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CHARACTERISTICS OF miRNA INTERACTION WITH mRNA IN 5'UTR, CDS AND 3'UTR OF CANDIDATE GENES OF ESOPHAGEAL AND STOMACH CANCER

miRNAs demonstrate a class of small, non-coding RNAs that can regulate the expression of genes, and are associated with approximately all known physiological and pathological processes, especially cancer. Expression of many genes is regulated by binding of miRNA with mRNA, therefore it is required to identify candidate genes of esophageal and stomach cancers and to what extent they can interact with miRNA. To determine the important miRNAs biding sites in genes, involved in the development of esophageal and stomach cancers, there were used the MirTarget program. The article presents the results of studying the characteristics of the interaction of miRNAs with mRNAs of 121 genes involved in the development of esophageal and stomach cancer. From the 68 candidate genes, participating in the development of esophageal cancer, only 54 genes were targets for miRNAs. 148 miRNAs have binding sites at 5'UTR, CDS, and 3'UTR, and the average free binding energy (ΔG) of miRNAs with mRNAs was -126 kJ/mole, -121 kJ/mole and -111kJ/mole, respectively. 20 miRNAs and mRNA genes associations with a free energy of interaction more than -125 kJ/mole are recommended for the diagnosis of esophageal cancer. From the 106 candidate genes, participating in the development of stomach cancer, 86 genes were targets for miRNAs. 253 miRNAs have binding sites at 5'UTR, CDS and 3'UTR and the average free binding energy (ΔG) of miRNAs with mRNAs was -124 kJ/mole, -116 kJ/mole and -110 kJ/mole, respectively. 28 miRNAs associations with mRNAs are recommended for the diagnosis of stomach cancer that have a free energy of interaction more than -125 kJ/mole. 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, groups of miRNA and mRNA associations of candidate genes are recommended to develop methods for early diagnosis of esophageal and stomach cancer. The 768 previously unreported binding sites for 3071 miRNAs, which may be the main ones in the regulation of genes responsible for the development of esophageal and stomach cancers was established.

Key words: mRNA, miRNA, genes, oncological diseases, esophageal cancer, stomach cancer.

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Өңеш және асқазанның қатерлі ісігінің кандидатты гендердің mRNA-ның 5'UTR, CDS және 3'UTR-де miRNA-дың өзара әсерлесуі

miRNA кішімолекулалы, кодтамайтын RNA класын көрсетеді, олар гендердің экспрессиясын реттейді және барлық белгілі физиологиялық және патологиялық процестермен, әсіресе қатерлі ісікпен байланысты. Көптеген гендердің экспрессиясы miRNAның mRNAмен байланысуымен

реттеледі, сондықтан өңеш және асқазан қатерлі ісігінің кандидаты гендерін және олардың miRNAмен өзара әсерлесуінің дәрежесін анықтау қажет. Өңеш пен асқазанның қатерлі ісігі дамуына қатысатын гендердің miRNAмен маңызды байланысу сайттарын анықтау үшін MirTarget бағдарламасы қолданылды. Жұмыста өңеш пен асқазанның қатерлі ісігі дамуына қатысатын miRNA мен 121 гендердің mRNA өзара байланысу ерекшеліктерін зерттеу нәтижелері көрсетілген. Өңеш қатерлі ісігінің дамуына қатысатын 68 кандидаттық гендерден 54 гендер miRNA нысаналары болып келеді. 148 miRNA-дар үшін 5'UTR, CDS және 3'UTR байланысу сайттар бар және байланысудың бос энергиясы (ΔG) -126 kJ/mole, -121 kJ/mole және -111 kJ/mole тең, тиісінше. Өңештің қатерлі ісігін диагностикалау үшін, mRNA мен miRNA-дың 20 ассоциациялары ұсынылады, олардың байланысуының бос энергиясы -125 kJ/mole жоғары. Асқазанның қатерлі ісігінің дамуына қатысатын 106 кандидаттық гендерден 86 гендер miRNA нысаналары болып келеді. 253 miRNA 5'UTR, CDS және 3'UTR байланысу сайттары бар және байланысудың орташа бос энергиясы -124 kJ/mole, -116 kJ/mole және -110 kJ/mole тең, тиісінше. Асқазанның қатерлі ісігін диагностикасы үшін mRNA мен miRNA-дың 28 ассоциациялары ұсынылады, олардың байланысуының бос энергиясы -125 kJ/mole жоғары. Екі немесе одан да көп miRNA байланыстыру сайттар қамтитын көптеген гендердің mRNA-дары өздерінің нуклеотидті тізбектерінің қабаттасуымен кластерлерді құрайды. Алынған нәтижелер негізінде miRNA мен кандидатты гендердің mRNA-дың ассоциациялар топтары өңеш пен асқазанның қатерлі ісігінің алдын ала диагностикалау әдістерін дамыту үшін ұсынылады. 3071 miRNA-ның бұрын белгісіз 768 байланыстыру сайттары анықталды, олар өнеш және асқазан қатерлі ісігінің дамуына жауапты гендер реттеуінің басты шарты болуы мүмкін.

Түйін сөздер: mRNA, miRNA, гендер, онкологиялық аурулар, өнештің қатерлі ісігі, асқазанның қатерлі ісігі.

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Характеристики взаимодействия miRNA с 5'UTR, CDS И 3'UTR mRNA кандидатных генов рака пищевода и желудка

miRNA представляют класс небольших, некодирующих RNA, которые могут регулировать экспрессию генов и связаны с приблизительно всеми известными физиологическими и патологическими процессами, особенно с раком. Экспрессия многих генов регулируется связыванием miRNA с mRNA, поэтому необходимо идентифицировать гены-кандидаты рака пищевода и желудка и то, в какой степени они могут взаимодействовать с miRNA. Для определения важных сайтов связывания miRNA в генах, участвующих в развитии рака пищевода и желудка, была использована программа MirTarget. В работе представлены результаты изучения характеристик взаимодействия miRNA с mRNA 121 гена, участвующих в развитии рака пищевода и желудка. Из 68 генов-кандидатов, участвующих в развитии рака пищевода, мишенями miRNA являлись 54 гена. 148 miRNA имели сайты связывания в 5'UTR, CDS и 3'UTR и средняя свободная энергия связывания (ΔG) miRNA с mRNA равнялась -126 kJ/mole, -121 kJ/mole и -111 kJ/mole, соответственно. Для диагностики рака пищевода рекомендованы 20 ассоциаций miRNA с mRNA, имеющих свободную энергию взаимодействия более -125 kJ/mole. Из 106 генов-кандидатов, участвующих в развитии рака желудка, мишенями miRNA являлись 86 генов. 253 miRNA имели сайты связывания в 5'UTR, CDS и 3'UTR и средняя свободная энергия связывания (ΔG) miRNA с mRNA равнялась -124 kJ/mole, -116 kJ/mole и -110 kJ/mole, соответственно. Для диагностики рака желудка рекомендованы 28 ассоциаций miRNA с mRNA, имеющих свободную энергию взаимодействия более -125 kJ/mole. mRNA большинства генов, содержащих два и более сайтов связывания miRNA с наложением их нуклеотидных последовательностей, образуют кластеры. На основе полученных результатов рекомендуются группы ассоциаций miRNA и mRNA кандидатных генов для разработки методов ранней диагностики рака пищевода и желудка. Были установлены ранее не изученные 768 сайтов связывания 3071 miRNA, которые могут быть основными в регуляции генов, ответственных за развитие рака пищевода и желудка.

Ключевые слова: mRNA, miRNA, гены, онкологические заболевания, рак пищевода, рак желудка.

Introduction

Small RNAs that bind to mRNA and inhibit protein synthesis are called microRNAs (Bartel, 2004: 281). This name inadequately reflects the function of such RNAs. The size of these small RNAs of 17-27 nucleotides is 6-9 nanometers and, therefore, they are nanoscale, rather than microscale RNAs. These RNAs inhibit mRNA and therefore can be briefly termed miRNA: mRNA inhibitory RNA. The next misconception is that miRNA binds only in the 3'UTR of mRNA (Bartel, 2009: 215). Note that miRNAs interact with mRNA according to their physicochemical properties. Therefore, existing programs for searching for miRNA binding sites in mRNA are unreasonably limited to the 3'UTR domain. This restriction certainly does not take into account the miRNA binding sites in 5´UTR and CDS. Many studies have shown that miRNA binding sites exist in 5'UTR and CDS. However, this misconception continues to dominate the search for miRNA binding sites (Bartel, 2009: 215; Cipolla, 2014: 1). Another misconception is that the main role in binding miRNA to mRNA is played by 6-10 nucleotides, the so-called "seed" located in the 5'-end of miRNA (Bartel, 2009: 215). This assumption is incorrect for several reasons. miRNA arose many millions of years ago and during this time the entire nucleotide sequence remains practically unchanged (Cipolla, 2014: 1). The nucleotide sequences of the miRNA binding sites in mRNA are generally also conservative for millions of years (Naeli, 2017: 1).

Gastrointestinal (GI) tract cancer is one of the three most common oncological diseases in the world with a high mortality rate (Syngal, 2015: 223; Torre, 2015: 87). Esophageal cancer is the most invasive disease associated with inclusive poor prognosis. Efforts to identify diagnostic/prognostic markers have proven to be unsuccessful for translation into clinics. Esophageal cancer (EC) usually is found as either adenocarcinoma or squamous cell carcinomas (Mathé, 2009: 6192; Zeng, 2016: 232; Rustgi, 2014: 2499). Stomach cancer (SC) takes the second place among the leading causes of death from oncology diseases all over the world and continues to grow. Stomach cancer refers to cancer originating from any part of the stomach and mainly includes four histological types: adenocarcinoma, lymphoma, carcinoid tumor and gastrointestinal stromal tumor (Lin, 2012: 3081). Results on the study of gene expression, the sequencing of complete cancer genomes and the study of epigenetic disorders have shown that it is very difficult to determine the basic set of genes for each type of cancer (el-Rifai, 2002: 273). Stomach and esophageal adenocarcinomas are often considered as a single entity, even though differences exist in epidemiology, clinical presentation, molecular biology and treatment options (Fornaro, 2018: 90). Stomach and esophageal cancers are as main cancers of the gastrointestinal tract, which are associated with poor diagnosis and survival. 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. Among several biomarkers tissue specific and circulating miRNAs have emerged as powerful candidates in the diagnosis of stomach and esophageal cancers (Jamali, 2018: 1; Abbas, 2018: 1688). Recently, alteration in miRNA expression has emerged as an important hallmark of cancer. Different miRNAs can function as tumor suppressors or oncogenes in cancer cells, and the dysregulation of certain miRNAs may contribute to human cancer (Garofalo, 2011: 25; Zhou, 2017: 3893; Wang, 2018: 2018). An individual miRNA could potentially alter complex cellular processes such as cell growth, cell cycle, apoptosis and invasion. The recent emergence of observations on the role of miRNAs in cancer and their functions has induced many investigations to examine their relevance to esophageal and stomach cancer (Feng, 2018: 1595; Chen, 2018: 68; Cao, 2018: 1958; Guanen, 2018: 350).

