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CHARACTERISTICS OF miRNA INTERACTION WITH mRNA OF CANDIDATE GENES OF THE NON-SMALL CELL LUNG CANCER

Lung cancer is one of the most common malignant diseases. It is expected to involve more than 200 candidate genes in the development of the non-small cell lung cancer (NSCLC). The present study aimed to study the interaction characteristics of the 137 NSCLC candidate genes and miRNAs. The data obtained show that a significant number of genes are targets of two or more mRNAs. The mRNA of some genes contain binding sites for several miRNAs with overlapping nucleotide sequences, which have been termed clusters. The properties of clusters of non-small cell lung cancer genes, containing binding sites of several miRNAs, were studied for the first time. The properties of clusters with a large number of miRNA binding sites in the 5'UTR are considered. The mRNA of CHKA, E2F1, HMGA2, PTEN, HTRA2, ING1, MTA3, SMARCA4, NFATC2 genes contain clusters of binding sites for the largest number of miRNAs. Several clusters are available in the CDS mRNA of CEBPA, NOTCH3, TRIO, LATS2, RB1 and ZBTB7A genes. 32 genes are targets of 49 miRNAs, binding sites of which are localized in the 3'UTR. The CDK6 and IRS1 genes have clusters with the largest number of miRNA binding sites. The AKT1, EMP and HMGA2 genes have clusters of 34 nt, 40 nt and 41 nt in length, respectively, of the binding sites of three miRNAs. Each mRNA of BCAR1, ITGA11, MYLK, PTK6, PXN, SOX2 and SSX2 genes has clusters of the binding sites of two miRNAs. It is assumed that the expression of the examined genes will depend more on the miRNA interacting with mRNA with a higher free binding energy.

Key words: miRNA, mRNA, Non-Small Cell Lung Cancer, target genes.

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miRNA-дың өкпе қатерлі ісігінің ұсақ емес жасушалы субтипінің кандидатты гендерінің mRNA-мен өзара әрекетінің сипаттамалары

Өкпе қатерлі ісігінің ұсақ емес жасушалы субтипінің дамуына 200-ден көп кандидатты гендердің қатысуы қарастырылады. Зерттеудің мақсаты 137 өкпе қатерлі ісігінің ұсақ емес жасушалы субтипінің кандидатты гендерінің және miRNA-дың өзара әрекетінің сипаттамаларын зерттеу болды. Алынған мәліметтер гендердің көп саны екі немесе одан да көп miRNA нысаналары болып табылатынын көрсетті. Кейбір гендердің mRNA-дары кластерлер деп аталатын бір-бірімен қиыстырылған нуклеотидті тізбектері бар бірнеше miRNA байланыстыратын сайттарын қамтиды. Алғаш рет бірнеше miRNA-ның байланыстыратын сайттары бар өкпе қатерлі ісігінің ұсақ емес субтипінің гендер кластерлерінің қасиеттері зерттелген. 5'UTR-де көптеген miRNA байланыстыру сайттары бар кластерлердің қасиеттері қарастырылады. CHKA, E2F1, HMGA2,

PTEN, HTRA2, ING1, MTA3, SMARCA4, NFATC2-тің гендердің mRNA-ры көпшілік miRNA-ға арналған байланыстыру сайттарды қамтиды. CEBPA, NOTCH3, TRIO, LATS2, RB1 және ZBTB7A гендерінің mRNA CDS-да бірнеше кластерлер бар. 32 ген 49 miRNA нысандары болып табылады, олардың байланыстыру сайттары 3'UTR аймағында локализацияланған. CDK6 мен IRS1 гендері ең көп miRNA байланыстыру сайттары бар кластерлерді қамтиды. AKT1, EMP және HMGA2 гендерінде ұзындығы 34 nt, 40 nt and 41 nt, тиісінше, кластерлер бар, үш miRNA-ның байланыстыру сайттарынан тұрады. BCAR1, ITGA11, MYLK, PTK6, PXN, SOX2 және SSX2 гендерінің әрбір mRNA-да екі miRNA-ның байланыстыру сайттарынан тұратын кластерлері бар. Зерттелетін гендердің экспрессиясы miRNA-мен mRNA-дың байланысудың жоғары бос энергиясына тәуелді.

Түйін сөздер: miRNA, mRNA, ұсақ емес жасушалы өкпе қатерлі ісігі, нысана гендер.

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Характеристики взаимодействия miRNA с mRNA кандидатных генов немелкоклеточного рака легкого

Рак легких является одним из наиболее распространенных злокачественных заболеваний. В развитии немелкоклеточного рака легкого предполагается участие более 200 кандидатных генов. Целью исследования было изучить характеристики взаимодействия 137 кандидатных генов немелкоклеточного рака легкого и miRNA. Полученные данные показывают, что значительное число генов являются мишенями двух и более miRNA. mRNA некоторых генов содержат сайты связывания нескольких miRNA с наложением нуклеотидных последовательностей, которые были названы кластерами. Впервые изучены свойства кластеров генов немелкоклеточного рака легкого, содержащие сайты связывания нескольких miRNA. Рассмотрены свойства кластеров с большим числом сайтов связывания miRNA в 5'UTR. mRNA генов CHKA, E2F1, HMGA2, PTEN, HTRA2, ING1, MTA3, SMARCA4, NFATC2 содержат кластеры сайтов связывания для наибольшего количества miRNA. Несколько кластеров имеется в CDS mRNA генов CEBPA, NOTCH3, TRIO, LATS2, RB1 и ZBTB7A. 32 гена являются мишенями 49 miRNA, сайты связывания с которыми локализованы на участке 3'UTR. Гены CDK6 и IRS1 обладают кластерами с наибольшим числом сайтов связывания miRNA. В генах AKT1, EMP и HMGA2 имеются кластеры длиной 34 нт, 40 нт and 41 нт, соответственно, из сайтов связывания трёх miRNA. Каждая mRNA генов BCAR1, ITGA11, MYLK, PTK6, PXN, SOX2 и SSX2 имеют кластеры из сайтов связывания двух miRNA. Предполагается что экспрессия рассмотренных генов будет зависеть в большей степени от miRNA взаимодействующей с mRNA с большей свободной энергией связи.

Ключевые слова: miRNA, mRNA, немелкоклеточный рак легкого, гены-мишени.

