bind different miRNAs. Diagrams of evolutionary conservation of olygopeptides encoding by miRNA binding sites were created by the program WebLogo (https://www.weblogo.berkeley.edu/). The information about microRNA expression was taken from Human miRNA tissue atlas (*https://ccb-web.cs.uni-saarland.de/tissueatlas*) and TiGER: Tissue-specific Gene Expression and Regulation (bioinfo.wilmer.jhu.edu/tiger/). Positions of miRNA binding sites were compared with single nucleotide polymorphisms (SNPs) of human titin gene (https://www.ncbi.nlm.nih.gov/snp/?term=TTN*)*.

Results and their discussion

We have studied binding of 6271 human miRNAs with mRNA of human titin gene. There were found 23 binding sites of 18 miRNAs with value of ΔG/ΔG_m that is more than 85% (Table «a»). These sites have "bubble" in the structure of miRNA-mRNA duplex with the exception of hsa-miR-11-28905-3p and hsa-miR-14-24215-3p. The density of miRNA binding sites in mRNA sequence of human titin is approximately equal to one site for five thousands nucleotides. hsa-miR-6861-5p, hsa-miR-494-5p, hsa-miR-374b-3p, hsa-miR-374c-3p, hsa-miR-34a-3p and hsa-miR-4495 are synthesized by intergenic regions. hsa-miR-578, hsa-miR-3714, hsa-miR-1278, hsa-miR-544b, hsa-miR-4738-3p, hsa-miR-136-3p and hsa-miR-4693-5p are synthesized by host genes (*CPE, PLCL2, CDKN1A, CDK4, CDK6, CDC73, UMPS, UNK, RTL1* and *RP11,* respectively) [9-11]. hsa-miR-19-36945-3p, hsa-miR-1-1585-3p, hsa-miR-11-28905-3p, hsa-miR-14-24215-3p and hsa-miR-12-32366-3p are novel human miRNAs and absent in the miRBase [12]. hsa-miR-6861-5p and hsa-miR-14-24215-3p have three binding sites each. These sites are located from 177th exon to 197th exon inclusively and contain so called PEVK-repeats. The rest miRNAs have only one binding site each. Only hsa-miR-374b-3p, hsa-miR-374c-3p, hsa-miR-3714, hsa-miR-4738-3p, hsa-miR-136-3p and hsa-miR-4495 have relatively high level of expression in heart and muscle tissues in comparison with other tissues [13-16]. In this way, no one binding site of these miRNAs doesn't coincide of known pathological mutations of human titin gene.

Four mouse miRNAs (mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p and mmu-miR-494-5p) are orthologous to corresponding human miRNAs (Table «b»). Differences of mouse miRNA nucleotide sequences and corresponding human sequences are one-three nucleotides. For example, the sequence of hsa-miR-34a-3p has a cytosine (C) in the beginning that is absent in the sequence of mmu-miR-34a-3p. So the length of hsa-miR-34a-3p is 22 nucleotides while the length of mmu-miR-34a-3p is 21 nucleotides. The sequence of hsa-miR-136-3p has nucleotide C in the beginning that is absent in the sequence of mmu-miR-136-3p. But it hasn't an uracil (U) that is present in the sequence of mmu-miR-136-3p. So the length of these miRNAs is the same. hsa-miR-374c-3p is three nucleotides longer (22 nucleotides) than mmu-miR-374c-3p (19 nucleotides) because hsa-miR-374c-3p has nucleotide C in the beginning and nucleotides A (adenine) and U at the end that are absent in the sequence of mmu-miR-374c-3p. hsa-miR-494-5p has nucleotide U at the end of sequence that makes it longer than mmu-miR-494-5p. The length of hsa-miR-494-5p is 23 nucleotides and the length of mmu-miR-494-5p is 22 nucleotides.

We have calculated interaction between mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p, mmu-miR-494-5p and mRNA of human titin gene. Only mmu-miR-494-5p bound with mRNA of this gene with value of ΔG/ΔG_m that is not lower than 90 %. It has ΔG/ΔG_m that is equal 93 % (Table «a»).