Analysis of information to study the involvement of candidate genes in the development of gastrointestinal tract cancer shows that the number of publications on this problem increases in recent years. We have previously studied the characteristics of intronic human miRNAs and features of their interaction with mRNA (Berillo, 2013: 1374). But the present study aimed to identify not previously used miRNAs binding sites in mRNA of genes involved in the development of cancer of the esophagus and stomach and the clusters of miRNA binding sites and their properties. Studying of clusters of miRNA binding sites in mRNA of genes involved in the development of cancer of the esophagus and stomach in *Homo sapiens* are valuable for identification the role of these genes and miRNAs in oncogenesis.

Materials and Methods

The information about the role and function of genes participating in the development of esophageal and stomach cancer were taken from GeneBank databases and publications. The mRNA nucleotide sequences of the human genes were derived from GeneBank (http://www.ncbi.nlm.nih.gov). The

68 mRNAs of genes associated with development of esophageal cancer and 106 mRNAs of genes associated with development of stomach cancer were used in the study. The nucleotide sequences of 3701 miRNAs were taken from the article of Londin E. et. al (Londin, 2015: 1106).

Searching of miRNA's target genes was performed by MirTarget program, created in our laboratory. This program defines the beginning of miRNA and mRNA binding sites; 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 interaction. There were calculated the $\Delta G/\Delta G_m$ (%) ratio for each binding site, where ΔG_m is equal to free energy of interaction of miRNA with fully complementary nucleotide sequence. The miRNA-mRNA binding sites were taken with $\Delta G/\Delta G_m$ ratio higher than 86%. There were used the criterion that takes into account the length of miRNA, on which the energy ΔG also varied, which was differ for different miRNA lengths. Those, having the same ΔG/ΔGm value for miRNAs of 17 nt and 25 nt, the energy of binding of miRNA 25 nt was 1.47 times higher with respect to the absolute value of miRNA energy with 17 nucleotides length. This value makes it possible to reduce 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 esophageal and stomach cancer 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 esophageal and stomach cancer.

Table 1 – The list of candidate genes involved in the development of esophageal and stomach cancer

Candidate genes for esophageal cancer only: *CKS1B* (23301842); COL7A1 (18331784); DLG1 (25991909); DMD (28900487); DRD2 (16850143); FOXP2* (27382302); HOXC4 (17659465); HOXC5 (17659465); L7A* (17457978); PDE4D (23536305); PLEC (28900487); RGS22 (21533872); S1PR2 (18426913); SHANK2 (27058444); SP4 (19406933).*
Candidate genes for stomach cancer only: *AKT2 (25771729)*; *ARID4B (24570593)*; *BAALC (20841507)*; *CELSR3 (29085454)*; *CYLD (26711782)*; *COL3A1 (25500430)*; *DAPK3 (22160140)*; *EBF3 (21387304)*; *EPHB2 (17295683); ERBB4 (16187281); F13A1* (24159917); FGF14* (17071588); FKBP5 (22459275)*; *GAB1 (25743471); GFI1B (23528308)*; *GIPR (8243312)*; *HDAC4 (21725604)*; *IGF1R (14595755)*; *JMJD1C* (17549425); KDM1A (24914365)*; *KIAA1199 (19434458)*; *LARP7* (22488152); MGAT5 (29143776)*; *MTMR3 (28447759)*; *MTUS1* (24299308); MX2* (12082013); MYO5B (23456500)*; *PFKFB3 (27983531)*; *PKD1 (22217708)*; *POU2F2 (26019213)*; *PPP2CA (28904398)*; *PRDM2 (11544182)*; *PRKCA (28121923)*; *PRMT1 (26472729)*; *PTOV1 (20353268)*; *RASSF3 (26456015)*; *ROBO1 (28323002)*; *SEMA5A (23661031)*; *SKP1* (21190721); SLIT1 (27082735)*; *SLIT2* (27082735)*; *SLIT3 (27082735)*; *SND1 (25965817)*; *SREBF1 (25270091)*; *SREBF2 (19323650)*; *TACR1 (26852958)*; *TNKS (20811689)*; *TRRAP (18570183)*; *UGCG (29409484)*; *VPS13B (21733561)*; *WWP2 (19139817)*; *ZDHHC14 (24807047)*; *ZNF141* (17071588).*
Candidate genes for both esophageal and stomach cancers: *BBC3 (29966654)*; *CD58* (26774142); CDC16 (12029633)*; *CKS2 (26137251)*; *CPE (24716593); CUL2* (20712528); CYP19A1* (23110082); DCC (20150623); DDIT3* (26384350); DICER1 (24649159); DIS3L2* (22306653); DNMT3A (27789275); DTL* (29235520); EPCAM (24422715)*; *ERCC1 (25610304)*; *ETS1 (14562368)*; *EVL (29069803)*; *FAT2 (28930282)*; *FBXW7 (26886596)*; *FZD3 (24255701)*; *GDF15* (25867265); HNF4A (19468668)*; *IGF2 (19843644)*; *IHH (20307590)*; *ITCH (18552861)*; *ITGAL (24217965)*; *LAMB3 (29285246)*; *MAD1L1 (27895742)*; *MAP2K4 (23874846)*; *MCM7 (27476776)*; *MRE11A (23504502)*; *NFE2L2 (28900487)*; *NGFR (25244921)*; *NOTCH1 (28900487)*; *NPIP* (3528183); PARP1 (28789382); PAX5 (29099287); PPFIA1 (24009147); PTK2* (29285246); PTPRJ (25634668); PVR (20514215); RUNX1 (22171576); SFRP1 (21567192); STMN1 (28977901); TCF4 (25767603); TNFAIP6 (27072986); TNFRSF1B (20646319); TP63* (12447998); TPM3 (28138712); TRAF2 (24362534); TRPC6 (19651628); TRPV4 (27687509); UBC* (25820571).*
Note: In parentheses are shown the sources of information about candidate genes in PubMed. * – indicates the mRNAs, that are not targets for miRNA with chosen criteria.

From the table, we can observe that candidate genes can be either for esophageal or stomach cancer, or common. Part of the genes that are characteristic of only one can be used as selective markers. Some genes that are specific to only one type of disease can be used as selective markers. At the same time, there are genes involved in both types of disease. They also have a prognostic value. Genes that are not targets of miRNAs with ΔG/ΔGm value higher than 86%, show that their expression level is independent of miRNAs. And consequently, it is impossible to make associations of genes and mRNA on their basis. It was found that 13% of 121 candidate genes are not regulated by miRNA, and therefore their expression could not be suppressed.

1. The characteristics of miRNAs interaction with mRNAs of candidate genes, involved in the development of esophageal cancer

To determine the miRNAs that play a key role in the regulation of the translation of the protein coding genes involved in the development of EC, it was used a technique to search for the sites of interaction of miRNAs with high complementarity throughout the site sequence. As a result of the study of the 3701 miRNAs binding in mRNA of 68 protein-coding human genes, 54 mRNAs were identified as targets with given interaction criteria. miRNA binding sites with characteristics are represented in Tables 2-4.

Table 2 shows the results of a study of the interaction of miRNAs with mRNAs of genes in the 5'UTR. The mRNA of *EPCAM* gene has cluster of binding sites of miR-2-7088-3p and miR-10-25141-3p from 237 nt to 267 nt with ΔG value -117 kJ/mole and -119 kJ/mole, respectively.

mRNAs of *CDC16, HNF4A, IHH, NGFR, PAX5, PDE4D* and *RUNX1* genes have binding sites only for single miRNAs. The mRNA *PLEC* gene has three binding sites that form a cluster from 27 nt to 58 nt with average energy ΔG equal to -124 kJ/mole.

The mRNA of *PTPRJ* gene has seven binding sites for six miRNAs: miR-20-45152-5p, miR-2-3313-3p, miR-22-46979-5p and miR-1-155-3p, that form a cluster from 162 nt to 190 nt with a length 29 nt and average ΔG value equal to -132 kJ/mole; miR-12-10048-5p and miR-17-41183-5p form an another cluster from 206 nt to 236 nt with a length 31 nt and ΔG = -120 kJ/mole.

The average free energy binding of all miRNAs with mRNAs in the 5'UTR region was equal to -126 kJ/mole. 24 miRNAs bound with mRNAs of corresponding target genes, and the number of miRNA associations with mRNA having free energy of interaction greater than -125 kJ/mole is 10. These associations are recommended as markers for early diagnosis of esophageal cancer.

Table 3 shows the results of a study of the interaction of miRNAs with the mRNA of 29 genes in the CDS, each of which binds from one to several miRNAs. Some of these mRNAs bind six or more miRNAs. mRNA of *COL7A1, SHANK2* and *TNFRSF1B* genes have by two clusters of binding sites in CDS. Several miRNAs have some targets of studied genes. mir-1-2121-3p, miR-19-21199-3p and miR-19-33623-3p have binding sites in the CDS of mRNA of *BBC3* gene. These miRNAs are important as they have binding sites with mRNAs of studied genes.

mRNAs of *CPE, DCC, DICER1, DNMT3A, ETS1, EVL, FAT2, FBXW7, HOXC4, ITCH, MAD1L1, MAP2K4, NGFR, RGS22, S1PR2* and *TPM3* genes have binding site for single miRNAs.