Introduction

Lung cancer is one of the most common malignant diseases. Lung cancer consists of two main subtypes: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC) that are classified according to their physiological phenotypes (Pao W., 2011). NSCLC may be mainly divided into two pathological subtypes, adenocarcinoma and squamous cell carcinoma (Houston K., 2014). Emerging studies demonstrated that genes and miRNAs played fundamental roles in prognosis, diagnosis, treatment and metastatic potential of lung cancer subtypes. *CBX3* and *CRABP2* genes expression was markedly increased in lung cancer tissues and especially *CRABP2* may be promising candidate genes in lung adenocarcinoma (Han SS., 2014). The expression of *APOC1* gradually increased from early to late stage in lung cancer patients (Ko HL., 2014). The level of *Rb1* expression in NSCLC was inversely correlated with those of miR-155 and *MCRS1* (Liu M., 2015). *ING5* was offered as a potent tumor suppressor in lung cancer growth and metastasis (Zhang F., 2015). *RACK1* is a candidate gene associated with the prognosis of patients with early stage non-small cell lung cancer (Choi YY., 2015). *ARHGDIB* and *HOXD3* demonstrated significant associations with the overall survival of lung cancer patients (Huang T., 2015). *SFRP1* was specifically indicated to target β-catenin, and thus might be targeted by epigenetic

therapy in NSCLC cell lines (Taguchi YH., 2016). *NEK2, DLGAP5* and *ECT2* were found to be highly expressed in tumor samples of lung cancer patients and might be used as promising biomarkers for the early detection of lung cancer, as well as predicting the prognosis of lung cancer patients (Shi YX., 2017). Human epidermal growth factor receptor 2 (*HER2* or *ErbB2*) can be overexpressed, amplified and/or mutated in malignant tumors, and is a candidate for therapeutic targeting. However, molecular associations and clinical significances of these alterations were controversial in lung cancer (Kim EK., 2017). Combination of *CALR* and *PDIA3* might serve as an efficient biomarker and improved the prediction of NSCLC prognosis significantly (Wang K., 2017). *TOP2A, MELK* and *CENPF* genes might contribute to high proliferation and early metastatic spread of SCLC cells (Mizuno K., 2017). *CNTN6* and *LBX2* genes were commonly downregulated in all the 4 stages of NSCLC (Wang J. 2017). *A2ML1, CCNE1, COBL, ESCO2, GPR115, MMP10, OVOL1* and *SCGB1A1* genes may be used as potential prognostic biomarkers of lung squamous cell carcinoma (Zhang W., 2018). *AFAP1-AS1, BLACAT1, LOC101928245,* and *FENDRR* were most differentially expressed lncRNAs in lung adenocarcinoma (Ding X., 2018). *TTF1, SP-A,* Napsin A*, MUC5AC, CDX2* and *CK5* genes allows to identify prognostic subgroups among lung ADC (Tabbò F., 2018). Previously, we studied the associations of miRNAs and mRNAs of genes involved in the development of non-small cell lung cancer (Niyazova R.Y., 2015). The data on the functions of genes increases every year, and accordingly increases the number of candidate genes. The present study aimed to identify the interaction characteristics of potential candidate genes and miRNAs and identification the clusters of binding sites and their properties.

Materials and methods

The nucleotide sequences of 243 candidate genes of the BC subtypes were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). 3701 miRNAs were taken from the publication of Londin E. et al. (Londin, 2015: 1106-1115). The miRNAs binding sites in 5'-untranslated regions (5'UTRs), coding domain sequences (CDSs) and 3'-untranslated regions (3'UTRs) of several genes were predicted using the MirTarget program (Ivashchenko, 2016: 237-240). This program defines the following features of binding: a) the origin of the initiation of miRNA binding to mRNAs; b) the localization of miRNA binding sites in the 5'UTRs, CDSs and the 3'UTRs of the mRNAs; c) the free energy of hybridization (ΔG, kJ/mole); and d) the schemes of nucleotide interactions between the miRNAs and the mRNAs. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its perfect complementary nucleotide sequence). The miRNA binding sites located on the mRNAs had ΔG/ΔGm ratios of 90% or more. The program identifies the positions of the binding sites on the mRNA, beginning from the first nucleotide of the mRNA's 5'UTR. The MirTarget program found hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The distances between A and C were equal to those between G and C, A and U, and G and U (Kool, 2001: 1-22; Leontis, 2002: 3497-3531). The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively. Table 1 shows sources of information on candidate genes of NSCLC subtype of lung cancer.

Results and Discussion

It is expected to involve more than 200 candidate genes in the development of the NSCLC subtype of lung cancer. Tables 1-3 show characteristics of the interaction of miRNA with mRNA of 137 NSCLC candidate genes. The data obtained from this study show that a significant number of genes are targets for two or more miRNAs. Among these genes, those that contain mRNA binding sites with overlapping of nucleotide sequences are of particular interest. These groups of miRNA binding sites are called clusters.

In the article considered the properties of clusters with a large number of miRNA binding sites in the 5'UTR (Table 1). Ten mRNAs of the candidate genes have clusters for two miRNA binding sites and six clusters for three miRNA binding sites. The mRNA of *CHKA* gene have 13 miRNA binding sites, three binding sites for miR-2-3313-3p, and two binding sites for miR-19-21199-3p and miR-1-155-3p. The total cluster length of all binding sites is 362 nt, and the cluster has a length of 44 nt, which is 8.2 times smaller than the total length. The 5'UTR of the *CHKA* gene is 214 nt, that is, less than the total length of all binding sites. Due to the compact arrangement of the 12 miRNAs binding sites, they occupy only 21% of the 5'UTR length.

Table 1 – Characteristics of miRNAs interaction in the 5'UTR mRNA of NSCLC candidate gene

