IRSTI 34.15.23; 34.15.25; 31.27.31; 76.03.31; 76.29.49 DOI: https://doi.org/10.26577/eb-2019-3-b10

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CHARACTERISTICS OF MIRNA INTERACTION WITH MRNA CANDIDATE GENES OF ESOPHAGEAL ADENOCARCINOMA

miRNAs is a class of nano-sized non-coding RNAs that regulate the expression of genes, and associated with many physiological and pathological processes, especially cancer. The expression of many genes is regulated by the binding of their mRNA with miRNA, so it is required to identify which candidate genes of oncogenesis and to what extent can interact with miRNA. The purpose of this work was to establish the characteristics of the interaction of known 3707 miRNA with mRNA of 38 candidate esophageal adenocarcinoma genes. It has been identified, that 84 miRNAs have binding sites in 31 mRNAs of genes at 5'UTR, CDS, and 3'UTR and the average free binding energy (ΔG) of miRNAs with mRNAs was -121 kJ/mole, -118 kJ/mole and -113 kJ/mole, respectively. 19 associations of miRNAs and mRNA of genes with a free energy of interaction more than -120 kJ/mole are recommended for the diagnosis of esophageal adenocarcinoma. The mRNAs of most genes containing two or more miRNA binding sites with overlapping of their nucleotide sequences form clusters. Based on the obtained results, associations of miRNA and mRNA of candidate genes are recommended to develop methods for early diagnosis of esophageal adenocarcinoma.

Key words: mRNA, miRNA, genes, oncological diseases, esophageal adenocarcinoma.

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Өңеш аденокарцинома кандидатты гендердің mRNA-ның miRNA-мен өзара әсерлесуінің сипаттамалары

miRNA – гендік экспрессияны реттейтін және өлшемі көптеген физиологиялық және патологиялық процестермен, атап айтқанда қатерлі ісік ауруымен байланысты нано-өлшемді, кодталмаған RNA класы. Көптеген гендердің экспрессиясы олардың mRNA-лары мен miRNA-ның байланыстыру арқылы реттеледі, сондықтан онкогенездің қай кандидаттық гендерін және miRNA-мен қандай дәрежеде әрекеттесетінін анықтау қажет. Бұл жұмыстың мақсаты белгілі 3707 miRNA-дың 38 кандидатты өңеш аденокарцинома гендерінің mRNA-мен өзара әсерлесуінің дәрежесін анықтау болды. 84 miRNA-ның 31 mRNA-да гендерінде 5'UTR, CDS және 3'UTR-де байланыстыратын сайттар бар екендігі анықталды, ал mRNA-мен бірге осы miRNA-ның орташа бос байланыс энергиясы (ΔG) -121 кДж/моль, -118 кДж/моль және -113 кДж/мольға сәйкесінше болды. Өңештің аденокарциномасын диагностикалау үшін 19 mRNA гендерімен еркін әрекеттесу энергиясы -120 кДж / мольден асатын 19 miRNA бірлестігі ұсынылады. Екі немесе одан да көп miRNA – байланыстыратын сайттар бар нуклеотидтер тізбегі қабаттасатын гендердің көпшілігінің mRNA-сы кластерлерді қалыптастырады. Алынған нәтижелер негізінде, miRNA-мен mRNA-ға кандидат гендерімен ассоциациясына өңеш аденокарциномасын ерте диагностикалау әдісін жасау ұсынылады.

Түйін сөздер: mRNA, miRNA, гендер, онкологиялық аурулар, өңеш аденокарцинома.