The mRNA of *PLEC* gene has 10 binding sites, that form the following clusters: 1) miR-17-39011-3p, miR-13-32613-3p and miR-2-6862-5p form a cluster, localized from 5200 nt to 5231 nt with a length 32 nt and ΔG = -124 kJ/mole. 2) miR-9-25082-3p and miR-9-22187-3p cluster located in segment from 6335 nt to 6360 nt with an average ΔG value equal to -122 kJ/mole. 3) miR-19-41910-5p, miR-13-32613-3p and miR-5-15548-3p localize from 7013 nt to 7058 nt form a cluster with a length 46 nt and average ΔG = -129 kJ/mole.

The mRNA of *PARP1* gene has cluster from 1275 nt to 1302 nt with a whole length equal to 28 nt and average energy range equal to -111 kJ/mole. The mRNA of *HOXC5, ITGAL, PPFIA1, SP4, TRAF2, TRPC6* and *TRPV4* genes have binding sites for two miRNAs.

The average free energy of binding of all miRNAs with mRNAs in the CDS was equal to -121 kJ/mole. The number of miRNA associations with mRNA having free energy of interaction greater than -125 kJ/mole is eight. All of them can serve as markers in the development of methods for early diagnosis of esophageal cancer.

Characteristics of site location in the 3'UTR were selected by similar way as in 5'UTR and CDS. Of the *BBC3, ITCH, PVR, RUNX1, S1PR2, STMN1* target genes in the 3'UTR, their mRNAs form the associations with 4 miRNAs (table 4).

Table 2 – Characteristics of miRNA interaction with mRNA in the 5'UTR of genes involved in the development of esophageal cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
CDC16	miR-5-15733-3p	89	-134	90	24
DCC	miR-19-36133-3p	440	-121	92	22
	miR-12-32603-3p	482	-115	92	23
DICER1	miR-13-34605-5p	86	-115	92	21
	miR-5-15733-3p	86	-132	89	24
	miR-2-4989-3p	157	-115	90	22
DNMT3A	miR-2-6128-5p	44	-129	88	24
	miR-8-23997-5p	88	-102	94	19
DRD2	miR-14-32135-5p	10	-117	93	21
	miR-19-43527-5p	24	-125	89	23
EPCAM	miR-2-7088-3p	237	-117	92	21
	miR-10-25141-3p	244	-119	89	23
	miR-17-39405-5p	314	-115	89	23
ETS1	miR-4-11022-5p	20	-117	89	23
	miR-13-32878-3p	40	-113	91	21
	miR-11-29831-3p	57	-134	89	24
HNF4A	miR-9-26042-5p	53	-125	92	22
IHH	miR-7-21068-3p	23	-129	88	24
MCM7	miR-7-20142-5p	26	-119	89	23
	miR-8-23353-3p	111	-121	90	22
	miR-16-39014-5p	846	-106	91	21
NGFR	miR-19-43963-5p	12	-119	92	22
PAX5	miR-2-6328-5p	331	-119	90	23
PDE4D	miR-17-39416-3p	66	-121	92	22
PLEC	miR-7-23800-3p	27÷35 (2)	-121 ÷ -125	89÷92	23
	miR-17-39570-5p	36	-127	94	22
PTPRJ	miR-14-37452-3p	32	-125	91	23
	miR-7-15849-3p	85	-110	96	18
	miR-20-45152-5p	162	-134	90	24
	miR-2-3313-3p	163÷165 (2)	-138	87	25
	miR-22-46979-5p	166	-123	89	23
	miR-1-155-3p	168	-125	91	22
	miR-12-10048-5p	206	-117	92	20
	miR-17-41183-5p	213	-123	89	23
	miR-9-27797-5p	240	-125	88	24
	miR-9-20317-3p	267	-132	89	24
RUNX1	miR-5-14114-5p	1417	-123	89	23
SFRP1	miR-19-33623-3p	111	-132	89	24
	miR-11-28567-3p	175	-123	89	23
TRPC6	miR-9-25488-3p	346	-113	93	20
	miR-10-11155-3p	392	-115	90	22
Note: Here and in the tables below the number of miRNAs binding sites is indicated in parentheses					

Table 3 – Characteristics of miRNA interaction with mRNA in the CDS of genes involved in the development of esophageal cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
BBC3	miR-22-45004-5p	408	-115	95	21
	mir-1-2121-3p	455	-138	88	25
	miR-19-21199-3p	457	-138	88	25
	miR-19-33623-3p	458	-132	89	24
	miR-22-42699-3p	869	-113	90	23
COL7A1	miR-8-23997-5p	4008	-102	94	19
	miR-10-26838-3p	4491	-110	90	22
	miR-6-17644-5p	4669	-123	91	23
	miR-13-32983-5p	4677	-119	89	23
	miR-11-26584-3p	5567	-110	90	23
	miR-1-2558-3p	6143	-113	90	22
	miR-7-20724-5p	7016	-104	92	20
	miR-9-23731-3p	7503	-113	88	24
	miR-11-30599-3p	8089	-123	88	24
CPE	miR-3-3472-5p	355	-115	90	22
DCC	miR-10-27508-3p	2971	-102	91	22
DICER1	miR-12-32603-3p	4492	-117	93	23
DNMT3A	miR-5-14479-5p	681	-115	90	23
ETS1	miR-11-28259-3p	1295	-108	96	20
EVL	miR-X-48174-3p	864	-132	93	24
FAT2	miR-7-19687-3p	12856	-115	89	23
FBXW7	miR-4-13692-3p	1243	-108	93	22
HOXC4	miR-19-30988-5p	364	-129	90	23
HOXC5	miR-22-45959-3p	275	-115	92	22
	miR-5-16341-5p	316	-115	89	23
ITCH	miR-17-34996-5p	725	-110	90	23
ITGAL	miR-1-1982-3p	2292	-100	90	22
	miR-2-6532-3p	2712	-108	89	23
MAD1L1	miR-3-9978-3p	1965	-113	90	22
MAP2K4	miR-7-20203-3p	91	-123	92	22
NGFR	miR-3-9952-3p	1309	-115	89	23
NOTCH1	miR-2-7838-5p	46	-123	91	22
	miR-7-20621-3p	2480	-108	91	21
	miR-21-44879-5p	4415	-119	93	23
	miR-9-25099-3p	4972	-115	90	22
	miR-6-19010-3p	5181	-119	89	23
	miR-18-41949-5p	6766	-123	92	22
PARP1	miR-19-36095-3p	1275	-119	90	23
	miR-17-12514-5p	1282	-102	91	20
PLEC	miR-17-39011-3p	5200	-119	90	23
	miR-13-32613-3p	5201	-132	93	24

Continuation of table 3

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
	miR-2-6862-5p	5208	-121	89	23
	miR-9-25082-3p	6335	-125	88	24
	miR-9-22187-3p	6337	-119	89	23
	miR-22-40302-3p	6503	-119	90	22
	miR-14-34560-3p	6503	-119	89	24
	miR-19-41910-5p	7013	-129	88	24
	miR-13-32613-3p	7022	-132	93	24
	miR-5-15548-3p	7035	-127	91	23
PPFIA1	miR-5-17240-3p	63	-119	89	23
	miR-21-43879-3p	171	-115	93	20
RGS22	miR-3-11123-5p	3500	-98	90	22
S1PR2	miR-11-30639-3p	1169	-115	90	23
SHANK2	miR-10-13751-3p	4664	-125	95	21
	miR-17-40012-5p	4666	-113	91	21
SP4	miR-17-39416-3p	215	-121	92	22
	miR-2-6184-3p	1130	-119	92	23
TNFRSF1B	miR-17-36319-3p	1156	-127	90	24
	miR-16-37909-3p	1157	-110	91	21
TPM3	miR-5-14479-5p	335	-117	92	23
TRAF2	miR-17-41486-3p	1178	-110	91	21
	miR-10-27780-3p	1395	-106	88	24
TRPC6	miR-17-39143-3p	470	-125	91	24
	miR-5-15829-5p	527	-110	91	22
TRPV4	miR-15-36320-5p	1371	-121	90	23
	miR-1-1855-3p	2078	-104	89	23

In mRNA of *ETS1* gene it was found the interesting evidence: miR-15-36862-3p and miR-10-29282-3p have 12 and 11 multiple binding sites, respectively. They located from 3875 nt to 3931 nt. The effect of each of the miRNAs will depend on the ratio of their concentrations, and overall the expression of the *ETS1* gene will be determined by the total concentration of miR-15-36862-3p and miR-10-29282-3p, since they have close free energies interaction (ΔG are equal to -108 kJ/mole and -107 kJ/mole, respectively) with mRNA of *ETS1* gene. The same miR-15-36862-3p and miR-10-29282-3p form a clustered binding site with a length 33 nt located from 5454 nt to 5487 nt in the 3'UTR of mRNA of *RUNX1* gene.

The mRNA of *S1PR2* has seven miRNA binding sites in the 3'UTR.The binding sites of miR-2-4804-5p and miR-17-39935-3p are located in cluster from 2763 nt to 2795 nt with a length 33 nt and with average ΔG = -110 kJ/mole. miR-19-42814-5p and miR-10-29282-3p form cluster from 3191 nt to 3218 nt with ΔG value equal to -105 kJ/mole.

The mRNA of *DMD* gene has cluster from 11762 nt to 11791 nt with average ΔG = -104 kJ/mole. mRNA of *CKS2, DLG1, ERCC1, FAT2, IHH, MRE11A, NGFR, TRPC6* and *TRPV4* genes have binding sites only for single miRNAs. *TNFRSF1B* gene has a cluster from 2321 nt to 2365 nt with an average ΔG value equal to -112 kJ/mole.

The *IGF2* gene has clustered binding sites from 2286 nt to 2351 nt with average ΔG = -108.3 kJ/mole and also in position of 2442-2463 nt with ΔG = -108.8 kJ/mole. The average free energy of binding of miRNAs with all mRNAs in the 3'UTR was equal to -111 kJ/mole.