Gene	Characteristics of binding
ACTN4	miR-19-41803-3p, 86, 90, -113, 22
ACVRL1	miR-19-44097-5p, 165, 92, -123, 23
ADAM23	miR-12-32997-5p, 67 ÷ 81 (3), 89 ÷ 94, -125 ÷ -132, 23; miR-1-3554-3p, 73, 89, -121, 23; miR-8-22944-3p, 143, 93, -115, 20
ALK	miR-2-4035-3p, 51 ÷ 52 (2), 90 ÷ 100, -117 ÷ -129, 23
BAD	miR-11-29089-3p, 237, 90, -113, 22
BCAR1	miR-20-22562-3p, 127, 89, -134, 24; miR-19-35955-3p, 136, 89, -117, 23
BMI1	miR-7-18337-3p, 173, 91, -123, 23; miR-17-39011-3p, 222 ÷ 228 (3), 89 ÷ 92, -117 ÷ -121, 23; miR-3-8809-3p, 251, 90, -117, 22
BMP4	miR-9-26506-3p, 33, 90, -110, 22
CASP9	miR-1-2631-5p, 100, 91, -106, 21; miR-17-38067-3p,180,93, -132,23; miR-14-37896-3p, 272,93,-115,21
CD151	miR-19-21199-3p, 24, 89, -140, 25; miR-3-8100-5p, 20, 88, -129, 24; miR-3-9461-3p, 30, 89, -121, 23
CD44	miR-16-40163-5p, 129, 90, -121, 23
CDC42	miR-2-4804-5p, 115 ÷ 116 (2), 93 ÷ 97, -117 ÷ -121, 24; miR-19-43871-5p, 120, 91, -110, 22
CDC6	miR-10-27065-3p, 7, 92, -115, 21
CDH1	miR-14-36073-5p, 57, 89, -123, 23
CDK6	miR-17-21769-5p, 261, 91, -106, 21
CHKA	miR-2-3313-3p, 51 ÷ 54 (3), 87 ÷ 89, -138 ÷ -142, 25; miR-3-8100-5p, 53, 90, -132, 24; miR-2-4453-3p, 54, 92, -121, 21; miR-19-21199-3p, 56 ÷ 57 (2), 88, -138, 25; miR-1-155-3p, 57 ÷ 62 (2), 91, -125, 22; miR-20-43381-5p, 61, 94, -123, 21; miR-19-43966-3p, 64, 89, -125, 23; miR-7-15849-3p, 65, 100, -115, 18; miR-22-23987-3p, 66, 92, -121, 21; miR-5-8853-5p, 69, 93, -117, 20; miR-9-13610-3p, 69, 97, -127, 21; miR-13-18339-3p, 70, 88, -125, 24; miR-1-367-3p, 95, 89, -125, 23
CYLD	miR-15-37972-3p, 15, 90, -110, 22
CYR61	miR-20-45753-5p, 80, 94, -127, 22; miR-7-15849-3p,125,96,-110,18; miR-12-26632-3p,180,89,-125, 23
DDR1	miR-5-14114-5p, 73, 91, -125, 23
DIABLO	miR-9-25031-3p, 409 ÷ 426 (2), 92, -119, 21
DKK3	miR-19-42426-5p, 93, 92, -125, 23
DLX5	miR-1-1418-3p, 46, 92, -117, 20; miR-11-18690-5p, 82, 90, -110, 22
DPP4	miR-3-9273-3p, 369, 93, -108, 20
DVL1	miR-20-45152-5p, 23, 89, -132, 24
E2F1	miR-4-11239-3p, 84, 93, -115, 20; miR-20-45152-5p, 84 ÷ 85 (2), 90 ÷ 100, -134 ÷ -149, 24; miR-2-4453-3p, 87, 94, -123, 21; miR-1-1714-3p, 90, 93, -117, 20; miR-12-5800-5p, 90, 93, -113, 20; miR-19-21199-3p, 90, 88, -138, 25; miR-8-24509-3p, 90, 100, -108, 17; miR-2-3313-3p, 91, 87, -138, 25
ECT2	miR-17-34996-5p, 231, 93, -115, 23
EFEMP1	miR-19-41434-3p, 331, 93, -113, 21; miR-5-14114-5p, 333, 89, -123, 23; miR-17-39672-3p, 338, 91, -113, 21; miR-20-44786-5p, 425, 93, -121, 21
EGR1	miR-15-32047-5p, 13, 90, -132, 24; miR-2-3313-3p, 13, 91, -144, 25; miR-22-46979-5p, 14, 91, -125, 23; miR-10-13655-3p, 15,91,-123,22; miR-15-38504-5p,90,92,-138,24; miR-1-123-3p, 216, 89, -123, 24
EIF2AK2	miR-11-29089-3p, 17, 90, -113, 22; miR-9-20317-3p, 106 ÷ 116 (4), 90, -134, 24; miR-17-39416-3p, 111 ÷ 114 (2), 92, -121, 22; miR-5-15733-3p, 116, 90, -134, 24; miR-1-3679-3p, 455, 90, -113, 23; miR-1-3679-5p, 455, 90, -113, 23
EIF4B	miR-3-10329-5p, 100, 92, -125, 24
ENG	miR-16-37746-3p, 6, 91, -123, 23
EPAS1	miR-16-37573-3p, 273, 93, -119, 21; miR-17-37993-5p, 418, 90, -115, 22

Continuation of table 1

Gene	Characteristics of binding
EPB41L3	miR-5-15611-5p, 7, 90, -117, 22
EPHA2	miR-17-39972-5p, 56, 93, -108, 20
EPHA3	miR-20-28747-5p, 32, 90, -110, 22
EPHB6	miR-19-41131-3p, 21, 94, -136, 23; miR-17-38067-3p, 75, 90, -127, 23
ERBB3	miR-1-163-3p, 114 ÷ 115 (2), 91 ÷ 93, -110 ÷ -113, 21
ERRFI1	miR-3-9439-3p, 4, 96, -108, 18; miR-5-15733-3p, 4, 90, -134, 24; miR-11-25459-3p, 100, 90, -119, 23
EZH2	miR-2-6831-5p, 112, 95, -113, 20; miR-5-15733-3p, 120, 93, -138, 24; miR-19-36133-3p, 122, 90, -119, 22; miR-3-9439-3p, 126, 98, -110, 18; miR-13-32613-3p, 127, 88, -125, 24
FEN1	miR-21-45306-5p, 320, 89, -121, 23
FOXO1	miR-14-14807-5p, 120, 91, -110, 21; miR-12-17229-5p, 228, 91, -110, 22; miR-1-654-3p, 305, 92, -115, 20; miR-22-40302-3p, 356, 90, -119, 22
GATA2	miR-8-25030-3p, 189, 89, -117, 23; miR-11-28671-3p, 210, 90, -115, 22; miR-8-4989-5p, 413, 93, -115, 20; miR-12-5800-5p, 415, 95, -115, 20
HMGA2	miR-2-3313-3p, 99, 87, -138, 25; miR-17-38391-3p, 312, 90, -115, 23; miR-3-9317-3p, 314, 90, -110, 22; miR-19-43373-3p, 539, 93, -119, 21; miR-15-32047-5p, 541 ÷ 544 (2), 88 ÷ 91, -129 ÷ -134, 24; miR-1-265-3p, 542, 91, -125, 22; miR-17-41168-3p, 542, 95, -117, 20; miR-3-9301-5p, 542, 93, -115, 20; mir-1-2121-3p, 544, 93, -146, 25; miR-19-33623-3p, 544 ÷ 545 (2), 89 ÷ 96, -132 ÷ -142, 24; miR-1-155-3p, 550, 95, -132, 22; miR-10-26815-5p, 575, 88, -121, 24; miR-11-18690-5p, 585, 90, -110, 22; miR-1-1819-3p, 788, 89, -123, 23
HNRNPD	miR-12-31754-5p, 141, 89, -117, 23
HTRA2	miR-22-46979-5p, 239, 91, -125,23; miR-15-32047-5p, 241, 90, -132, 24; miR-17-40348-5p, 243, 89, -121, 23; miR-19-21199-3p, 243, 89, -140, 25; miR-2-4005-5p, 243, 94, -140, 24
ING1	miR-8-22077-3p, 361, 95, -127, 22; miR-19-34067-3p, 531, 89, -119, 23; miR-11-28698-5p, 595, 92, -127, 23, miR-2-4005-5p, 657, 89, -132, 24; miR-1-275-3p, 664, 90, -127, 23; miR-19-43966-3p, 665, 89, -125, 23; miR-1-155-3p, 667, 92, -127, 22; miR-3-9441-3p, 667, 91, -127, 23; miR-17-40274-3p, 820, 90, -113, 22
JAK1	miR-11-29827-3p, 66, 90, -129, 24
KIF14	miR-14-35852-5p, 223, 91, -113, 22
KRAS	miR-17-39416-3p, 17 ÷ 29 (2), 92 ÷ 94, -121 ÷ -123, 22; miR-9-20317-3p, 37, 89, -132, 24; miR-13-32878-3p, 96, 91, -113, 21
LATS2	miR-2-4453-3p, 92, 92, -121, 21
LRIG3	miR-4-11437-3p, 170, 89, -125, 23; miR-7-20431-3p, 170, 90, -113, 22
MAPK1	miR-17-39570-5p, 11, 97, -132, 22
MAPK3	miR-17-36936-3p, 84, 90, -113, 22
MTA3	miR-17-39416-3p, 110, 92, -121, 22; miR-5-15733-3p, 127, 90, -134, 24; miR-12-32603-3p, 145, 90, -113, 23; miR-22-40302-3p, 146, 90, -119, 22; miR-16-37579-3p, 153, 91, -110, 21; miR-1-1819-3p, 158, 89, -123, 23; miR-19-43342-3p, 162, 90, -119, 22
MYC	miR-19-44540-3p, 14, 93, -132, 23; miR-3-9461-3p, 16, 89, -121, 23; miR-2-5150-5p, 22, 91, -113, 21; miR-19-44191-3p, 232, 87, -132, 25; miR-17-39987-3p, 296, 90, -110, 22
MYLK	miR-13-18339-3p, 59, 91, -129, 24; miR-17-40321-3p, 75, 90, -113, 22
NFATC2	miR-20-45152-5p, 4, 89, -132, 24; miR-1-1714-3p, 10, 92, -115, 20; miR-2-3313-3p, 10, 87, -138, 25; miR-19-21199-3p, 10 ÷ 12 (2), 88 ÷ 91, -138 ÷ -142, 25; miR-17-40348-5p, 12, 91, -123, 23; miR-10-13655-3p, 13, 94, -127, 22; miR-20-43381-5p,17,92,-121,21; miR-19-43966-3p,179,89,-125, 23
NFKB1	miR-10-13655-3p, 126, 94, -127, 22; miR-17-40141-3p, 223, 92, -115, 20; miR-9-13610-3p, 231, 95, -125, 21; miR-19-42137-5p, 292, 89, -117, 23
NKX2	miR-11-18690-5p, 306, 95, -117, 22
ORAI3	miR-12-34513-5p, 136, 88, -121, 24
PDK1	miR-2-4736-5p, 2, 92, -121, 21; miR-5-16438-3p, 3, 92, -121, 22