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Характеристики взаимодействия miRNA с mRNA кандидатных генов аденокарциномы пищевода

miRNA класс нано-размерных, не кодирующих РНК, которые регулируют экспрессию генов, и связаны со многими физиологическими и патологическими процессами, в частности с раком. Экспрессия многих генов регулируется связыванием их mRNA с miRNA, поэтому требуется выявить, какие кандидатные гены онкогенеза и в какой степени могут взаимодействовать с miRNA. Цель настоящей работы заключалась в установлении характеристик взаимодействия известных 3707 miRNA с mRNA 38 кандидатных генов аденокарциномы пищевода. Установлено что, 84 miRNA имеют сайты связывания в 31 mRNA генов в 5'UTR, CDS, и 3'UTR, а средняя свободная энергия связывания (ΔG) этих miRNAs с mRNA составила -121 кДж/моль, -118 кДж/моль и -113 кДж/моль, соответственно. 19 ассоциаций miRNA с mRNA генов со свободной энергией взаимодействия более -120 кДж/моль рекомендуются для диагностики аденокарциномы пищевода. mRNAs большинства генов, содержащих два или более сайтов связывания miRNA с наложением их нуклеотидных последовательностей, образуют кластеры. На основании полученных результатов, ассоциации miRNA с mRNA генов-кандидатов рекомендуются для разработки метода ранней диагностики аденокарциномы пищевода.

Ключевые слова: mRNA, miRNA, гены, онкологические заболевания, аденокарцинома пищевода.

Introduction

Esophageal cancer (EC) usually is found as either adenocarcinoma (EAC) or squamous cell carcinomas (ESCC) (Mathé, 2009: 6192; Zeng, 2016: 232; Rustgi, 2014: 2499). EAC form in the mucus-forming glandular cells and ordinarily develops in the lower one third of the esophagus near the stomach. Barrett's esophagus (Hvid-Jensen, 2011: 365) is the presumed metaplastic precursor of EAC (Desai, 2012: 970; Wani, 2009: 502). There is a persistent need for improving our understanding of the molecular basis of this disease. Finding new biomarkers that cover various aspects of the diseases could provide a choice of suitable therapies and better monitoring of patients with these cancers. Recently, alteration in miRNA expression has emerged as an important hallmark of cancer (Garofalo, 2011: 25; Zhou, 2017: 3893; Wang, 2018: 2018). However, the correlation between changes in expression of genes and miRNAs is not evidence that these genes are targets of these miRNAs (Atambaeva, 2017: 428; Ivaschenko, 2014: 1; Ivaschenko, 2014: e11). It is impossible to experimentally determine how a known miRNA can interact with more than 30,000 of genes and their isoforms. Therefore, it is required to predict the target genes of certain miRNAs and then test them experimentally. miRNAs can affect cancer pathogenesis, playing a crucial role as either oncogenes or tumor suppressors (Hata, 2015: 121). miRNA could potentially alter complex cellular processes such as cell growth, cell cycle, apoptosis and invasion (Wu, 2016: 12061; Poy, 2004: 226; Karp, 2005: 1288; Cheng, 2005: 1290). Identification of specific miRNAs and their target genes, participating in carcinogenesis allows to better understanding the mechanism of regulation of genes expression (Rath, 2016: 112). The recent emergence of observations on the role of miRNAs in cancer and their functions has induced many investigations to examine their relevance to EAC.

Analysis of information to study the involvement of candidate genes in the development of EAC shows that the number of publications on this problem increases in recent years. The present study is aimed to identify miRNA binding sites in mRNA of genes involved in the development of EAC and the clusters of miRNA binding sites in mRNA and their properties. Further, research of these miRNAs would provide a diagnostic strategy based on prevention or treatment of EAC.

Materials and methods

The information about the role and function of genes participating in the development of EAC were taken from GenBank databases and publications. The 38 mRNAs of genes associated with development

of EAC were used in the study. mRNA nucleotide sequences of the human genes were derived from GenBank (http://www.ncbi.nlm.nih.gov) by use of Lextractor script. The nucleotide sequences of 3707 miRNAs were taken from Londin et al. (Londin, 2015: 1106).