Table 4 – Characteristics of miRNA interaction with mRNA in the 3'UTR of genes involved in the development of esophageal cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
BBC3	miR-17-42375-5p	999	-119	89	23
	miR-19-41914-3p	1130	-121	95	21
	miR-11-12657-3p	1625	-108	91	21
	miR-6-18764-3p	1681	-123	89	24
CKS2	miR-3-11351-5p	556	-79	97	17
DLG1	miR-9-24591-3p	4250	-108	89	23
DMD	miR-3-5147-5p	11762÷11766 (2)	-100÷-102	90÷92	22
	miR-101-27078-5p	11768	-110	91	23
DRD2	miR-7-2273-5p	2039	-115	93	21
	miR-9-24471-3p	2581	-115	90	23
ERCC1	miR-17-9289-5p	1544	-127	94	24
ETS1	miR-6-20721-5p	3284	-96	90	22
	miR-15-36862-3p	3875÷3908 (12)	-108	89	23
	miR-10-29282-3p	3888÷3908 (11)	-104÷-108	89	23
FAT2	miR-19-43329-3p	13551	-123	91	24
FZD3	miR-20-20331-5p	6810	-106	91	21
	miR-14-35161-5p	9897	-117	89	24
	miR-2-4826-5p	10100÷01 (2)	-113	90	23
HOXC4	miR-11-18690-5p	1258	-110	90	22
	miR-13-35476-3p	1387	-117	90	22
HOXC5	miR-17-39477-3p	828	-113	91	21
	miR-2-6824-3p	935	-110	90	22
IGF2	miR-101-27078-5p	2286÷2351 (6)	-108÷-113	89÷93	23
	miR-3-5147-5p	2301	-104	94	22
	miR-3-5147-5p	2345	-104	94	22
	miR-101-27078-5p	2404÷2412 (2)	-110	91	23
	miR-3-5147-5p	2345	-104	94	22
	miR-101-27078-5p	2442÷2463 (3)	-108÷-113	89÷93	23
	miR-3-5147-5p	2457	-104	94	22
	miR-101-27078-5p	2520÷2539 (3)	-108	89	23
	miR-101-27078-5p	2571	-108	89	23
	miR-101-27078-5p	2620	-108	89	23
	miR-101-27078-5p	2655÷2672 (3)	-108÷-110	89÷91	23
	miR-101-27078-5p	2704÷2725 (2)	-108	89	23
	miR-101-27078-5p	2837	-110	91	23
	miR-9-24619-3p	4292	-115	90	23
IHH	miR-6-19324-3p	1940	-121	89	23
ITCH	miR-14-35161-5p	3672	-119	90	24
	miR-22-45335-5p	3727	-113	90	23
	miR-19-42529-3p	5645	-110	90	22

Continuation of table 4

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
	miR-22-45335-5p	5899	-115	92	23
ITGAL	miR-10-11641-3p	4703	-119	89	23
	miR-22-45335-5p	4943	-113	90	23
MRE11A	miR-11-18690-5p	2966	-110	90	22
NGFR	miR-6-18910-3p	1996	-108	93	20
PAX5	miR-15-37146-3p	2136	-110	90	22
	miR-17-36319-3p	2864	-127	90	24
PLEC	miR-7-17280-5p	14501	-119	90	22
	miR-17-39642-5p	14781	-115	90	22
	miR-17-40730-3p	15022	-119	89	23
PVR	miR-17-39935-3p	3528	-104	91	21
	miR-8-17639-3p	3573	-110	91	22
	miR-7-21133-5p	5105	-121	89	24
	miR-19-43240-3p	5734	-113	93	21
RUNX1	miR-4-11239-3p	3123	-115	93	20
	miR-1-2558-3p	3368	-117	93	22
	miR-15-36862-3p	5454-5464 (2)	-108÷-113	89÷93	23
	miR-10-29282-3p	5464	-108	93	23
S1PR2	miR-2-4804-5p	2763-2764 (2)	-110÷-115	88÷92	24
	miR-17-39935-3p	2774	-104	91	21
	miR-19-42814-5p	3191-3195 (2)	-104	89	23
	miR-10-29282-3p	3192-3194 (2)	-104÷-106	89÷91	23
STMN1	miR-5-17240-3p	1096	-119	89	23
	miR-7-13347-5p	1730	-106	91	22
	miR-10-26483-5p	1744	-113	91	22
	miR-2-5355-3p	1987	-119	93	22
TNFRSF1B	miR-2-4826-5p	2321-2322 (2)	-113	90	23
	miR-17-8001-3p	2342	-110	90	23
	miR-9-24929-3p	3040	-119	92	23
TPM3	miR-17-34996-5p	3181	-110	90	23
	miR-10-26483-5p	3540	-117	95	22
	miR-8-11096-5p	4451	-117	93	22
TRPC6	miR-6-15855-3p	3877	-96	92	21
TRPV4	miR-11-31858-5p	2714	-110	93	20

Table 5 shows the patterns of miRNA interaction with mRNA of the candidate genes involved in the development of esophageal cancer. The results show that nucleotides forming non-canonical pairs participate in the interaction of miRNA with mRNA: G-U and A-C. Due to this, the interaction of all nucleotides is taken into account, which increases the free energy of the interaction of miRNA with mRNA. miR-10-29282-3p has 12 binding sites in the 3'UTR mRNA of *ETS1* gene with identical characteristics.

Table 5 – Schemes of the interaction of miRNA with mRNA of candidate genes of the esophageal cancer

PTPRJ; miR-14-37452-3p; 5'UTR;32; -125; 91; 23 5'-AGCGGGAGCAGCCGCGGGAGCCG-3' 3'-UCGCUCCCGUCGGCGCCCCCGGU-5'	*PTPRJ*; miR-2-3313-3p; 5'UTR; 163; -138; 87; 25 5'-UGUGGCCGCGGCCGCCGCCGCCGCU-3' 3'-GCCCCGGCGGCGGCGGCGGCGGCGG-5'
PLEC; miR-19-42706-5p; CDS; 6157; -125; 91; 23 5'-AGGAGGCGGAGAACGAGCGCCUG-3' 3'-UCCUCCGCCUCCCGCUCGCGGGC-5'	*TRAF2*; miR-17-41486-3p; CDS; 1178; -110; 91; 21 5'-CCGCCAUCUUCUCCCCAGCCU-3' 3'-GGCGGUAGAAGGGAGGCCGGA-5'
ITCH; miR-17-34996-5p; 725; CDS; -110; 90; 23 5'-GCAACCUCUGCCUCCCGGGUUUA-3' 3'-CGUUAGAGAAGGAGAGCCCAAGU-5'	*ITCH*; miR-14-35161-5p; 3672; 3'UTR; -119; 90; 24 5'-GCACUCUGGGAGGCCGAGGCAGGA-3' 3'-UGUGAAACCCUCUCGCUCCGUCCU-5'
IGF2; miR-101-27078-5p; 2295; 3'UTR; -104; 86; 23 5'-GCACACACACGCACACACAUGCA-3' 3'-UGUGUGUGCGUAUGUGUGCAUGU-5'	*IGF2*; miR-101-27078-5p; 2341; 3'UTR; -113; 93; 23 5'-ACACACACGCACACACAUGCACA-3' 3'-UGUGUGUGCGUAUGUGUGCAUGU-5'
ETS1; miR-10-29282-3p; 3888; 3'UTR; -104; 89; 23 5'-GUGUGUGUGUGUGUGUGUGUG-3' 3'-CACACACGCAUAUAUACACACAU-5'	*RUNX1;* miR-10-29282-3p; 5464; 3'UTR; -108; 92; 23 5'-GUGUGUGCGUGUGUGUGUGUG-3' 3'-CACACACGCAUAUAUACACACAU-5'
Note. Here and in Tables 5 and 9, the first line shows: the name of the gene; miRNA; mRNA region; beginning of the miRNA binding site, nt; the value of ΔG, kJ/mole; the value of ΔG/ΔGm,%; the length of miRNA, nt.	

2. The characteristics of miRNAs interaction with mRNAs of candidate genes, involved in the development of stomach cancer

To determine miRNA, the targets of which are the genes responsible for the development of SC, it was conducted a search for binding sites in 106 mRNA human genes responsible for stomach oncogenesis. As a result of the study, it was found that 85 of the 106 genes have binding sites with high affinity for miRNA (Table 6).

The degree of interaction of miRNA with mRNA is determined by the amount of free energy (ΔG) of their binding. For this indicator, several miRNAs can be distinguished.

Binding sites of miR-17-39555-3p, miR-1-654-3p and miR-14-18322-3p in mRNA *EPHB2* gene located from 74 nt to 121 nt. The free energy of interaction of these miRNAs is average ΔG value equal to -115 kJ/mole.

The mRNA of *ROBO1* has cluster from 596 nt to 616 nt with an average energy range equal to -114 kJ/mole. miR-22-23987-3p and miR-5-8853-5p have the same binding sites in mRNA of *ROBO1* with ΔG value equal to -123 kJ/mole and -117 kJ/mole, respectively.

Identified miR-3-8100-5p, miR-9-5204-5p, miR-5-8853-5p binding sites in mRNA of *SLIT3* gene located from 220 nt to 256 nt with average ΔG/ΔGm value was 91%, and an average ΔG value equal to 124 kJ/mole. The degree of complementarity of the interaction of miRNA with mRNA is highest for the *SLIT3* and miR-5-8853-5p association. It should be remembered that the effectiveness of miRNA interaction with mRNA is determined not only by the degree of complementarity of nucleotides in the binding site, but also by the nucleotide interaction energy. Consequently, the more G-C-pairs are formed, the stronger binding of miRNA with mRNA will be (Table 6). mRNA of *EPHB2* gene has a cluster from 74 nt to 121 nt with an average energy of hybridization equal to -115 kJ/mole. mRNA of *SLIT3* gene has two clusters, in segment from 26 nt to 55 nt with ΔG = -122 kJ/mole and from 220 nt to 256 nt with ΔG = -124 kJ/mole.

mRNA of *ARID4B, BAALC, CDC16, FKBP5, GIPR, HNF4A, IHH, KDM1A, KIAA1199, NGFR, PAX5, PKD1, PPP2CA, PTOV1, RASSF3, SEMA5A* and *TACR1* genes have the binding sites with single miRNA.