Continuation of table 1

Gene	Characteristics of binding
PTEN	miR-20-43459-5p, 75, 92, -115, 20; miR-5-15564-3p, 486, 91, -125, 22; miR-17-39416-3p, 531, 90, -119, 22; miR-3-9439-3p, 533, 96, -108, 18; miR-5-15733-3p, 533, 89, -132, 24; miR-7-20203-3p, 536, 90, -121, 22; miR-17-40141-3p, 705, 92, -115, 20; miR-17-41310-3p, 708, 96, -110, 18; miR-3-9461-3p, 790, 89, -121, 23
PTGIS	miR-19-44127-3p, 13, 90, -134, 24
PTGS2	miR-9-23969-3p, 108, 92, -123, 21
PXN	miR-11-29831-3p, 2 ÷ 3 (2), 89,-134, 24; miR-17-39227-3p,4,89,-123,23; miR-10-8203-5p,7,92,-115, 20
RAF1	miR-3-8604-5p, 303, 90, -115, 22
RBL2	miR-5-16634-3p, 8, 90, -121, 22
REPS2	miR-9-20317-3p, 64, 91, -136, 24; miR-5-15733-3p, 67 ÷ 73 (2), 89 ÷ 91, -132 ÷ -136, 24; miR-17-39416-3p, 71, 90, -119, 22; miR-3-9439-3p, 73, 96, -108, 18
RHOA	miR-16-38447-3p, 16, 90, -121, 23; miR-19-21199-3p, 97, 88, -138, 25
RND3	miR-2-6824-3p, 100, 90, -110, 22
RRM1	miR-10-28238-3p, 123, 93, -108, 20
S100A2	miR-9-24929-3p, 133, 89, -115, 23
SMARCA4	miR-10-26812-3p, 85, 91, -132, 24; miR-20-44754-3p, 86, 89, -123, 23; miR-5-14873-3p, 87, 94, -125, 22; miR-11-29999-3p-2, 96, 90, -129, 24; miR-3-8775-3p, 98, 88, -123, 24
SOX2	miR-19-43069-3p, 344, 89, -117, 23
STK11	miR-7-21139-3p, 47, 90, -134, 24; miR-19-43345-3p, 113, 92, -125, 24; miR-22-16963-5p, 254, 91,-127,22
TACC3	miR-3-8242-5p, 70, 89, -119, 23
TGFBR3	miR-7-19076-5p, 49, 91, -110, 22
TJP2	miR-1-2077-5p, 32, 93, -110, 20; miR-10-27065-3p, 140, 92, -115, 21; miR-8-977-5p, 140 ÷ 141 (2), 89 ÷ 91, -125 ÷ -127, 23
TWIST1	miR-1-3558-3p, 153, 91, -113, 22; miR-5-14114-5p, 157, 91, -125, 23; miR-19-43426-5p, 173, 93, -121, 21; miR-16-37196-5p, 233, 93, -115, 20
USP1	miR-2-4600-5p, 574, 90, -117, 23
USP15	miR-2-3313-3p, 24, 89, -142, 25; miR-17-35260-3p, 28, 90, -115, 22
USP7	miR-1-265-3p, 9, 92, -127, 22; miR-19-21199-3p, 14 ÷ 104 (8), 88 ÷ 95, -138 ÷ -149, 25; mir-1-2121-3p, 15 ÷ 96 (7), 88 ÷ 95, -138 ÷ -149, 25; miR-19-33623-3p, 15 ÷ 99 (5), 89 ÷ 93, -132 ÷ -138, 24; miR-20-22562-3p,15 ÷ 103 (3),90 ÷ 97,-136 ÷ -146,24; miR-3-9461-3p,17,89,-121,23; miR-20-43381-5p, 18 ÷ 105 (2), 94 ÷ 98, -123 ÷ -129, 21; miR-4-6496-3p, 23 ÷ 110 (3), 92 ÷ 95, -119 ÷ -123, 21; miR-3-9137-3p, 29, 89, -123, 23; miR-2-4005-5p, 44 ÷ 94 (2), 90 ÷ 91, -134 ÷ -136, 24; miR-8-4989-5p, 45 ÷ 95 (2),93,-115, 20; miR-9-25099-3p, 68,90,-115,22; miR-15-32047-5p, 89 ÷ 98 (4), 88 ÷ 91, -129 ÷ -134, 24; miR-17-41168-3p, 90 ÷ 93 (2), 93 ÷ 95, -115 ÷ -117, 20; miR-20-45152-5p, 92, 89, -132, 24; miR-3-8100-5p, 92, 91, -134, 24; miR-2-3313-3p, 93 ÷ 98 (4), 88 ÷ 92, -140 ÷ -146, 25; miR-2-4453-3p, 93 ÷ 101 (3), 94, -123, 21; miR-17-40348-5p, 94, 92, -125, 23; miR-10-13655-3p, 95 ÷ 104 (5), 91, -123, 22; miR-1-155-3p, 95 ÷ 107 (4), 92 ÷ 98, -127 ÷ -136, 22; miR-17-42540-3p, 95 ÷ 121 (2), 92, -115, 20; miR-8-24509-3p, 96, 100, -108, 17; miR-19-43966-3p, 99, 89, -125, 23; miR-16-13062-5p, 102 ÷ 108 (2), 90 ÷ 91, -134 ÷ -136, 24; miR-5-8853-5p, 104, 93, -117, 20; miR-22-16963-5p, 104 ÷ 110 (3), 91 ÷ 92, -127 ÷ -129, 22; miR-22-23987-3p, 104 ÷ 110 (2),94,-123, 21; miR-4-11923-3p, 105, 95, -127, 22; miR-16-38418-5p, 108 ÷ 130 (2), 95, -115, 19; miR-5-16438-3p, 116, 90, -119, 22; miR-22-16699-3p, 121 ÷ 122 (2), 92, -117, 20; miR-5-12460-5p, 135, 90, -129, 24
VCP	miR-19-41131-3p, 17 ÷ 18, 90 ÷ 91, -129 ÷ -132, 23
YBX1	miR-2-4453-3p, 81, 92, -121, 21; miR-2-4736-5p, 82, 92, -121, 21; miR-22-23987-3p, 92, 130, -121, 21
ZBTB7A	miR-12-17704-3p, 104, 94, -132, 23; miR-7-22377-3p, 107, 97, -132, 22; miR-19-30988-5p, 109, 90, -129, 23; miR-5-16165-5p, 112, 95, -115, 20
ZFX	miR-15-32047-5p, 27, 88, -129, 24; miR-19-21199-3p, 29, 88, -138, 25; miR-1-356-5p, 31, 93, -136, 23
Note: miRNA; the beginning of binding site; the ΔG/ΔGm (%); the free energy change (ΔG, kJ/mole); length of miRNA (nt)	