Search for miRNA's target genes was performed by MirTarget program, created in our laboratory (Ivashchenko, 2014: 237). This program defines the start of miRNA binding sites in mRNA; localization of binding sites in 5'-untranslated region (5'UTR), protein coding region (CDS), and 3'-untranslated region (3'UTR); free energy of interaction (ΔG, kJ/mole) and scheme of miRNA-mRNA nucleotides (nt) interaction. The ΔG/ΔGm (%) ratio was calculated for each binding site, where ΔG_m is equal to the free energy of miRNA interaction with fully complementary nucleotide sequence. miRNA-mRNA binding sites with ΔG/ΔGm ratio higher than 88% were selected. However, this criterion does not include the length of miRNA, on which ΔG energy also varies, depending on the miRNA lengths. Thus, in miRNAs with the same ΔG/ΔGm value, but varying lengths of 17 nt and 25 nt, correspondingly, the energy of binding of mRNA for miRNA with the length of 25 nt was 1.47 times higher than for miRNA with the length of 17 nt. ΔG/ΔGm value leads to the reduction in the number of false-positive miRNAs with a length of less than 20 nt. The position of binding sites is indicated from the first nucleotide of the 5'UTR in mRNA. The unique property of MirTarget program include consideration of nucleotide interaction in miRNA with mRNA of target genes not only between adenine (A) and uracil (U), guanine (G) and cytosine (C), but also between A and C, G and U via single hydrogen bond (Kool, 2001: 1; Leontis, 2002: 3497). The distance between A-C and G-U is equal to distance value between G-C and A-U.

Results and discussion

The search of genes responsible for the development of EAC performed by the existed fragmented data because there is no available unified database of genes. To create the database of genes, we took as a basis the information available in the NCBI (National Center for Biotechnology Information) and through a search of PubMed. Table 1 presents the information about the candidate genes involved in the development of EAC. The list of candidate genes was formed from publications based on laboratory research.

Table 1 – Database of EAC candidate genes

Gene	PMID	Gene	PMID	Gene	PMID
ALDH1A2	25447851	*ERBB2*	24151090	*NOX5**	26901778
*APOBEC1**	25085003	*ERBB3*	24151090	*OXT**	26406593
AR	26467701	*ESR1*	26406593	*OXTR*	26406593
ARID1A	28440661	*FKBP5*	26467701	*PARP1*	23757351
AXIN2	26297437	*FOXF1*	26383589	*ROCK2*	26901778
BARX1	26383589	*FOXM1*	25889361	*RUNX3*	25229459
BTG3	25701359	*FOXP1*	26383589	*SEPP1**	22715394
*CD55**	26202380	*GDF7*	26783083	*SMAD4*	24952744
CDK9	28404924	*GPBAR1**	28293080	*SOX2*	28692180
*CDKN2A**	25280564	*IGFBP2*	26317790	*TBX5**	26783083
CEP72	27527254	*LEP*	24569475	*TP53*	26733670
CTSE	25348778	*LGALS9*	28586026	*VDR**	25910066
DKK3	26093488	*MUC1*	28212575		

Note: * – indicates mRNAs, that are not targets for miRNA with chosen criteria

It was found that nine of 38 candidate genes are not targeted by miRNAs with ΔG/ΔGm value higher than 86%, show that their expression level is independent of miRNAs.

The distribution of miRNA binding sites in mRNA was uneven. Most of the miRNA had binding sites in different parts of the mRNA and could bind independently of each other. However, some miRNAs had overlapped nucleotide sequences in binding sites, forming clusters. Table 2 shows the characteristics of the interaction of miRNA in 5'UTR mRNA genes involved in the development of EAC. ID00792.3p-miR and ID00744.3p-miR targeted only mRNA of *ALDH1A2* gene. mRNA of *ARID1A* gene has cluster of binding sites in position from 295 nt to 331 nt with a whole length equal to 37 nt and an average ΔG = -111 kJ/mole. *ARID1A* could act as a tumor suppressor and has an important role in carcinogenesis in many organs (Wu, 2014: 655). ARID1A protein loss was also identified in Barrett's esophagus, a precancerous lesion of esophageal adenocarcinoma, and frequency of loss was higher in lesions with more severe dysplasia (Streppel, 2014: 347).