The mRNA of *ETS1* gene has cluster from 20 nt to 81 nt with an average energy of hybridization equal to 121 kJ/mole.

Table 6 – Characteristics of miRNA interaction with mRNA in the 5'UTR of genes involved in the development of stomach cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ARID4B	miR-21-40861-3p	389	-110	90	22
BAALC	miR-19-43576-5p	197	-108	91	21
CDC16	miR-5-15733-3p	89	-134	90	24
CELSR3	miR-20-44217-3p	166	-127	92	23
	miR-17-35537-3p	168	-115	90	21
	miR-9-27797-5p	271	-125	88	24
CYLD	miR-15-37972-3p	15	-110	90	22
	miR-X-37327-3p	52	-115	93	20
DCC	miR-19-36133-3p	440	-121	92	22
	miR-12-32603-3p	482	-115	92	23
DICER1	miR-13-34605-5p	86	-115	92	21
	miR-5-15733-3p	86	-132	89	24
	miR-2-4989-3p	157	-115	90	22
DNMT3A	miR-2-6128-5p	44	-129	88	24
	miR-8-23997-5p	88	-102	94	19
EPCAM	miR-2-7088-3p	237	-117	92	21
	miR-10-25141-3p	244	-119	89	23
	miR-17-39405-5p	314	-115	89	23
EPHB2	miR-17-39555-3p	74	-108	96	18
	miR-1-654-3p	82	-115	92	20
	miR-14-18322-3p	100	-123	92	21
ETS1	miR-4-11022-5p	20	-117	89	23
	miR-13-32878-3p	40	-113	91	21
	miR-11-29831-3p	57	-134	89	24
FKBP5	miR-19-42169-5p	11	-115	89	23
GIPR	miR-12-31754-5p	36	-119	90	23
HNF4A	miR-9-26042-5p	53	-125	92	22
IHH	miR-7-21068-3p	23	-129	88	24
KDM1A	miR-10-28030-3p	54	-127	91	23
KIAA1199	miR-16-37525-3p	54	-119	90	22
MCM7	miR-7-20142-5p	26	-119	89	23
	miR-8-23353-3p	111	-121	90	22
	miR-16-39014-5p	846	-106	91	21
MTMR3	miR-14-35410-5p	130	-115	92	22
	miR-19-38260-3p	135	-113	90	22
MYO5B	miR-8-23323-3p	24	-123	91	23
	miR-19-36992-3p	248	-110	93	20
NGFR	miR-19-43963-5p	12	-119	92	22
PAX5	miR-2-6328-5p	331	-119	90	23
PKD1	miR-16-38906-3p	161	-127	88	24
PPP2CA	miR-9-22179-3p	368	-110	93	21
PTOV1	miR-10-13751-3p	79	-121	92	21

Continuation of table 6

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
PTPRJ	miR-14-37452-3p	32	-125	91	23
	miR-7-15849-3p	85	-110	96	18
	miR-20-45152-5p	162	-134	90	24
	miR-2-3313-3p	163	-138	87	25
	miR-2-4453-3p	165	-119	90	21
	miR-22-46979-5p	166	-123	89	23
	miR-1-155-3p	168	-125	91	22
	miR-12-10048-5p	206	-117	92	20
	miR-17-41183-5p	213	-123	89	23
	miR-9-27797-5p	240	-125	88	24
	miR-9-20317-3p	267	-132	89	24
RASSF3	miR-16-7974-5p	21	-121	90	22
ROBO1	miR-2-7838-5p	501	-123	91	22
	miR-22-23987-3p	596	-123	94	21
	miR-5-8853-5p	596	-117	93	20
	miR-2-6409-5p	599	-102	96	17
RUNX1	miR-5-14114-5p	1417	-123	89	23
	miR-17-40012-5p	1435-1436 (2)	-110	90	21
SEMA5A	miR-1-2602-3p	411	-117	93	22
SFRP1	miR-X-46434-5p	105	-113	90	21
	miR-19-33623-3p	111	-132	89	24
	miR-11-28567-3p	175	-123	89	23
SLIT3	miR-9-15689-5p	26	-129	91	24
	miR-19-43373-3p	34	-115	90	21
	miR-5-14202-5p	137	-123	91	22
	miR-3-8100-5p	220	-129	88	24
	miR-9-5204-5p	226	-123	92	22
	miR-5-8853-5p	236	-119	95	20
TACR1	miR-3-9000-5p	511	-104	91	21
TRPC6	miR-9-25488-3p	346	-113	93	20
	miR-10-11155-3p	392	-115	90	22
UGCG	miR-15-38620-5p	132	-119	90	22
	miR-1-654-3p	194	-117	93	20
ZDHHC14	miR-X-44972-5p	159	-117	92	20
	miR-17-39593-3p	427	-136	89	24

Analysis of clusters localization relatively to genes by use of such instruments, as MirTarget program, give ability to identify the localization of clustered binding sites, to classify which miRNA has ability to concur for binding site, what is additional functional characteristics of such clusters. Additional characteristics of nucleic acid sequences for description of clustered binding sites, such values as (ΔG, kJ/mole) and (ΔG/ΔGm, %) allow more precise determination of what miRNAs will have the advantage and allow qualitatively solve new problems of analysis of binding sites by their indicators. The development of methods for locating binding sites in mRNA genes facilitates the

determination of more accurate patterns of miRNA functioning for the regulation of gene transcription.

The average free energy of binding of miRNA with mRNA in the 5'UTR of all mRNA is -124 kJ/mole. The number of miRNA associations with mRNA having a free interaction energy of more than -125 kJ/mole is 14. All of them can serve as markers in the development of methods for the early diagnosis of stomach cancer.

Table 7 shows the results of a study of the interaction of miRNAs with mRNAs of 48 genes in the CDS regions, each of which have binding sites for one to several miRNAs. mRNA of *BBC3* and *PRDM2* genes have three clustered binding sites of miRNA. In mRNA of *PRDM2* gene the average free energy value is equal to -112 kJ/mole. mRNA of *ARID4B, DAPK3* and *KDM1A* have the consequentially located multiple binding sites with miRNA with free energy range between -108 kJ/mole to -132 kJ/mole.

In the CDS of mRNA *BBC3* gene identified three miRNAs binding sites with overlapped nucleotide sequences. They all form a cluster from 455 nt to 482 nt with average ΔG value equal to -136 kJ/mole. 11 miRNAs binding sites identified in mRNA of *PKD1* gene, and two miRNAs have had the same starting nucleotide. mRNAs of *BAALC, COL3A1, EPHB2, ITGAL, PARP1, PPFIA1, PRDM2, SREBF1, TRAF2, TRPC6,* and *TRPV4* have two binding sites for miRNAs in each gene.

Table 7 – Characteristics of miRNA interaction with mRNA in the CDS of genes involved in the development of stomach cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ARID4B	miR-9-26506-3p	2134-37 (2)	-110	90	22
BAALC	miR-1-3822-3p	207	-121	92	22
	miR-18-41032-5p	305	-123	92	22
BBC3	miR-22-45004-5p	408	-115	95	21
	mir-1-2121-3p	455	-138	88	25
	miR-19-21199-3p	457	-138	88	25
	miR-19-33623-3p	458	-132	89	24
	miR-22-42699-3p	869	-113	90	23
CELSR3	miR-21-43422-5p	374	-113	90	22
	miR-3-8100-5p	1710	-129	88	24
	miR-17-40348-5p	1714	-121	89	23
	miR-10-27065-3p	4533	-115	92	21
	miR-16-38755-3p	4712	-119	89	24
COL3A1	miR-22-45188-5p	3517	-113	93	22
	miR-6-19625-5p	3593	-117	92	23
CPE	miR-3-3472-5p	355	-115	90	22
DAPK3	miR-11-24912-5p	813-14 (2)	-108	91	21
DCC	miR-10-27508-3p	2971	-102	91	22
DICER1	miR-12-32603-3p	4492	-117	93	23
DNMT3A	miR-5-14479-5p	681	-115	90	23
EBF3	miR-10-26815-5p	1629	-121	88	24
EPHB2	miR-12-31830-3p	155	-127	91	24
	miR-17-40348-5p	169	-121	89	23
ETS1	miR-11-28259-3p	1295	-108	96	20
EVL	miR-X-48174-3p	864	-132	93	24
FAT2	miR-7-19687-3p	12856	-115	89	23
FBXW7	miR-4-13692-3p	1243	-108	93	22
GFI1B	miR-11-29046-5p	436	-100	90	22