In the cluster organization of miRNA binding sites, the problem of competition between miRNA for binding with mRNA is peculiarly solved. If miRNA within the RISC complex binds to mRNA in a cluster of 44 nt in length, another miRNA in the RISC complex will no longer be able to communicate with it. Thus, the expression of *CHKA* gene will depend more on miRNA interacting with mRNA with a higher free binding energy. In considered example, miR-2-3313-3p has three binding sites in the cluster. There is a cluster in the mRNA of *E2F1* gene with a length of 33 nt including eight miRNAs binding sites. The total length of these binding sites is 196 nt, that is more of the length of the 5'UTR which equal to 140 nt. With the cluster organization of binding sites for eight miRNAs, they occupy only 24% of the length of the 5'UTR. Among these miRNAs, miR-20-45152-5 has the greatest free energy, which has two binding sites in mRNA of *E2F1* gene.

The mRNA of *HMGA2* gene can bind 16 miRNAs. The binding sites of miR-17-38391-3p with miR-3-9317-3p and miR-10-26815-5p with miR-11-18690-5p form two clusters. Binding sites for seven miRNAs with a total length of 205 nt are organized into a cluster 32 nt long, which is 4% of the length of the 5'UTR mRNA of *HMGA2* gene.

The mRNA of *PTEN* gene can bind 9 miRNAs. The binding sites of miR-17-39416-3p and miR-17-40141-3p form two clusters. Binding sites for four miRNAs with a total length of 86 nt are organized into a cluster 27 nt long, which is 0.3% of the length of the 5'UTR of *PTEN* gene.

Each of the mRNA of *HTRA2, ING1, MTA3* and *SMARCA4* genes have clusters of the binding sites of five mRNAs. Seven miRNAs can bind to the mRNA of *NFATC2* gene, wherein miR-19-21199-3p has two binding sites and interacts with free energy -138 ÷ -142 kJ/mole.

The *USP7* gene is unique, the mRNA of which has a cluster of binding sites of 33 miRNAs with a length of 151 nt, with the 5'UTR length of 199 nt. The total length of all 33 miRNA binding sites is 1798 nt, which is nine times more than the length of the 5'UTR.

Table 2 presents data on the characteristics of the interaction of miRNA in the CDS mRNA of the candidate NSLC genes.

mRNA of *CEBPA, NOTCH3* genes can bind with 17 miRNAs. The miRNAs binding sites in the mRNA of *CEBPA* gene form three clusters. The 13 miRNA binding sites form a 35 nt long cluster. The miRNA binding sites in mRNA of *CEBPA* gene form one cluster with a total length of 50 nt.

The mRNA of *TRIO* gene can bind 14 miRNAs. The binding sites of seven miRNAs are organized in a cluster of 306 nt in length.

Eight miRNAs have clusters of binding sites in mRNA of *LATS2, RB1* and *ZBTB7A* genes.

Table 2 – Characteristics of miRNAs interaction in the CDS mRNA of NSCLC candidate gene

Gene	Characteristics of binding
ABCC10	miR-14-35246-5p, 3430, 88, -121, 24
ACLY	miR-11-24912-5p, 1084, 91, -108, 21
ADAM23	miR-5-3563-5p, 337, 94, -129, 22; miR-4-13274-3p, 340, 91, -113, 21
AKT1S1	miR-12-30944-5p, 824, 91, -110, 22
ALK	miR-9-23745-3p, 1036, 95, -115, 20; miR-16-38700-5p, 1337, 92, -125, 23; miR-3-10496-3p, 3079, 89, -117, 23; miR-1-2802-3p, 3395, 90, -113, 22; miR-8-22818-3p, 5212, 96, -108, 20
ANP32A	miR-12-32603-3p, 687 ÷ 741 (2), 92 ÷ 93, -115 ÷ -117, 23; miR-9-26506-3p, 751, 91, -113, 22; miR-9-25099-3p, 778, 90, -115, 22
BCAR1	miR-17-42375-5p, 662, 89, -119, 23; miR-3-6141-3p, 888, 92, -100, 20
BIRC6	miR-8-23323-3p, 221, 89, -121,23; miR-18-41949-5p, 264, 90, -121, 22; miR-2-5674-3p, 266, 89, -123, 23; miR-9-20317-3p, 266, 90, -134, 24
BMP4	miR-19-44061-5p, 906, 91, -106, 21
CARD10	miR-16-37330-3p, 6, 92, -117, 21; miR-19-38260-3p, 24, 90, -113, 22; miR-12-32603-3p, 1167, 90, -113, 23; miR-1-875-3p, 1820, 90, -115, 22
CCDC6	miR-10-29090-5p, 245 ÷ 246 (2), 91 ÷ 100, -110 ÷ -121, 21; miR-9-27797-5p, 338, 88, -125, 24; miR-11-29839-5p, 1505, 89, -115, 23