Than we have calculated binding of 18 human miRNAs and mmu-miR-34a-3p, mmu-miR-136-3p, mmu-miR-374c-3p, mmu-miR-494-5p with mRNA of mouse titin gene. Only eight miRNAs bind with mRNA of mouse titin gene with values of ΔG/ΔG_m equal 85 % and more (hsa-miR-19-36945-3p, hsa-miR-11-28905-3p, mmu-miR-34a-3p, hsa-miR-1278, hsa-miR-544b, hsa-miR-34a-3p, hsa-miR-4693-5p and hsa-miR-4495) (Table «c»).

Table «a» – The characteristics of human and mouse miRNA binding sites in the mRNA of human titin gene, *$\Delta G/\Delta G_m$ ratio is more than 85 %*

miRNA	Position in the mRNA	Position in exon[1]	Energy[2] (kJ/mole)	$\Delta G/\Delta G_m$ (%)	Length of miRNA, nt	Exon	Schemes of miRNA-mRNA interaction[3]
hsa-miR-494-5p	1301	161	-108	90	23	7	5′ – UGAGAGAGACAACGCUGACAACCU – 3′ |||| ||||||||| |||||||| 3′ – UCUCU-UCUGUUGUGCCUGUUGGA – 5′
mmu-miR-494-5p	1302	162	-108	93	22	7	5′ – GAGAGAGACAACGCUGACAACCU – 3′ |||| ||||||||| |||||||| 3′ – CUCU-UCUGUUGUGCCUGUUGGA – 5′
hsa-miR-578	1960	72	-98	90	21	11	5′ – ACAGUCCCGGGAGCUCAAGAAG – 3′ |||||||||| ||| ||||||| 3′ – UGUUAGGAUC-UCGUGUUCUUC – 5′
hsa-miR-19-36945-3p	3271	204	-102	92	20	18	5′ – AGUGCUGUAAAUGAGGCUGGA – 3′ ||||||| |||||||||||| 3′ – UCACGAC-UCUACUCCGACCC – 5′
hsa-miR-1-1585-3p	8609	3	-96	92	21	36	5′ – UCAAGAUCAUUAAAAAGCCAAA – 3′ |||||| ||| |||||||||| 3′ – AGUUCUUGUA-UUUUCCGGUUU – 5′
hsa-miR-374b-3p	17239	110	-98	90	22	58	5′ – AAAGAUAACACAAUCCUGCGAAG – 3′ || |||||||||| ||||| ||| 3′ – UUACUAUUAUGUU-GGACGAUUC – 5′
hsa-miR-374c-3p	17241	112	-98	90	22	58	5′ – AGAUAACACAAUCCUGCGAAGUG – 3′ | ||||||||| ||||| ||||| 3′ – UAUAUUAUGUU-GGACGAUUCAC – 5′
hsa-miR-11-28905-3p	17446	38	-117	89	23	59	5′ – UCCCUCCGGGGAGGCACAGCUGC – 3′ ||||||||| |||||||||||| 3′ – AAGGAGGCCGCCCUGUGUCGACG – 5′
hsa-miR-3714	17450	42	-110	90	22		5′ – UCCGGGGAGGCACAGCUGCCUUC – 3′ | |||||| ||| ||||||||| 3′ – UGUCCCCUC-GUGACGACGGAAG – 5′
hsa-miR-34a-3p	22116	208	-104	91	22	75	5′ – AGGGCAGUAUUCCUGCGAGAUUG – 3′ |||||||||| ||||| ||||| 3′ – UCCCGUCAUAUGAACGA-CUAAC – 5′
hsa-miR-1278	24928	197	-98	90	22	85	5′ – AUAGAGGAUUAUGCACAGUACAG – 3′ ||||| ||| |||||||||||| | 3′ – UAUCUACUA-UACGUGUCAUGAU – 5′
hsa-miR-544b	26044	179	-104	93	22	89	5′ – CUGGAAAUGCACAAUCUCAGUGU – 3′ |||||||||||||||||||| || 3′ – AAUCUUUACGUGUUGGAGUC-CA – 5′