Continuation of table 7

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
GIPR	miR-7-20718-5p	688	-113	90	23
ITCH	miR-17-34996-5p	725	-110	90	23
ITGAL	miR-1-1982-3p	2292	-100	90	22
	miR-2-6532-3p	2712	-108	89	23
KDM1A	miR-9-22187-3p	179	-119	89	23
	miR-16-39014-5p	208	-106	91	21
	miR-1-2180-3p	341-342 (2)	-127÷-136	94-100	22
	miR-19-43437-5p	601	-117	92	23
KIAA1199	miR-17-35122-5p	364	-113	91	21
MAD1L1	miR-3-9978-3p	1965	-113	90	22
MAP2K4	miR-7-20203-3p	91	-123	92	22
MYO5B	miR-3-9744-5p	1422	-117	89	23
NGFR	miR-3-9952-3p	1309	-115	89	23
NOTCH1	miR-2-7838-5p	46	-123	91	22
	miR-7-20621-3p	2480	-108	91	21
	miR-21-44879-5p	4415	-119	93	23
	miR-9-25099-3p	4972	-115	90	22
	miR-6-19010-3p	5181	-119	89	23
	miR-18-41949-5p	6766	-123	92	22
PARP1	miR-19-36095-3p	1275	-119	90	23
	miR-17-12514-5p	1282	-102	91	20
PFKFB3	miR-22-23015-3p	1877	-106	91	21
PKD1	miR-17-39570-5p	213	-123	91	22
	miR-19-44070-3p	295	-117	89	23
	miR-11-29553-3p	974	-119	92	21
	miR-2-6831-5p	2059	-110	93	20
	miR-10-8412-5p	3010	-132	91	23
	miR-8-25030-3p	3323	-119	90	23
	miR-11-30592-3p	5986	-117	90	23
	miR-9-15689-5p	6448	-125	88	24
	miR-1-869-3p	11047	-102	92	22
	miR-8-21107-5p	11450	-110	90	22
	miR-11-31032-3p	12009	-119	89	23
	miR-19-41434-3p	12767	-110	91	21
	miR-9-25624-3p	12767	-108	91	21
POU2F2	miR-19-43819-5p	1145	-110	90	22
	miR-1-2802-3p	1308	-113	90	22
	miR-8-23986-3p	1315	-129	90	24
PPFIA1	miR-5-17240-3p	63	-119	89	23
	miR-21-43879-3p	171	-115	93	20
PRDM2	miR-9-26506-3p	1661-64 (2)	-110÷-113	90-91	22
	miR-12-32603-3p	1666	-113	90	23
PRKCA	miR-19-42303-3p	1789	-115	89	23

Continuation of table 7

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
PTOV1	miR-18-41128-3p	191	-113	91	21
	miR-1-1007-3p	227	-115	89	23
	miR-1-2030-3p	1131	-110	90	22
	miR-19-37450-3p	1393	-108	94	21
PTPRJ	miR-17-35260-3p	408	-117	92	22
SLIT1	miR-22-38710-5p	504	-110	91	21
SND1	miR-10-26483-5p	1344	-110	90	22
	miR-9-25082-3p	1993	-125	88	24
	miR-2-6494-3p	2234	-102	92	21
SREBF1	miR-8-23525-5p	323	-113	93	20
	miR-8-23111-3p	546	-117	90	22
SREBF2	miR-1-2002-3p	564	-121	90	22
TNFRSF1B	miR-17-36319-3p	1156	-127	90	24
	miR-16-37909-3p	1157	-110	91	21
TNKS	miR-7-19239-3p	74	-125	89	23
	miR-11-29000-5p	454	-119	89	23
	miR-1-3558-3p	2944	-110	90	22
TPM3	miR-5-14479-5p	335	-117	92	23
TRAF2	miR-17-41486-3p	1178	-110	91	21
	miR-10-27780-3p	1395	-106	88	24
TRPC6	miR-17-39143-3p	470	-125	91	24
	miR-5-15829-5p	527	-110	91	22
TRPV4	miR-15-36320-5p	1371	-121	90	23
	miR-1-1855-3p	2078	-104	89	23
TRRAP	miR-19-33623-3p	1735	-132	89	24
VPS13B	miR-1-4248-3p	3692	-110	93	21

mRNAs of *CPE, DCC, DICER1, DNMT3A, EBF3, ETS1, EVL, FAT2, FBXW7, GFI1B, GIPR, ITCH, KIAA1199, MAD1L1, MAP2K4, MYO5B, NGFR, PFKFB3, PRKCA, PTPRJ, SLIT1, SREBF2, TPM3, TRRAP* and *VPS13B* genes have binding sites for single miRNAs with high affinity.

The average free energy of binding of miRNA with mRNA in the CDS of all mRNA is -116 kJ/mole. The number of miRNA associations with mRNA having free interaction energy is -125 kJ/mole and more is 12 (Table 7). All of them can serve as markers in the development of methods for early diagnosis of SC.

The characteristics of the interaction of miRNA with mRNAs of 47 target genes in the 3'UTR are given in Table 8. The mRNA of some genes can bind 2 or more miRNAs. There were six binding sites for different miRNAs in the 3'UTR of *AKT2* gene, two of which form a cluster from 2248 nt to 2279 nt. Four miRNAs have binding sites in mRNA of *BBC3* gene.

mRNA of *CKS2, CYLD, EBF3, ERBB4, FAT2, HDAC4, IHH, ITGAL, MRE11A, MTMR3, NGFR, PFKFB3, PRMT1, PTOV1, POU2F2, SLIT1, SND1, SREBF1, TRPC6* and *TRPV4* genes has binding sites for single miRNAs.

mRNAs of *CELSR3, EPHB2, ERCC1, KIAA1199, PAX5, PRDM2, POU2F2, PRKCA, SEMA5A, SFRP1, SREBF2, VPS13B* and *WWP2* genes have binding sites for two miRNAs.

Table 8 – Characteristics of miRNA interaction with mRNA in the 3'UTR of genes involved in the development of stomach cancer

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
AKT2	miR-17-42375-5p	1935	-119	89	23
	miR-8-24024-3p	2248	-123	89	24
	miR-15-38767-3p	2255	-121	88	24
	miR-12-32747-3p	2551	-110	93	20
	miR-19-39818-3p	3095	-102	91	20
	miR-1-75-3p	3926	-117	90	22
BBC3	miR-17-42375-5p	999	-119	89	23
	miR-19-41914-3p	1130	-121	95	21
	miR-11-12657-3p	1625	-108	91	21
	miR-6-18764-3p	1681	-123	89	24
CELSR3	miR-16-37914-3p	10515	-119	86	25
	miR-19-43804-3p	10520	-110	91	21
CKS2	miR-3-11351-5p	556	-79	97	17
CYLD	miR-10-29282-3p	6075	-106	91	23
EBF3	miR-19-41383-3p	2255	-115	90	23
EPHB2	miR-10-28283-5p	3606	-123	85	25
	miR-17-39907-3p	4700	-108	91	21
ERBB4	miR-21-40861-3p	10515	-113	91	22
ERCC1	miR-4-13310-3p	1042	-104	91	20
	miR-17-9289-5p	1544	-127	94	24
ETS1	miR-6-20721-5p	3284	-96	90	22
	miR-15-36862-3p	3875÷3908 (12)	-108	89	23
	miR-19-42814-5p	3876÷3913 (15)	-100÷-102	85÷87	23
	miR-10-29282-3p	3888÷3908 (11)	-104÷-108	89	23
FAT2	miR-19-43329-3p	13551	-123	91	24
FKBP5	miR-2-5355-3p	1434	-115	90	22
	miR-10-26483-5p	4870	-110	90	22
	miR-11-29602-5p	5727	-106	91	21
	miR-17-39935-3p	6364	-104	91	21
	miR-10-26483-5p	6367	-110	90	22
	miR-3-3567-3p	6487	-102	91	21
	miR-22-45902-3p	7306	-110	91	22
FZD3	miR-20-20331-5p	6810	-106	91	21
	miR-14-35161-5p	9897	-117	89	24
	miR-2-4826-5p	10100÷01(2)	-113	90	23
HDAC4	miR-16-40163-5p	4605	-121	90	23
IGF1R	miR-10-28238-3p	4199	-106	91	20
	miR-2-4736-5p	5113	-121	92	21
IGF2	miR-101-27078-5p	2286÷2351 (6)	-108÷-113	89÷93	23
	miR-3-5147-5p	2301	-104	94	22

Continuation of table 8

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
	miR-3-5147-5p	2301	-104	94	22
	miR-3-5147-5p	2345	-104	94	22
	miR-3-5147-5p	2457	-104	94	22
	miR-101-27078-5p	2404÷2412 (2)	-110	91	23
	miR-3-5147-5p	2345	-104	94	22
	miR-101-27078-5p	2442÷2463 (3)	-108÷-113	89÷93	23
	miR-3-5147-5p	2457	-104	94	22
	miR-101-27078-5p	2520÷2539 (3)	-108	89	23
	miR-101-27078-5p	2571	-108	89	23
	miR-101-27078-5p	2620	-108	89	23
	miR-101-27078-5p	2655÷2672 (3)	-108÷-110	89÷91	23
	miR-101-27078-5p	2704÷2725 (2)	-108	89	23
	miR-101-27078-5p	2837	-110	91	23
	miR-9-24619-3p	4292	-115	90	23
IHH	miR-6-19324-3p	1940	-121	89	23
ITCH	miR-14-35161-5p	3672	-119	90	24
	miR-22-45335-5p	3727	-113	90	23
	miR-19-42529-3p	5645	-110	90	22
	miR-22-45335-5p	5899	-115	92	23
ITGAL	miR-10-11641-3p	4703	-119	89	23
KIAA1199	miR-11-29796-3p	5267	-106	93	20
	miR-11-28087-3p	5269	-113	91	22
MGAT5	miR-10-24586-5p	2381	-106	91	22
	miR-17-40389-5p	4119	-110	93	20
	miR-10-29282-3p	4308-4334 (14)	-104÷ -106	89÷91	23
	miR-15-36862-3p	4308-4332 (13)	-108	89	23
MRE11A	miR-11-18690-5p	2966	-110	90	22
MTMR3	miR-3-8863-5p	4427	-102	91	21
NGFR	miR-6-18910-3p	1996	-108	93	20
PAX5	miR-15-37146-3p	2136	-110	90	22
	miR-17-36319-3p	2864	-127	90	24
PFKFB3	miR-5-15245-3p	3363	-104	91	20
PKD1	miR-6-17487-3p	13941÷42 (2)	-113÷ -117	90÷93	23
	miR-6-17605-3p	13952	-108	91	21
POU2F2	miR-17-17436-3p	2305	-108	91	20
	miR-5-14706-3p	5031	-102	91	20
PRDM2	miR-15-36862-3p	6149	-113	93	23
	miR-2-6238-3p	6254	-102	92	22
PRKCA	miR-8-23744-5p	6764	-119	90	23
	miR-20-20331-5p	6874	-106	91	21
PRMT1	miR-10-17673-3p	1255	-117	92	21