Continuation of table 2

Gene	Characteristics of binding
CDH1	miR-2-6803-5p, 484, 90,-119,22; miR-1-3928-3p, 573, 90, -110, 22; miR-15-37333-3p, 2454, 92,-100,20
CDT1	miR-22-45959-3p, 97, 90, -113, 22; miR-20-24547-3p, 138, 90, -121, 22; miR-1-124-5p, 199, 89, -134, 24; miR-5-3563-5p, 200, 91, -125, 22; miR-5-14705-3p, 202, 95, -123, 21; miR-10-13751-3p, 204, 94, -123, 21; miR-21-45472-3p, 210, 89, -125, 23; miR-4-14131-5p, 287, 88, -121, 25; miR-14-33186-5p, 578, 90, -132, 24
CEBPA	miR-19-28028-5p, 233, 89, -132, 24; miR-21-23994-3p, 236, 91, -113, 21; miR-19-33623-3p, 648, 89, -132, 24; mir-1-2121-3p, 648 ÷ 651 (2), 89 ÷ 91, -140 ÷ -142, 25; miR-2-3313-3p, 651 ÷ 654 (2), 87 ÷ 88, -138 ÷ -140, 25; miR-19-21199-3p, 651 ÷ 660 (4), 88 ÷ 96, -138 ÷ -151, 25; miR-12-5800-5p, 653, 95, -115, 20; miR-2-4453-3p, 654, 95, -125, 21; miR-10-13655-3p, 654 ÷ 657 (3), 92 ÷ 94, -125 ÷ -127, 22; miR-1-155-3p, 654 ÷ 660 (3), 91 ÷ 94, -125 ÷ -129, 22; miR-4-6496-3, 657, 92, -119, 21; miR-5-8853-5p, 657, 93, -117, 20; miR-19-43966-3p, 658, 89, -125, 23; miR-4-11923-3p, 661, 92, -123, 22; miR-20-43381-5p, 661, 97, -127, 21, miR-20-40417-3p, 777, 96, -113, 19; miR-22-16963-5p, 782, 91, -127, 22
CHFR	miR-1-3633-5p, 1537, 90, -121, 22; miR-19-21199-3p, 386 ÷ 395 (4), 88 ÷ 92, -138 ÷ -144, 25
CHKA	mir-1-2121-3p, 386 ÷ 423 (4), 88 ÷ 96, -138 ÷ -151, 25; miR-1-155-3p, 389, 91, -125, 22; miR-3-8100-5p, 397, 93, -136, 24; miR-19-33623-3p, 422 ÷ 423 (2), 89 ÷ 94, -132 ÷ -140, 24; miR-20-22562-3p, 429 ÷ 430 (2), 92, -138, 24
CTDSPL	miR-16-20705-3p, 852, 89, -123, 24
DAPK1	miR-2-6184-3p, 4436, 89, -115, 23
DDR1	miR-8-21738-3p, 570, 90, -110, 22; miR-3-10387-3p, 2409, 91, -110, 22; miR-3-10387-5p, 2409, 91, -110, 22; miR-7-23800-3p, 2871, 91, -123, 23
DIABLO	miR-1-2802-3p, 1465, 90, -113, 22
DLX5	miR-1-1488-5p, 489, 88, -108, 24; miR-22-44023-3p, 1015, 92, -121, 21
DVL1	miR-9-23297-3p, 1087, 93, -106, 20
E2F1	miR-20-23817-3p, 290 ÷ 291 (2), 100, -153, 24; miR-10-5299-5p, 290 ÷ 291 (2), 95, -115, 19; miR-17-40141-3p, 295, 93, -117, 20; miR-1-1714-3p, 381, 95, -119, 20; miR-17-36033-3p, 446, 87, -129, 25; miR-22-44137-3p, 764, 89, -115, 23; miR-22-44137-5p, 764, 89, -115, 23; miR-16-36797-3p, 1382, 93, -115, 22
EGFR	miR-3-7886-3p, 1779, 88, -127, 24
EGR1	miR-9-25082-3p, 467, 90, -127, 24; miR-22-40302-3p, 470, 92, -121, 22; miR-11-18690-5p, 806, 90, -110, 22; miR-5-14103-5p, 813, 93, -108, 20; miR-2-6184-3p, 1682, 89, -115, 23
EIF4E	miR-8-18887-3p, 2148, 92, -93, 21
ENG	miR-16-37839-3p, 508, 89, -115, 23; miR-11-28996-5p, 1159, 90, -115, 23
ENO1	miR-17-39143-3p, 1554, 88, -121, 24
EPAS1	miR-17-40452-5p, 1446, 90, -117, 22; miR-5-15664-3p, 1928, 92, -104, 20
EPB41L3	miR-19-36133-3p, 154, 95, -125, 22
EPHA2	miR-2-6640-3p, 204, 90, -119, 23; miR-22-46516-3p, 211, 92, -117, 21; miR-17-40725-5p, 756, 90, -110, 22; miR-22-40730-3p, 1148, 90, -113, 22; miR-2-6494-3p, 2706, 92, -102, 21
EPHB6	miR-12-30818-5p, 1607, 88, -123, 24; miR-12-32998-3p, 3815, 90, -113, 22
FGF2	miR-8-22077-3p, 344, 92, -123, 22; miR-17-36033-3p, 383, 87, -129, 25
FOXO1	miR-9-20317-3p, 655 ÷ 658 (2), 91 ÷ 94, -136 ÷ -140, 24; miR-5-15564-3p, 660, 91, -125, 22; miR-5-15733-3p, 661, 89, -132, 24; miR-3-9439-3p, 667, 96, -108, 18; miR-2-3313-3p, 669, 89, -142, 25; miR-15-39028-5p, 745, 91, -123, 23; miR-3-9461-3p, 749, 91, -123, 23
FUBP1	miR-12-30416-5p, 1503, 90, -115, 22
GATA2	miR-5-17240-3p, 859, 89, -119, 23; miR-4-12483-3p,865,90,-115,22; miR-16-38537-3p,877,89,-125,24
HNRNPD	miR-11-29998-3p, 366, 89, -125, 23
HSPB1	miR-1-265-3p, 320, 92, -127, 22
HTRA2	miR-8-22077-3p, 962, 90, -121, 22; miR-1-1670-5p, 1185, 90, -110, 22
ID1	miR-8-977-5p, 117, 91, -127, 23; miR-4-6496-3p, 122, 93, -121, 21; miR-11-29553-3p, 123,92, -119, 21
ILK3	miR-3-11072-3p,318,90,-113,22; miR-20-44572-3p,730,90,-113,22; miR-22-44471-3p,1388,91, -102,21