Continuation of table «a»

hsa-miR-14-24215-3p	37245	61	-104	93	22	177-178*	5′ – AGAAGCUCCAAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	37324	59	-115	92	22	178-179*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-14-24215-3p	37998	61	-102	91	22	186-187*	5′ – AGAAGCUCCGAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	38077	59	-115	92	22	187-188*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-14-24215-3p	38751	61	-104	93	22	195-196*	5′ – AGAAGCUCCAAUUGUCCCAGUG – 3′ 3′ – UCUUCGAGGUUUAUAGGGUCAU – 5′
hsa-miR-6861-5p	38830	59	-115	92	22	196-197*	5′ – CCAGAAGCCCCACCUGCCACAGU – 3′ 3′ – GGACCUCGGGGUGGAUGG-GUCA – 5′
hsa-miR-136-3p	71469	1528	-102	91	22	326	5′ – GGACCCACCUGAGAACGAUGGUG – 3′ 3′ – UCUGAGUAAACUCU-GCUACUAC – 5′
hsa-miR-12-32366-3p	71984	2043	-108	90	22		5′ – UGGCUCUGGAUCCCAUUGACCCA – 3′ 3′ – ACCGAGAC-UGGGGUCACUGGAU – 5′
hsa-miR-4738-3p	74955	5014	-113	93	22		5′ – UCCUCCUGGCACUCCAGUUGUCA – 3′ 3′ – AGGAGGUCCGCGAGGUCAA-AGU – 5′
hsa-miR-4693-5p	92464	86	-108	94	23	339	5′ – GGUGGCAGUGAAAUUCAACAGUAU – 3′ 3′ – ACACUGUCACUUUAAGU-GUCAUA – 5′
hsa-miR-4495	93909	1531	-93	90	21		5′ – AGCAGGAAGCCCAUUUACCAUU – 3′ 3′ – UCGUUUUUCGGACAAAUG-UAA – 5′

Notes:
1. Distance from exon beginning (from the first exon beginning if miRNA binding site is located on the border of two exons).
2. Energy of miRNA-mRNA interaction.
3. In each scheme upper sequence is the sequence of mRNA site, lower sequence is miRNA strand.
* – miRNA binding site is located on the border of two exons.

Table «b» – Differences between orthological human and mouse miRNAs binding with mRNAs of human and mouse titin genes

MiRNAs of human	MiRNAs of mouse
hsa-miR-34a-3p 22 nt. **C**AAUCAGCAAGUAUACUGCCCU	mmu-miR-34a-3p 21 nt. AAUCAGCAAGUAUACUGCCCU
hsa-miR-136-3p 22 нт. **C**AUCAUCGUCUCAAAUGAGUCU	mmu-miR-136-3p 22 nt. AUCAUCGUCUCAAAUGAGUCU**U**
hsa-miR-374c-3p 22 нт. **C**ACUUAGCAGGUUGUAUUAU**AU**	mmu-miR-374c-3p 19 nt. ACUUAGCAGGUUGUAUUAU
hsa-miR-494-5p 23 нт. AGGUUGUCCGUGUUGUCUUCUC**U**	mmu-miR-494-5p 22 nt. AGGUUGUCCGUGUUGUCUUCUC
Note: different nucleotides of corresponding human and mouse miRNA sequences are signed by bold letters.	

Table «c» – The characteristics of human and mouse miRNA binding sites in the mRNA of mouse titin gene, *$\Delta G/\Delta G_m$ is equal or more than 85 %*