Table 2 – Characteristics of miRNAs binding sites in 5`UTR mRNA of genes involved in the development of EAC

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ALDH1A2	ID00792.3p-miR	10	-132	95	22
	ID00744.3p-miR	122	-125	89	23
AR	ID01739.3p-miR	621	-115	95	21
ARID1A	ID02751.3p-miR	206	-125	92	23
	ID01257.3p-miR	295	-113	93	20
	ID00414.3p-miR	303	-108	93	20
	ID02428.3p-miR	310	-113	91	22
BARX1	ID01675.5p-miR	61	-121	92	21
BTG3	ID01123.3p-miR	155	-127	88	24
	ID02803.5p-miR	186	-108	91	21
	ID00278.3p-miR	230	-125	91	23
CDK9	ID02823.3p-miR	25÷48 (2)	-117 ÷ -119	90÷92	22
	ID02094.3p-miR	62	-113	90	22
	ID01158.3p-miR	86	-119	98	20
DKK3	ID03462.5p-miR	73	-123	91	22
	ID01632.5p-miR	93	-125	92	23
ERBB3	ID00099.3p-miR	114	-113	93	21
FKBP5	ID01708.5p-miR	11	-115	89	23
FOXM1	ID01615.3p-miR	68	-115	90	22
FOXP1	ID00387.3p-miR	108	-125	89	23
	ID03332.3p-miR	130	-134	90	24
GDF7	ID03054.3p-miR	125	-113	90	22
	ID02682.5p-miR	339	-113	93	20
	ID01112.3p-miR	446	-113	93	20
	ID00009.3p-miR	546	-115	92	20
	ID01910.3p-miR	546	-115	92	20
	ID02781.3p-miR	547	-121	97	20
ROCK2	ID01184.3p-miR	248	-117	93	20
SMAD4	ID00577.3p-miR	160	-106	94	20
	ID00961.3p-miR	248	-127	90	23
SOX2	ID01749.3p-miR	344	-117	89	23

The cell cycle is a highly conservative and highly regulated biological system that controls cell proliferation and differentiation. Changes in the regulatory proteins (cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors) lead to a loss of normal control of the cell cycle, are a hallmark of many types of cancer, including EAC (Mikhail, 2015: 1185). We have identified *CDK9* gene as a candidate gene for EAC. ID02823.3p-miR and ID02094.3p-miR form a cluster of binding sites in mRNA of *CDK9* gene in position from 25 nt to 83 nt with a whole length 59 nt and an average energy of interaction equal to -116 kJ/mole.

mRNAs of *AR, BARX1, ERBB3, FKBP5, FOXM1, ROCK2* and *SOX2* genes in 5'UTR were targeted by single miRNAs. Nuclear *AR* and high expression of *FKBP5* is associated with decreased survival in EAC (Smith, 2016: 433). mRNA of *GDF7* gene were targeted by six miRNAs, three of which formed the cluster of binding sites. All three miRNAs formed a cluster in position from 546 nt to 567 nt with the length of 22 nt. The total length of all three sites was 60 nt. The formation of a cluster of binding sites for *GDF7* gene in the 5'UTR indicates a greater ability of this gene for compaction, which causes the competition of miRNAs data for the binding site. The average binding energy for these three miRNAs was -117 kJ/mole.

Du with coauthors (Du, 2015: 31) identified that *BTG3* gene expression was significantly downregulated in EAC tissues compared with adjacent normal tissues. *BTG3* is a direct transcriptional target gene of p53 (Ou, 2007: 3968), which affects its role in tumor suppression and DNA damage response. mRNA of *BTG3* gene was targeted by three miRNAs and these binding sites did not overlap, and each of them could independently bind to the mRNA. mRNA of *DKK3* gene had cluster of binding sites from 73 nt to 115 nt with an average ΔG = -124 kJ/mole.

The average free energy of interaction of all miRNA binding sites in 5'UTR is equal to -121 kJ/mole. The number of binding sites with ΔG value higher than -120 kJ/mole is equal to 11. The associations of these miRNAs with their target genes are recommended to be used as markers for the diagnosis of EAC.

Table 3 shows the results of miRNAs binding with mRNAs of candidate genes of EAC in CDS. The *AR* gene was targeted by three miRNAs whose binding sites were located in CDS. mRNA of *ARID1A* gene was targeted six miRNAs. Those binding sites were not overlapped, and each miRNA could independently bind with mRNA of *ARID1A* gene.