Continuation of table 2

Gene	Characteristics of binding
IRS1	miR-3-7886-3p, 412, 88, -127, 24; miR-8-23997-5p, 1418, 94, -102, 19; miR-22-45452-5p, 2084, 92, -102, 20; miR-10-26714-5p, 2085, 89, -121, 24; miR-19-37450-3p, 3446, 93, -106, 21
JAK1	miR-3-10699-5, 2532, 96, -108, 21
LATS2	miR-5-14233-3p, 1673, 88, -125, 24; miR-1-124-5p, 1705, 90, -136, 24; mir-1-2121-3p, 1851, 91, -142, 25; miR-17-40081-5p, 1851, 91, -134, 23; miR-19-33623-3p, 1851, 89, -132, 24; miR-1-28-5p, 1854,90, -134, 24; miR-11-28484-5p, 1856, 90, -117, 22; miR-22-16963-5p, 1857 ÷ 1863, 91 ÷ 92, -127 ÷ -129, 22; miR-9-25031-3p, 1861, 98, -127, 21; miR-2-2621-5p, 1864, 95, -134, 22
MAP2K4	miR-7-20203-3p, 91, 92, -123, 22
MAPK1	miR-5-14873-3p, 243, 90, -121, 22; miR-9-20317-3p, 243, 90, -134, 24; miR-17-39416-3p, 244, 92, -121, 22; miR-19-41910-5p, 246, 90, -132, 24; miR-12-33610-3p, 246, 91, -136, 24
MAPK3	miR-1-2802-3p, 1144, 93, -117, 22; miR-19-42375-3p, 1144, 91, -110, 21
MYLK	miR-3-9317-3p, 5995, 91, -113, 22
NFKB1	miR-11-29785-3p, 2249, 91, -108, 21; miR-11-29785-5p, 2249, 91, -108, 21
NKX2	miR-7-7134-3p, 431, 93, -113, 20; miR-9-22187-3p, 1041, 89, -119, 23; miR-5-15704-3p, 1048, 97, -123, 21; miR-3-8419-3p, 1289, 89, -121, 23
NNMT	miR-1-2236-3p, 1014, 90, -113, 22
NOTCH3	miR-20-45152-5p, 85, 93, -138, 24; miR-15-32047-5p, 85 ÷ 86 (2), 88, -129, 24; miR-2-3313-3p, 85 ÷ 92 (3), 89 ÷ 93, -142 ÷ -149, 25; miR-11-1939-5p, 87, 92, -115, 20; miR-2-4005-5p, 88 ÷ 113 (2), 89, -132, 24; miR-9-5204-5p, 89, 90, -121, 22; miR-12-5800-5p, 90, 93, -113, 20; miR-19-8151-3p, 90 ÷ 115 (2), 92, -117, 21; miR-11-28656-5p, 91, 89, -125, 23; miR-2-4453-3p, 94, 92, -121, 21; miR-19-43966-3p, 126, 94, -132, 23; miR-11-32084-3p, 4257, 91, -110, 22; miR-21-44879-5p, 4305, 92, -117, 23; miR-8-23353-3p, 6288, 90, -121, 22; miR-5-15599-5p, 6340, 90, -110, 22; miR-2-6803-5p, 6799, 90, -119, 22; miR-3-10473-5p, 6921, 92, -104, 20
ORAI3	miR-5-14747-3p, 927, 91, -113, 21
PDCD6	miR-10-27918-3p, 126, 91, -123, 23; miR-1-163-3p, 457, 91, -110, 21
PDK1	miR-17-10097-3p, 145, 89, -119, 23; miR-8-4989-5p, 147, 93, -115, 20
PLAUR	miR-17-40348-5p, 233, 91, -123, 23
POU3F2	miR-9-20317-3p, 375 ÷ 420 (3), 89 ÷ 91, -132 ÷ -136, 24; miR-17-39416-3p, 379 ÷ 418 (2), 90 ÷ 95, -119 ÷ -125, 22; miR-1-1819-3p, 385, 89, -123, 23; miR-12-33610-3p, 392 ÷ 393 (2), 89, -132, 24; miR-2-6772-3p, 400, 91, -134, 24; miR-11-29463-5p, 404, 88, -125, 24; miR-2-3313-3p, 879, 87, -138, 25; miR-12-5800-5p, 878, 93, -113, 20
PTK6	miR-19-44132-3p, 184, 90, -115, 22
PXN	miR-21-42431-3p, 879, 88, -123, 24
RAPGEF1	miR-18-40504-5p,1992,91,-106,22; miR-19-42673-3p,2125,90,-113,22; miR-2-6482-3p,2675,93,-119,21
RASSF5	miR-5-8853-5p, 160, 92, -115, 20; miR-19-43003-3p,294,90,-121,22; miR-17-39416-3p,318,90,-119, 22
RB1	miR-15-32047-5p, 192, 91, -134, 24; miR-2-3313-3p, 192, 91, -144, 25; miR-2-4453-3p, 192, 92, -121,21; miR-22-46979-5p, 193, 89, -123, 23; miR-3-8100-5p, 194, 93, -136, 24; miR-19-21199-3p, 195 ÷ 224 (3), 88 ÷ 92, -138 ÷ -144, 25; miR-1-155-3p, 195 ÷ 230 (3), 91 ÷ 92, -125 ÷ -127, 22; miR-3-9461-3p, 198, 91, -123, 23; miR-1-1714-3p, 224, 92, -115, 20
REPS2	miR-17-39416-3p, 178 ÷ 187 (3), 90 ÷ 94, -119 ÷ -123, 22; miR-5-15733-3p, 189, 89, -132, 24; miR-21-45056-3p, 1173, 92, -104, 20
ROS1	miR-11-31014-5p, 7102, 90, -100, 22; miR-11-32280-3p, 7102, 90, -100, 22
RRM2	miR-3-10227-5p, 112, 90, -119, 22
SMARCA4	miR-16-39450-3p, 656, 94, -123, 23; miR-4-12861-5p, 681, 90, -117, 22; miR-17-38067-3p, 972, 90, -127, 23; miR-9-25099-3p, 5080, 93, -119, 22; miR-9-26506-3p, 5086, 90, -110, 22; miR-5-15733-3p, 5191, 94, -140, 24; miR-4-12154-5p, 5194, 88, -127, 24
SOX2	miR-9-24392-5, 455, 93, -106, 20; miR-17-40730-3p, 1218, 90, -121, 23
SRSF1	miR-9-22187-3p, 501, 92, -123, 23
STK11	miR-5-14952-3p, 1157, 91, -106, 21

Continuation of table 2

Gene	Characteristics of binding
TACC3	miR-19-42195-3p, 337, 93, -113, 22
TEK	miR-14-35424-3p, 2003, 90, -119, 23
TJP2	miR-12-33740-3p, 1132, 90, -117, 22
TOPORS	miR-2-6128-5p, 208, 88, -129, 24
TPBG	miR-1-265-3p, 1262, 91, -125, 22; miR-16-16153-5p, 1269, 100, -108, 17
TRIO	miR-10-13655-3p, 26, 91, -123, 22; miR-9-23270-3p, 84, 90, -136, 24; miR-16-33136-3p, 85, 91, -123, 22; miR-8-21978-5p, 100, 88, -125, 24; miR-16-35596-3p, 4331, 93, -110, 23; miR-19-43584-3p, 5567, 92, -121, 23; miR-17-39416-3p,6875,92, -121, 22; miR-9-20317-3p, 6877, 89, -132,24; miR-5-15733-3p, 6883, 89, -132, 24; miR-1-1819-3p, 6884, 91, -125, 23; miR-9-22187-3p, 6907, 89, -119, 23; miR-5-15564-3p, 6927, 100, -138, 22; miR-9-25265-3p, 6934, 90, -119, 22; miR-11-28698-5p, 7145, 89, -123, 23; miR-11-28041-3p, 7181, 89, -123, 23
TWIST1	miR-7-20781-5p, 428, 90, -113, 22; miR-15-37572-3p, 486, 92, -125, 22; miR-16-33136-3p, 490, 91, -123, 22; miR-13-32613-3p, 602, 90, -127, 24
WWTR1	miR-4-11421-3p, 357, 89, -125, 23
YBX1	miR-11-28484-5p, 201, 90, -117, 22; miR-19-28028-5p, 201, 89, -132, 24; miR-5-14114-5p, 205, 89, -123, 23; miR-12-28419-3p, 272, 91, -108, 21
ZBTB7A	mir-1-2121-3p, 664 ÷ 671 (3), 88, -138, 25; miR-19-44540-3p, 667, 90, -127, 23; miR-19-21199-3p, 669 ÷ 670 (2), 88 ÷ 92, -138 ÷ -144, 25; miR-1-1714-3p, 670, 92, -115, 20; miR-10-5299-5p, 671 ÷ 672 (2), 95, -115, 19; miR-12-5800-5p, 672, 95, -115, 20; miR-1-155-3p, 673, 91, -125, 22; miR-2-3313-3p; 673 ÷ 674 (2); 87 ÷ 91; -138 ÷ -144, 25; miR-11-31496-5p, 1619, 90, -127, 23; miR-16-36024-3p, 1696, 91, -129, 23
Note: miRNA; the beginning of binding site; the ΔG/ΔGm (%); the free energy change (ΔG, kJ/mole); length of miRNA (nt)	

Of the 243 genes participating in the development of the NSCLC subtype of lung cancer, 32 genes are targets of 49 miRNAs whose binding sites are localized in the 3'UTR. Table 3 presents data on the characteristics of the miRNA interaction in the 3'UTR mRNA of the candidate genes. The data obtained show that a larger number of genes are targets of two or more mRNAs. Among them, mRNA of genes that contain binding sites with overlapping of nucleotide sequences are of great interest. Such groups of miRNA binding sites are called clusters. The five mRNAs have clusters of two miRNAs binding sites, four mRNAs have clusters of three miRNAs binding sites.