miRNA	Position in the mRNA, nt.	Energy[1] (kJ/mole)	$\Delta G/\Delta G_m$ (%)	Length of miRNA, nt	Exon	Position in exon[2]	Schemes of miRNA-mRNA interaction[3]
hsa-miR-19-36945-3p	3288	-100	90	20	18	204	5′ – AGCGCCGUGAAUGAGGCCGGG – 3′ ||||||| || ||||||||| 3′ – UCACGACUCU-ACUCCGACCC – 5′
hsa-miR-11-28905-3p	16760	-113	86	23	58	37 nt.	5′ – CUCCCUCCGUGGGGGCACAGCCGC – 3′ |||| |||| |||||||||||| 3′ – AAGG-AGGCCGCCCUGUGUCGACG – 5′
	16761					38 nt.	5′ – UCCCUCCGUGGGGGCACAGCCGC – 3′ |||||||| |||||||||||| 3′ – AAGGAGGCCGCCCUGUGUCGACG – 5′
mmu-miR-34a-3p	19463	-93	86	21	66	203 nt.	5′ – UGGACAGUAUACUUGCCAAGUU – 3′ |||||||||||||||||| || 3′ – UCCCGUCAUAUGAACGACU-AA – 5′
hsa-miR-1278	24243	-96	88	22	83	197 nt.	5′ – AUAGAGGAUUAUGCACAGUACGC -3′ ||||| ||| ||||||||||| 3′ – UAUCUACUA-UACGUGUCAUGAU -5′
hsa-miR-544b	25359	-100	89	22	87	179 nt.	5′ – CUGGAGAUGCACAGUCUCAGUGU – 3′ |||||||||||||||||||| || 3′ – AAUCUUUACGUGUUGGAGUC-CA – 5′
hsa-miR-34a-3p	27077	-100	87	22	93	208 nt.	5′ – AGGGCAGUACAACUGCUACAUUG – 3′ ||||||||||| |||||| |||| 3′ – UCCCGUCAUAUGAACGAC-UAAC – 5′
hsa-miR-4693-5p	90243	-98	85	23	282	86 nt.	5′ – GGUGGCAGUGAGAUUCAACACUAC – 3′ ||||||||||||||| || ||| 3′ – ACACUGUCACUUUAAGU-GUCAUA – 5′
hsa-miR-4495	96348	-91	88	21	295	245 nt	5′ – GGCAAGAAGACCGUUUACAUU – 3′ ||||||||| |||||||||| 3′ – UCGUUUUUCGGACAAAUGUAA – 5′

Notes:
1. Energy of miRNA-mRNA interaction; 2. Distance from exon beginning; 3. In each scheme upper sequence is the sequence of mRNA site, lower sequence is miRNA strand.

Table «d» – miRNA binding sites in mRNAs of human and mouse titin gene and their artificial miRNAs, different nucleotides are signed by bold letters

miRNA	Position, nt.	Nucleotide sequence of miRNA binding site	Artificial miRNA
hsa-miR-494-5p	1301	UGAGA**G**AGACAACGC**U**GACAACCU	AGGUUGUCAGCGUUGUCUCUCUCA
mmu-miR-494-5p	1302	GAGA**G**AGACAACGCUGACAACCU	AGGUUGUCAGCGUUGUCUCUCUC
hsa-miR-578	1960	ACAGUCCCGG**G**AGC**U**CAAGAAG	CUUCUUGAGCUCCCGGGACUGU
hsa-miR-19-36945-3p	3271	AGUGCUG**U**AAAUGAGGCUGGA	UCCAGCCUCAUUUACAGCACU
hsa-miR-1-1585-3p	8609	UCAAGAUCA**U**UAAAAAGCCAAA	UUUGGCUUUUUAAUGAUCUUGA
hsa-miR-374b-3p	17239	AA**A**GAUAACACAA**U**CCUGC**G**AAG	CUUCGCAGGAUUGUGUUAUCUUU
hsa-miR-374c-3p	17241	A**G**AUAACACAA**U**CCUGC**G**AAGUG	CACUUCGCAGGAUUGUGUUAUCU
hsa-miR-11-28905-3p	17446	UCCCUCCGG**G**GAGGCACAGCUGC	GCAGCUGUGCCUCCCCGGAGGGA
hsa-miR-3714	17450	**U**C**C**GGGGAG**G**CACAGCUGCCUUC	GAAGGCAGCUGUGCCUCCCCGGA
hsa-miR-34a-3p	22116	AGGGCAGUAU**U**CCUGCG**A**GAUUG	CAAUCUCGCAGGAAUACUGCCCU
hsa-miR-1278	24928	AUAGA**G**GAU**U**AUGCACAGUAC**A**G	CUGUACUGUGCAUAAUCCUCUAU
hsa-miR-544b	26044	CUGGAAAUGCACAAUCUCAG**U**GU	ACACUGAGAUUGUGCAUUUCCAG
hsa-miR-14-24215-3p	37245	AGAAGCUCCAA**U**UGUCCCAGUG	CACUGGGACAAUUGGAGCUUCU
hsa-miR-6861-5p	37324	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-14-24215-3p	37998	AGAAGCUCCGA**U**UGUCCCAGUG	CACUGGGACAAUCGGAGCUUCU
hsa-miR-6861-5p	38077	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-14-24215-3p	38751	AGAAGCUCCAA**U**UGUCCCAGUG	CACUGGGACAAUUGGAGCUUCU
hsa-miR-6861-5p	38830	CC**A**GAAGCCCCACCUGCC**A**CAGU	ACUGUGGCAGGUGGGGCUUCUGG
hsa-miR-136-3p	71469	GGACCCACCUGAGAA**C**GAUGGUG	CACCAUCGUUCUCAGGUGGGUCC
hsa-miR-12-32366-3p	71984	UGGCUCUG**G**AUCCCA**U**UGACCCA	UGGGUCAAUGGGAUCCAGAGCCA
hsa-miR-4738-3p	74955	UCCUCC**U**GGCACUCCAGUU**G**UCA	UGACAACUGGAGUGCCAGGAGGA
hsa-miR-4693-5p	92464	**G**GUGGCAGUGAAAUUCAA**C**AGUAU	AUACUGUUGAAUUUCACUGCCACC
hsa-miR-4495	93909	AGCAGGAAGCCCAUUUAC**C**AUU	AAUGGUAAAUGGGCUUCCUGCU
hsa-miR-19-36945-3p	30584	AGCGCCG**U**GAA**U**GAGGCCGGG	CCCGGCCUCAUUCACGGCGCU