Table 3 – Characteristics of miRNA binding sites in CDS mRNA of genes involved in the development of EAC

Gene	miRNA	Start of site, nt	ΔG (kJ/mole)	ΔG/ΔGm, %	Length, nt
*A*R	ID00372.5p-miR	1363	-127	94	24
	ID01398.3p-miR	1517	-115	90	22
	ID01261.5p-miR	1744	-113	95	20
ARID1A	ID01753.3p-miR	749	-104	91	21
	ID01473.3p-miR	1093	-125	89	23
	ID01508.5p-miR	1459	-129	90	23
	ID02945.5p-miR	4274	-108	91	21
	ID01565.5p-miR	4916	-115	93	21
	ID01819.5p-miR	7062	-119	89	23
AXIN2	ID01796.3p-miR	1526	-125	89	24
	ID00648.5p-miR	1765	-125	92	22
	ID02344.3p-miR	2086	-127	88	24
BARX1	ID01757.3p-miR	192	-117	93	21
	ID02052.5p-miR	311	-132	89	24
	ID02429.3p-miR	314	-121	89	23
	ID02079.5p-miR	500	-115	92	20
	ID00380.3p-miR	558	-117	89	23
BTG3	ID02017.3p-miR	664	-117	92	22

Continuation of table 3

Gene	miRNA	Start of site, nt	ΔG (kJ/mole)	ΔG/ΔGm, %	Length, nt
CEP72	ID01839.3p-miR	1363	-123	89	23
DKK3	ID03402.5p-miR	295	-117	92	22
ERBB2	ID00692.3p-miR	3815	-113	90	22
ESR1	ID02606.5p-miR	409	-106	91	22
	ID02556.3p-miR	1852	-115	90	23
FOXF1	ID00267.3p-miR	55	-113	91	21
	ID00407.3p-miR	718	-119	90	22
FOXM1	ID01279.5p-miR	1897	-115	93	22
GDF7	ID01508.5p-miR	699	-129	90	23
	ID00102.3p-miR	784	-117	95	20
	ID01419.3p-miR	876	-115	89	23
	ID01346.3p-miR	1576	-119	92	22
IGFBP2	ID01859.5p-miR	198	-117	90	22
MUC1	ID00645.5p-miR	507	-110	93	20
OXTR	ID01310.3p-miR	1328	-123	94	22
PARP1	ID00550.5p-miR	1265	-113	90	23
	ID01616.3p-miR	1275	-119	90	23
RUNX3	ID02259.5p-miR	593	-119	89	23
	ID00024.5p-miR	1406	-119	93	21
SOX2	ID03289.5p-miR	455	-106	93	20
	ID01259.3p-miR	1218	-121	90	23

mRNA of *BARX1* gene were targeted by five miRNAs, two of which form the cluster of binding sites from 311 nt to 336 nt with an average free energy of hybridization equal to -127 kJ/mole. mRNAs of *BTG3, CEP72, DKK3, ERBB2, FOXM1, IGFBP2, MUC1* and *OXTR* in CDS have had binding sites for single miRNAs. ID01508.5p-miR, ID00102.3p-miR, ID01419.3p-miR and ID01346.3p-miR targeted mRNA of *GDF7* gene. mRNA of *PAPR1* gene has cluster of binding sites in position from 1265 nt to 1297 nt with an average ΔG value equal to 116 kJ/mole.

The average free energy of binding of all miRNAs with mRNAs in CDS was equal to -118 kJ/mole. The 11 miRNAs were bound with mRNAs of five target genes with a free interaction energy more than -120 kJ/mole (Table 3), which allows us to recommend miRNAs as markers for the diagnosis of EAC.

Twelve mRNAs of genes were targeted by miRNAs in 3'UTR. mRNA of *FKBP5* gene of five miRNAs, two of them formed a cluster of binding sites in position from 6364 nt to 6388 nt with an average ΔG= -107 kJ/mole. mRNAs of *AR, ESR1, FOXM1, FOXP1* and *RUNX3* were targeted by single miRNAs.