Continuation of table 8

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
PTOV1	miR-17-39730-3p	1568	-106	94	20
PVR	miR-17-39935-3p	3528	-104	91	21
	miR-8-17639-3p	3573	-110	91	22
	miR-7-21133-5p	5105	-121	89	24
	miR-19-43240-3p	5734	-113	93	21
POU2F2	miR-17-17436-3p	2305	-108	91	20
RUNX1	miR-17-20229-5p	2954	-106	94	18
	miR-4-11239-3p	3123	-115	93	20
	miR-1-2558-3p	3368	-117	93	22
	miR-15-36862-3p	5464-5464 (2)	-108÷-113	89÷93	23
	miR-10-29282-3p	5464	-108	93	23
SEMA5A	miR-X-45975-5p	7923	-96	92	22
	miR-2-4826-5p	8045	-115	92	23
SFRP1	miR-9-24201-3p	1264	-113	91	22
	miR-10-26528-5p	2337	-121	88	24
SLIT1	miR-18-27691-3p	5412	-104	91	21
SND1	miR-3-8209-3p	2981	-106	91	21
SREBF1	miR-19-42491-3p	4420	-117	89	23
SREBF2	miR-17-38738-5p	4196	-117	90	22
	miR-1-2142-3p	4206	-123	92	23
STMN1	miR-5-17240-3p	1096	-119	89	23
	miR-7-13347-5p	1730	-106	91	22
	miR-10-26483-5p	1744	-113	91	22
	miR-2-5355-3p	1987	-119	93	22
TNFRSF1B	miR-2-4826-5p	2321-22 (2)	-113	90	23
	miR-17-8001-3p	2342	-110	90	23
	miR-9-24929-3p	3040	-119	92	23
TPM3	miR-17-34996-5p	3181	-110	90	23
	miR-10-26483-5p	3540	-117	95	22
	miR-8-11096-5p	4451	-117	93	22
TRPC6	miR-6-15855-3p	3877	-96	92	21
TRPV4	miR-11-31858-5p	2714	-110	93	20
VPS13B	miR-9-26530-3p	13482	-110	90	22
	miR-2-4804-5p	13561	-110	88	24
WWP2	miR-16-37839-3p	3838	-115	89	23
	miR-19-42814-5p	4433	-106	91	23

miR-2-4826-5p has binding sites in mRNAs of *FZD3* and *TNFRSF1B* genes with an energy value equal to -113 kJ/mole in both cases. The mRNA of *TNFRSF1B* gene has a cluster for two miRNAs in position from 2321 nt to 2365 nt. mRNA of *RUNX1* gene binds with miR-15-36862-3p and miR-10-29282-3p with an ΔG value equal to -113 kJ/mole and -108 kJ/mole, respectively.

The ΔG/ΔGm value, which characterizes the interaction of miRNA with mRNA presented in the table, does not exceed 95%.

The mRNA of *PKD1* gene has 3 binding sites that form a cluster with overlapped of nucleotide sequences with an average value of ΔG equal to -113 kJ/mole. miR-17-39935-3p and miR-10-26483-5p form a cluster in mRNA of *FKBP5* gene from 6364 nt to 6389 nt with a length 26 nt and ΔG = -107 kJ/mole.

The average free energy of binding of miRNA with mRNA in the 3'UTR of all mRNA is -110 kJ/mole. Only two miR-17-9289-5p and miR-17-36319-3p binds with mRNA of the target genes *ERCC1* and *PAX5* with energy of more than -125 kJ/mole and are therefore recommended as associations for diagnosis. Two more miRNA (miR-10-29282-3p and miR-15-36862-3p) have multiple binding sites in the *ETS1* and *MGAT5* genes and therefore are also suggested as associations for the diagnosis of stomach cancer.

Table 9 shows the patterns of miRNA interaction with the mRNA of the candidate genes involved in the development of stomach cancer. The results show that nucleotides forming non-canonical pairs participate in the interaction of miRNA with mRNA: G-U and A-C. Due to this, the interaction of all nucleotides is taken into account, which increases the free energy of the interaction of miRNA with mRNA.

Table 9 – Schemes of the interaction of miRNA with mRNA of candidate genes of the stomach cancer

DICER1; miR-5-15733-3p; 5'UTR;86; -132; 89; 24 5'-GGCGGCGGGGGCGGCGGCGCCGGG-3' |||||||| ||||| |||||||| 3'-CCGCCGCCGCCGCCGGCGCGGUUC-5'	*ROBO1*; miR-22-23987-3p; 5'UTR; 596; -123; 94; 21 5'-CCGCCGCCGCCGCGCCUGCCC-3' ||||||||||||||| |||| 3'-GGCGGCGGCGGCGUGGCCGGG-5'
KDM1A; miR-1-2180-3p; CDS; 342; -136; 100; 22 5'-CCUCCGCGGGCCUCGCCCCCCG-3' |||||||||||||||||||||| 3'-GGAGGCGCCCGGAGCGGGGGGC-5'	*NOTCH1*; miR-6-19010-3p; CDS; 5181; -119; 89; 23 5'-GCCGCCCCCGCCGGCGCAGCUGC-3' ||||||||||||||| |||||| 3'-CGACGGGGGCGGUUGUCUCGACG-5'
FZD3; miR-14-35161-5p; 9897; 3'UTR; -117; 89; 24 5'-ACACUUUGGGAGGCCGAGGCGGGC-3' ||||||||||||| ||||||||| 3'-UGUGAAACCCUCUCGCUCCGUCCU-5'	*MGAT5*; miR-10-29282-3p; 3'UTR;4308; -104; 89 5'-GUGUGUGUGUGUGUGUGUGUGUG-3' ||||||||||||||||||||||| 3'-CACACACGCAUAUAUACACACAU-5'
CELSR3; miR-19-43804-3p; 10520; 3'UTR; -110; 91; 21 5'-GAGGGGGAGGGAGAUAGAGGG-3' ||||||||||||||||||||| 3'-CUCCCCCUCCCUCUACCCCUC-5'	*CYLD*; miR-10-29282-3p; 6075; 3UTR; -106; 91; 23 5'-GUGUGUGUGUAUAUAUGUAUGUG-3' ||||||||||||||||||||||| 3'-CACACACGCAUAUAUACACACAU-5'
ETS1; miR-15-36862-3p; 3875; 3'UTR; -108; 89; 23 5'-GUGUGUGAAUGUUGUGUGUGUGU-3' ||||||| ||||||||||||| 3'-CGUACACGUACAACACACACACA-5'	*ETS1*; miR-19-42814-5p; 3887; 3'UTR; -102; 87; 23 5'-UGUGUGUGUGUGUGUGUGUGUGU-3' ||||||| ||| |||||||||| 3'-ACACACAAACAAACAUACACACG-5'

Conclusion

1. In each group of candidate esophageal and stomach cancer genes, it was identified miRNA and mRNA associations that had a free energy of interaction of -125 kJ/mole or more that could serve as markers for developing methods for early diagnosis of esophageal and stomach cancer.

2. The average free energy of binding of miRNA with mRNA of genes involved in esophageal and stomach cancer 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.

3. It was identified the location of miRNA binding sites in clusters containing two or more binding sites overlapping their nucleotide sequences. Such a compact arrangement of binding sites in mRNA significantly reduces the proportion of binding sites in mRNA nucleotide sequence. Overlapping miRNA binding sites creates competition between miRNA per binding site, since the RISC complex interacting with mRNA with more free energy will not allow binding to another RISC with miRNA having a weaker interaction with mRNA.

References

Bartel D.P. MicroRNAs: genomics, biogenesis, mechanism, and function // Cell. – 2004. – Vol. 116. – P. 281-97.

Bartel D.P. MicroRNAs: Target Recognition and Regulatory Functions // Cell. – 2009. – Vol. 136. – P. 215-233. DOI: https://doi.org/10.1016/j.

Cipolla G.A. A non-canonical landscape of the microRNA system // Frontiers in Genetics. – 2014. – Vol. 5. – P. 1-6. doi: 10.3389/fgene.2014.00337

Naeli P., Azad F. M., Malakootian Ma., Seidah N.G., Mowla S.J. Post-transcriptional Regulation of PCSK9 by miR-191, miR-222, and miR-224 // Frontiers in Genetics. – 2017. – Vol. 8. – P. 1-7. doi: 10.3389/fgene.2017.00189

Syngal S., Brand R.E., Church J.M., Giardiello F. M., Hampel H. L., Burt R.W. ACG Clinical Guideline: Genetic Testing and Management of Hereditary Gastrointestinal Cancer Syndromes // Am J Gastroenterol. – 2015. – Vol. 110. – P. 223-262.

Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A. Global cancer statistics, 2012 // CA Cancer J Clin. – 2015. – Vol. 65. – P. 87–108. doi: 10.3322/caac.21262.

Mathé E.A., Nguyen G.H., Bowman E.D., Zhao Y., Budhu A., Schetter A.J., Braun R., Reimers M., Kumamoto K., Hughes D., et al. MiRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: Associations with survival // Clin Cancer Res. – 2009. – Vol. 15. – P. 6192-6200.

Zeng H., Zheng R., Zhang S., Zuo T., Xia C., Zou X., Chen W. Esophageal cancer statistics in China, 2011: Estimates based on 177 cancer registries // Thorac Cancer. – 2016. – Vol. 7. – P. 232–237. doi: 10.1111/1759-7714.12322.

Rustgi A.K., El-Serag H.B. Esophageal Carcinoma // New Engl J Med. – 2014. – Vol. 371. – P. 2499-509. 10.1056/NEJMra1314530

Lin L.L., Huang H.C., Juan H.F. Discovery of biomarkers for gastric cancer: a proteomics approach // J. Proteom. – 2012. – Vol. 75. – P. 3081-3097.

el-Rifai W.P.S. Molecular and biologic basis of upper gastrointestinal malignancy. Gastric carcinoma // SurgOncolClin N Am. – 2002. – Vol. 11. – P. 273-91.

Fornaro L., Vasile E., Aprile G., Goetze T.O., Vivaldi C., Falcone A., Al-Batran S.E. Locally advanced gastro-oesophageal cancer: Recent therapeutic advances and research directions // Cancer Treat Rev. – 2018. – Vol. 69. – P. 90-100. doi: 10.1016/j.ctrv.2018.06.012.