These miRNAs have nine binding sites. Only hsa-miR-19-36945-3p bind with mRNA of mouse titin gene with value of $\Delta G/\Delta G_m$ equal to 90%. hsa-miR-4693-5p has the lowest value of $\Delta G/\Delta G_m$ ratio that is equal to 85%. It has binding site in the 282nd exon of mouse titin gene. hsa-miR-19-36945-3p, hsa-miR-11-28905-3p, hsa-miR-544b and hsa-miR-34a-3p have values of miRNA-mRNA interaction energy equal to -100 kJ/mole and lower. Other miRNAs have higher values of binding energy than these miRNAs. hsa-miR-11-28905-3p has two binding sites in the 58th exon with the lowest level of interaction energy (-113 kJ/mole) and ΔG/ΔGm ratio that is equal 86%. hsa-miR-4495 has the highest level of miRNA-mRNA interaction energy that is equal -91 kJ/mole. Binding site of this miRNA is located in the 295th exon of mouse titin gene. The single mouse miRNA binding site in mRNA of mouse titin gene is binding site of mmu-miR-34a-3p that is located in the 66th exon. It has value of $\Delta G/\Delta G_m$ that is equal 86 %. Binding site of hsa-miR-34a-3p is located very far from binding site of mmu-miR-34a-3p in the mRNA of mouse titin, in the 93rd exon, and has value of $\Delta G/\Delta G_m$ that is equal 87 %. hsa-miR-1278 has binding site in the 83rd exon of mouse titin gene and value of $\Delta G/\Delta G_m$ that is equal 88 %. hsa-miR-544b has binding site in the 87th exon of mouse titin gene and value of $\Delta G/\Delta G_m$ that is equal 89 %.