Notably, ID00037.3p-miR and ID00125.3p-miR occupied the same binding site, starting from 1671 nt in mRNA of *SOX2* gene. With the same ΔG/ΔGm values, the free interaction energy of ID00037.3p-miR (ΔG = -121 kJ/mole) is higher than the ΔG value of ID00125.3p-miR in mRNA of *SOX2* gene.

mRNA of *SMAD4* gene was targeted by six miRNAs, two of them form a cluster of binding sites from 4342 nt to 4371 nt with an average free energy of hybridization equal to -115 kJ/mole. mRNA of *LEP* gene have only two binding sites in 3`UTR that form a cluster of binding sites from 3087 nt to 3113 nt with an average ΔG= -113 kJ/mole.

The average free energy of binding of all miRNAs with mRNAs in the 3'UTR was equal to -113 kJ/mole. The number of associations with a free binding energy more than -120 kJ/mole is equal to three. The associations of these miRNAs with their target genes are recommended to be used as markers for diagnosis of EAC.

Table 4 – Characteristics of miRNA binding sites in 3`UTR mRNA of genes involved in the development of EAC

Gene	miRNA	Start of site, nt	ΔG (kJ/mole)	ΔG/ΔGm, %	Length, nt
AR	ID02403.3p-miR	4096	-108	89	23
AXIN2	ID01257.3p-miR	2960	-113	93	20
	ID02524.5p-miR	3702	-93	90	22
ESR1	ID03196.3p-miR	3339	-121	88	24
FKBP5	ID02017.3p-miR	1434	-115	90	22
	ID00367.5p-miR	4870	-110	90	22
	ID00625.5p-miR	5727	-106	91	21
	ID01360.3p-miR	6364	-104	91	21
	ID00367.5p-miR	6367	-110	90	22
	ID02175.3p-miR	7306	-110	91	22
FOXM1	ID00962.3p-miR	3260	-119	90	23
FOXP1	ID03465.3p-miR	3843	-117	90	22
LEP	ID03149.5p-miR	3087	-113	90	22
	ID01263.5p-miR	3092	-113	90	22
ROCK2	ID01640.5p-miR	6308	-117	89	24
	ID01836.5p-miR	6497	-115	92	23
RUNX3	ID02589.5p-miR	2956	-113	93	21
SMAD4	ID01838.5p-miR	4291	-113	90	24
	ID01656.3p-miR	4342	-115	89	23
	ID01404.5p-miR	4349	-115	93	23
	ID02732.3p-miR	7721	-123	91	23
	ID00106.5p-miR	7825	-106	91	22
	ID01592.3p-miR	8227	-117	89	23
SOX2	ID00037.3p-miR	1671	-121	90	23
	ID00125.3p-miR	1671	-113	90	22
TP53	ID00548.3p-miR	1393	-115	89	23
	ID02379.3p-miR	1397	-119	89	24
	ID01838.5p-miR	2459	-115	92	24
	ID00785.5p-miR	2520	-113	90	23

Table 5 shows the schemes of binding of miRNAs with mRNAs of EAC candidate genes. The interaction of nucleotides occurs along the entire length, except for the absence of hydrogen bonding between purines (A, G) or pyrimidines (C, U). Interactions of non-canonical pairs of nucleotides A-C and G-U are accounted by MirTarget program.

One of the misconceptions in many studies that miRNAs binds only (of predominantly) in 3'UTR of mRNAs (Ivashchenko, 2018: 36). However, miRNAs do not have the ability to distinguish binding sites in 5'UTR, CDS and 3'UTR. miRNA interacts with mRNA based on the physico-chemical properties of these molecules. Consequently, the interaction site can be localized in any region of mRNA and prohibitions on the location of such sites in the nucleotide sequence of the mRNA are still unknown. The conditions for the successful interaction of miRNA with mRNA are the energy characteristics and conformational properties of this interaction.