Jamali L., Tofigh R., Tutunchi S., Panahi G., Borhani F., Akhavan S., Nourmohammadi P., Ghaderian S.M., Rasouli M., Mirzaei H. Circulating microRNAs as diagnostic and therapeutic biomarkers in gastric and esophageal cancers // J Cell Physiol. – 2018. doi: 10.1002/jcp.26850.

Abbas M., Faggian A., Sintali D.N., Khan G.J., Naeem S., Shi M., Dingding C. Current and future biomarkers in gastric cancer // Biomed Pharmacother. – 2018. – Vol.103. – P. 1688-1700. doi: 10.1016/j.biopha.2018.04.178.

Garofalo M., Croce C.M. microRNAs: Master regulators as potential therapeutics in cancer // Annu Rev Pharmacol Toxicol. – 2011. – Vol. 51. – P. 25–43.

Zhou Y., Li R., Yu H., Wang R., Shen Z. microRNA-130a is an oncomir suppressing the expression of CRMP4 in gastric cancer // Onco Targets Ther. – 2017. – Vol. 10. – P. 3893-3905. doi: 10.2147/OTT.S139443.

Wang Z., Zhao Z., Yang Y., Luo M., Zhang M., Wang X., Liu L., Hou N., Guo Q., Song T., Guo B., Huang C. MiR-99b-5p and miR-203a-3p Function as Tumor Suppressors by Targeting IGF-1R in Gastric Cancer // Sci Rep. – 2018. – Vol. 8(1). – P. 10119. doi: 10.1038/s41598-018-27583-y.

Feng C., Xian Q., Liu S. Micro RNA-518 inhibits gastric cancer cell growth by inducing apoptosis via targeting MDM2 // Biomed Pharmacother. – 2018. – Vol. 97. – P. 1595-1602. doi: 10.1016/j.biopha.2017.11.091.

Chen M., Xia Y., Tan Y., Jiang G., Jin H., Chen Y. Downregulation of microRNA-370 in esophageal squamous-cell carcinoma is associated with cancer progression and promotes cancer cell proliferation via upregulating PIN1 // Gene. – 2018. – Vol. 661. – P. 68-77. doi: 10.1016/j.gene.2018.03.090.

Cao W., Wei W., Zhan Z., Xie D., Xie Y., Xiao Q. Regulation of drug resistance and metastasis of gastric cancer cells via the microRNA647-ANK2 axis // Int J Mol Med. – 2018. – Vol. 41(4). – P. 1958-1966. doi: 10.3892/ijmm.2018.3381.

Guanen Q., Junjie S., Baolin W., Chaoyang W., Yajuan Y., Jing L., Junpeng L., Gaili N., Zhongping W., Jun W. MiR-214 promotes cell meastasis and inhibites apoptosis of esophageal squamous cell carcinoma via PI3K/AKT/mTOR signaling pathway // Biomed Pharmacother. – 2018. – Vol. 105. – P. 350-361. doi: 10.1016/j.biopha.2018.05.149.

Berillo O., Régnier M., Ivashchenko A. Binding of intronic miRNAs with mRNAs of genes coding intronic microRNAs and proteins participating in tumourigenesis // Computers in Biology and Medicine. – 2013. – Vol. 43, № 10. – P. 1374-1381.

Londin E., Lohera P., Telonisa A.G., Quanna K., et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs // PNAS USA. – 2015. – Vol. 112, No. 10. – P. 1106-1115.

Kool E.T. Hydrogen bonding, base stacking, and steric effects in DNA replication // Annual Review of Biophysics and Biomolecular Structure. – 2001. – Vol. 30. – P. 1–22.

Leontis N.B., Stombaugh J., Westhof E. The non-Watson-Crick base pairs and their associated isostericity matrices // Nucleic Acids Research. – 2002. – Vol. 30, No. 16. – P. 3497–3531.

References

Abbas M., Faggian A., Sintali D.N., Khan G.J., Naeem S., Shi M., Dingding C. (2018) Current and future biomarkers in gastric cancer. Biomed Pharmacother., vol.103, pp. 1688-1700. doi: 10.1016/j.biopha.2018.04.178.

Bartel D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, vol. 116, pp. 281-97.

Bartel D.P. (2009) MicroRNAs: Target Recognition and Regulatory Functions. Cell, vol. 136, p. 215-233. doi: https://doi.org/10.1016/j.

Berillo O., Régnier M., Ivashchenko A. (2013) Binding of intronic miRNAs with mRNAs of genes coding intronic microRNAs and proteins participating in tumourigenesis. Computers in Biology and Medicine, vol. 43, no. 10, pp. 1374-1381.

Cao W., Wei W., Zhan Z., Xie D., Xie Y., Xiao Q. (2018) Regulation of drug resistance and metastasis of gastric cancer cells via the microRNA647-ANK2 axis. Int J Mol Med., vol. 41(4), pp. 1958-1966. doi: 10.3892/ijmm.2018.3381.

Chen M., Xia Y., Tan Y., Jiang G., Jin H., Chen Y. (2018) Downregulation of microRNA-370 in esophageal squamous-cell carcinoma is associated with cancer progression and promotes cancer cell proliferation via upregulating PIN1. Gene, vol. 661, pp. 68-77. doi: 10.1016/j.gene.2018.03.090.

Cipolla G.A. (2014) A non-canonical landscape of the microRNA system. Front Genet., vol. 5, p. 1-6. doi: 10.3389/fgene.2014.00337.

el-Rifai W.P.S. (2002) Molecular and biologic basis of upper gastrointestinal malignancy. Gastric carcinoma. SurgOncolClin N Am., vol. 11, pp. 273-91.

Feng C., Xian Q., Liu S. (2018) Micro RNA-518 inhibits gastric cancer cell growth by inducing apoptosis via targeting MDM2. Biomed Pharmacother., vol. 97, pp. 1595-1602. doi: 10.1016/j.biopha.2017.11.091.

Fornaro L., Vasile E., Aprile G., Goetze T.O., Vivaldi C., Falcone A., Al-Batran S.E. (2018) Locally advanced gastro-oesophageal cancer: Recent therapeutic advances and research directions. Cancer Treat Rev., vol. 69, pp. 90-100. doi: 10.1016/j.ctrv.2018.06.012.

Garofalo M., Croce C.M. (2011) microRNAs: Master regulators as potential therapeutics in cancer. Annu Rev Pharmacol Toxicol., vol. 51, pp. 25–43.

Guanen Q., Junjie S., Baolin W., Chaoyang W., Yajuan Y., Jing L., Junpeng L., Gaili N., Zhongping W., Jun W. (2018) MiR-214 promotes cell meastasis and inhibites apoptosis of esophageal squamous cell carcinoma via PI3K/AKT/mTOR signaling pathway. Biomed Pharmacother., vol. 105, pp. 350-361. doi: 10.1016/j.biopha.2018.05.149.

Jamali L., Tofigh R., Tutunchi S., Panahi G., Borhani F., Akhavan S., Nourmohammadi P., Ghaderian S.M., Rasouli M., Mirzaei H. (2018) Circulating microRNAs as diagnostic and therapeutic biomarkers in gastric and esophageal cancers. J Cell Physiol. doi: 10.1002/jcp.26850.

Kool E.T. (2001) Hydrogen bonding, base stacking, and steric effects in DNA replication. Annual Review of Biophysics and Biomolecular Structure, vol. 30, pp. 1–22.

Leontis N.B., Stombaugh J., Westhof E. (2002) The non-Watson-Crick base pairs and their associated isostericity matrices. Nucleic Acids Research, vol. 30, no. 16, pp. 3497–3531.

Lin L.L., Huang H.C., Juan H.F. (2012) Discovery of biomarkers for gastric cancer: a proteomics approach. J. Proteom., vol. 75, pp. 3081-3097.

Londin E., Lohera P., Telonisa A.G., Quanna K., et al. (2015) Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. PNAS USA, vol. 112, no. 10, pp. 1106-1115.

Mathé E.A., Nguyen G.H., Bowman E.D., Zhao Y., Budhu A., Schetter A.J., Braun R., Reimers M., Kumamoto K., Hughes D., et al. (2009) MiRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: Associations with survival. Clin Cancer Res., vol. 15, pp. 6192-6200.

Naeli P., Azad F. M., Malakootian Ma., Seidah N.G., Mowla S.J. (2017) Post-transcriptional Regulation of PCSK9 by miR-191, miR-222, and miR-224. Front Genet., vol. 8, pp. 1-7. doi: 10.3389/fgene.2017.00189.

Rustgi A.K., El-Serag H.B. (2014) Esophageal Carcinoma. New Engl J Med., vol. 371, pp. 2499-509. 10.1056/NEJMra1314530

Syngal S., Brand R.E., Church J.M., Giardiello F. M., Hampel H. L., Burt R.W. (2015) ACG Clinical Guideline: Genetic Testing and Management of Hereditary Gastrointestinal Cancer Syndromes. Am J Gastroenterol., vol. 110, pp. 223-262.

Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A. (2015) Global cancer statistics, 2012. CA Cancer J Clin., vol. 65, pp. 87–108. doi: 10.3322/caac.21262.

Wang Z., Zhao Z., Yang Y., Luo M., Zhang M., Wang X., Liu L., Hou N., Guo Q., Song T., Guo B., Huang C. (2018) MiR-99b-5p and miR-203a-3p Function as Tumor Suppressors by Targeting IGF-1R in Gastric Cancer. Sci Rep., vol. 8(1), pp. 10119. doi: 10.1038/s41598-018-27583-y.

Zeng H., Zheng R., Zhang S., Zuo T., Xia C., Zou X., Chen W. (2016) Esophageal cancer statistics in China, 2011: Estimates based on 177 cancer registries. Thorac Cancer, vol. 7, pp. 232–237. doi: 10.1111/1759-7714.12322.

Zhou Y., Li R., Yu H., Wang R., Shen Z. (2017) microRNA-130a is an oncomir suppressing the expression of CRMP4 in gastric cancer. Onco Targets Ther., vol. 10, pp. 3893-3905. doi: 10.2147/OTT.S139443.