One of the evidences of miRNA binding site existence is presence of it in mRNAs of orthologous genes. We have studied binding of 6271 human miRNAs with mRNAs of titin gene of 14 species of primates (*Homo sapiens, Pan troglodytes, Pongo abelii, Macaca fascicularis, Papio anubis, Pan paniscus, Colobus angolensis, Chlorocebus sabaeus, Rhinopithecus roxellana, Callithrix jacchus, Aotus nancymaae*, *Saimiri boliviensis*, *Gorilla gorilla, Nomascus leucogenys*). It was found that only hsa-miR-494-5p and hsa-miR-578 bind with mRNAs of primate titin gene with values of $\Delta G/\Delta G_m$ equal to 90% and more. Although orthologous miRNAs of various primate species are different, primates have conservative genes of miRNA synthesis and conservative miRNA binding sites. For example, Figure1: binding sites of hsa-miR-494-5p in titin mRNAs of primates encode conservative heptapeptide **RETTLTT**, in which the last aminoacid changed (threonin is changed to alanin or serin). Figure2: binding sites of hsa-miR-578 in titin mRNAs of primates encode conservative heptapeptide **TVPGAQE**. As we can see, flanking amino acid sequences are even more conservative than these olygopeptides themselves. Conservation of olygopeptides, encoding by miRNA binding sites, proves conservation of these mRNA regions. Characteristics of miRNA binding sites in mRNAs of primate titin gene are more similar than in mRNAs of human and mouse gene because of different evolutionary distance between these species.

miRNAs can't effectively block translation of titin because they are not absolutely complementary to their sites. But artificial miRNAs, that would be absolutely complementary to these sites and have $\Delta G/\Delta G_m$ ratio equal 100 %, could effectively bind with mRNAs of titin genes and block their translation (Table «d»). There are 25 miRNA binding sites in this table that have ΔG/ΔGm ratio equal 90% and more. Nucleotides of miRNAs, decreasing energy of miRNA-mRNA interaction, are signed by red color. For example, we need to change three nucleotides in the sequence of hsa-miR-374b-3p binding site that is located in the position 17241 of human titin gene to synthesize absolutely complementary artificial miRNA to this site. In other case, we need to change only one nucleotide in the sequence of mmu-miR-494-5p binding site located in position 1302 to get ideal artificial miRNA. In the case of hsa-miR-494-5p binding site, located in position 1301 of human titin gene, two nucleotides are needed for synthesis of complementary sequence for this site. In relation to mouse titin gene mRNA, it is needed to change two nucleotides in the sequence of hsa-miR-19-36945-3p located in position 30584 to get artificial miRNA.

Conclusions

We have found differences and similarities of characteristics of miRNA binding sites in mRNAs of human and mouse titin gene. Differences are the following:

1. Human titin mRNA has 23 miRNA binding sites whereas mouse titin mRNA has only nine sites.
2. 19 miRNAs (18 human miRNAs and one mouse miRNA) interact with mRNA of human titin but only eight miRNAs from this number bind with mRNA of mouse titin gene.
3. $\Delta G/\Delta G_m$ ratio of miRNA binding sites in the mRNA of human titin gene varies from 89% to 94% but in the mRNA of mouse titin gene it varies from 85% to 90%.
4. Orthologous human and mouse miRNAs have different nucleotides in their sequences.

Similarities of characteristics of miRNA binding sites in human and mouse titin mRNA are the following:

1. Positions of miRNA binding sites in the mRNA of mouse titin gene are very close to such positions in the mRNA of human titin.

Figure 1. Pinsky I., Labeit S., Labeit D., Ivashchenko A.

Figure 1 – Diagram of evolutional conservation of olygopeptides encoding by binding sites of hsa-miR-494-5p in mRNAs of primate titin genes. Horizontal axis is positions of amino acids in the sequence of this olygopeptide, vertical axis is a frequency of their appearing in this sequence

Figure 2. Pinsky I., Labeit S., Labeit D., Ivashchenko A.

Figure 2 – Diagram of evolutional conservation of olygopeptides encoding by binding sites of hsa-miR-578 in mRNAs of primate titin genes. Horizontal axis is positions of amino acids in the sequence of this olygopeptide, vertical axis is a frequency of their appearing in this sequence

2. All miRNA binding sites are located in protein-coding part of these mRNAs.

Thus characteristics of miRNA binding sites in mRNAs of human and mouse titin gene are different and it should be taken into account. So the model of mouse titin synthesis regulation by miRNAs is not completely adequate for human titin gene. Nevertheless, increasing of miRNA concentrations in relation to mRNA of mouse titin gene can cause the same effect on expression of this gene as in human. In something's totality, obtained results permit to suppose that expression of titin gene is weekly regulated by miRNAs in human and mouse organisms.

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