Table 5 – The schemes of miRNA interaction with mRNA of some genes participating in the development of EAC

Gene; miRNA; The start of site; Region; The characteristics of binding	Gene; miRNA; The start of site; Region; The characteristics of binding

ARID1A; ID00414.3p-miR; 303 nt; 5'UTR;
-108 kJ/mole; 93%; 20 nt
5' - GGG**G**CGGGGUGGGAGGGGG**G** - 3'
  | |||||||||||||||||
3' - CAC**U**GCCCCACCCUCCCCC**U** - 5'
	CDK9; ID02823.3p-miR; 25 nt; 5'UTR;
	-117 kJ/mole; 90%; 22 nt
	5' - **C**GCG**G**CCGCGGAGGG**G**CCUGGA - 3'
	  ||||||||||| ||||||||||
	3' - **A**CGC**U**GGCGCCGCCC**U**GGACCU - 5'

AR; ID01261.5p-miR; 1744 nt; CDS;
-113 kJ/mole; 95%; 20 nt
5' - GAGCGAGGGAGGCCUCGGGG - 3'
  ||| ||||||||||||||
3' - CUCCCUCCCUCCGGAGCCCC - 5'
	GDF7; ID01419.3p-miR; 876 nt; CDS;
	-115 kJ/mole; 89%; 23 nt
	5' - CGGGAG**G**GCUCCGGCCGGG**G**CAG - 3'
	  |||||||||||| |||| |||||
	3' - GCCCUC**U**CGAGGAUGGCAC**U**GUC - 5'

TP53; ID01838.5p-miR; 2460 nt; 3'UTR;
-110 kJ/mole; 88%; 24 nt
5' - **G**AUGG**G**GUCUCACAGUGUUGC**C**CA - 3'
  || ||||||||| ||||||||||
3' - **U**UUCC**U**CAGAGUGACACAACG**A**GU - 5'
	ROCK2; ID01836.5p-miR; 6497 nt; 3'UTR;
	-115 kJ/mole; 92%; 23 nt
	5' - G**C**CACUGCACUCCAGCCUGGGCA - 3'
	  ||||||||||||| ||||||||
	3' - A**A**GUGACGUGAGGUAGGACCCGU - 5'

Note: in bold type highlighted the non-canonical A-C and G-U pairs of nucleotides	

The given results demonstrate the important role of non-canonical pairs of nucleotides in the interaction of miRNA with mRNA of candidate genes involved in the development of EAC. For example, in the interaction of ID01838.5p-miR with mRNA of *TP53* gene, one pair of A-C and two pairs of G-U are formed. ID01419.3p-miR has binding sites in mRNA of *GDF7* gene and forms two pairs of G-U.

Conclusion

We studied the characteristics of the interaction of miRNA with mRNA of genes involved in the development of EAC. It was identified 19 key associations of miRNAs and mRNAs that have had a free energy of interaction of -120 kJ/mole or more that recommended both for the diagnostic markers of violations of the expression of these genes, and for the development of methods for the treatment of this subtype of disease at the molecular level.

The average free energy of miRNA binding in mRNA of genes involved in EAC development is greater in 5'UTR and CDS compared to 3'UTR, which suggests preferential binding of miRNA to 5'UTR and CDS of the studied genes. *ALDH1A2, ARID1A*, *BARX1*, *BTG3*, *DKK3*, *FOXP1*, *GDF7* and *SMAD4* genes was selected as candidate target genes for miRNAs having binding sites in 5'UTR of mRNA. *AR, ARID1A*, *AXIN2, BARX1, CEP72, GDF7, OXTR* and *SOX2* are candidate genes, having miRNAs binding sites in CDS, *ESR1, SMAD4* and *SOX2* are candidate genes in 3'UTR.

mRNA of these genes binds with miRNA with high affinity and, therefore, if the concentrations of the corresponding miRNAs are the same or exceed the concentrations of mRNAs, the effect of mRNA translation inhibition will be significant. Given that miRNAs are endogenous regulators of gene expression, these methods are quite feasible. The obtained results may be able to provide insights into pathogenesis mechanism and pave the way for the development of new diagnostic markers and therapeutic targets for patients with esophageal adenocarcinoma.

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