Table 3 – Characteristics of miRNAs interaction in the 3'UTR mRNA of NSCLC candidate gene

Gene	Characteristics of binding
AKT1	miR-10-27065-3p,2864,93,-117,21;miR-12-5800-5p,2866,93,-113,20;miR-13-36375-5p,2875,90,-119,23
BCAR1	miR-14-35410-5p, 3046, 90, -113, 22; miR-4-12483-3p, 3046, 92, -117, 22
CCDC6	miR-10-29282-3p, 5279, 89, -104, 23
CDH1	miR-2-4804-5p, 3413, 88, -110, 24
CDK6	miR-9-24961-3, 1678, 90, -98, 22; miR-10-29282-3p, 1896 ÷ 1920 (9), 89 ÷ 91, -104 ÷ -106, 23; miR-15-36862-3p, 1900 ÷ 1918 (7), 89 ÷ 95, -108 ÷ -114, 23; miR-4-13015-5p, 1901, 91, -102, 22; miR-9-9900-3p, 3041, 94, -102, 20; miR-8-23986-3p, 7773, 88, -127, 24
CDT1	miR-17-40078-3p, 2389, 88, -113, 24
CEBPA	miR-22-45967-3p, 2440, 92, -115, 22
CYLD	miR-10-29282-3p, 6075, 91, -106, 23
E2F1	miR-21-40861-3p, 2178, 90, -110, 22
EIF2AK2	miR-1-210-5p, 2654, 91, -102, 21
EMP3	miR-20-44484-3p, 748, 89, -119, 23; miR-16-40261-3p, 765, 92, -115, 20; miR-17-12804-3p, 767 ÷ 768 (2), 93, -113, 20

Continuation of table 3

Gene	Characteristics of binding
ENOX2	miR-3-5147-5p, 3059, 90, -100, 22
EPHA2	miR-2-6331-3p, 3699, 90, -117, 22
FOXO1	miR-3-10274-3p, 4489, 92, -93, 21
GATA2	miR-2-6824-3p, 2340, 97, -119, 22
HDAC2	miR-2-4804-5p, 5132 ÷ 5832 (2), 88 ÷ 90, -110 ÷ -113, 24
HMGA2	miR-2-6081-3p, 1255, 90, -113, 23; miR-13-35476-3p, 1261 ÷ 1268 (2), 90, -117, 22; miR-19-43804-3p, 1275, 95, -115, 21
IRS1	miR-10-29282-3p, 7321 ÷ 7425 (8), 89 ÷ 91, -104 ÷ -106, 23; miR-15-36862-3p, 7321 ÷ 7333 (2), 89, -108, 23; miR-19-42814-5p, 7334, 91, -106, 23; miR-8-23415-3p, 7351, 90, -98, 22
ITGA11	miR-101-27078-5p, 4598 ÷ 4634 (19), 89, -108, 23; miR-3-5147-5p, 4598 ÷ 4634 (19), 90, -100, 22
KRAS	miR-8-23744-5p, 3163, 92, -121, 23
MYLK	miR-10-29282-3p, 7649 ÷ 7651 (2), 89, -104, 23; miR-19-42814-5p, 7652, 89, -104, 23
PDSS2	miR-8-23744-5p, 2043 ÷ 2044 (2), 89 ÷ 90, -117 ÷ -119, 23; miR-1-1444-5p, 3351, 93, -106, 20
PTGIS	miR-10-22863-3p, 2569, 90, -113, 22; miR-2-4804-5p, 4356, 88, -110, 24
PTK6	miR-7-20563-3p, 2078, 89, -119, 24; miR-1-2142-3p, 2356, 90, -121, 23; miR-2-4826-5p, 2464 ÷ 2465 (2), 90, -113, 23; miR-17-40078-3p, 2480, 88, -113, 24
PXN	miR-13-28252-3p,1963,90,-117,22; miR-12-31811-3p,2313,89,-117,23; miR-3-9956-3p,2314,89,-121,24
RAPGEF1	miR-14-35683-5p, 3488, 91, -106, 22; miR-14-35556-3p, 4413, 91, -104, 21; miR-18-41332-3p, 6021, 89, -123, 23
RASSF2	miR-10-29282-3p, 1423, 91, -106, 23
RRM2	miR-2-4804-5p, 2664, 90, -113, 24
SOX2	miR-1-2142-3p, 1671, 90, -121, 23; miR-1-2602-3p, 1671, 90, -113, 22
SSX2	miR-101-27078-5p, 1104 ÷ 1129 (12), 89, -108, 23; miR-3-5147-5p, 1109 ÷ 1127 (10), 90, -100, 22
TP53	miR-3-8997-3p, 1397, 89, -119, 24; miR-2-4804-5p, 2459 ÷ 2460 (2), 88 ÷ 92, -110 ÷ -115, 24; miR-12-33375-5p, 2520, 90, -113, 23
ZBTB7A	miR-5-15026-5p, 2127, 89, -123, 23; miR-5-14687-5p, 2246, 93, -119, 21; miR-9-25031-3p, 2719, 92, -119, 21; miR-16-36024-3p, 2784, 91, -129, 23
Note. miRNA; the beginning of binding site; the ΔG/ΔGm (%); the free energy change (ΔG, kJ/mole); length of miRNA (nt)	

The *CDK6* and *IRS1* genes have clusters with the largest number of miRNA binding sites. The mRNA of *CDK6* gene contains binding sites of six miRNAs, miR-10-29282-3p has nine binding sites, and miR-15-36862-3p has seven binding sites.

The total length of all binding sites cluster is 390 nt, and the cluster length is 5 nt, which is 78 times smaller than the total length. The mRNA of *IRS1* gene contains four miRNA binding sites, wherein miR-10-29282-3p has eight binding sites, miR-15-36862-3p has two binding sites. The total cluster length of all binding sites is 275 nt, and the cluster length is 30 nt, which is 9.2 times less than the total length. The mRNA of *AKT1, EMP* and *HMGA2* genes have clusters of 11 nt, 20 nt and 20 nt in length, respectively, from the binding sites of the three miRNAs. Among these miRNAs, the largest free energy of binding is for miR-13-36375-5p (-119 kJ/mole, *AKT1*), miR-20-44484-3p (-119 kJ/mole, *EMP*) and miR-13-35476-3p (-117 kJ/mole), which has two binding sites in mRNA of *HMGA2* gene.

Each mRNA of *BCAR1, ITGA11, MYLK, PTK6, PXN, SOX2* and *SSX2* genes has clusters of two miRNAs binding sites. miR-101-27078-5p and miR-3-5147-5p have 19 binding sites in mRNA *ITGA11* gene, 12 and 10 binding sites in mRNA of *SSX2* gene.

The total length of all binding sites cluster for *ITGA11* gene is 855 nt, and the cluster length is 36 nt, which is 23.75 times less than the total length. The total cluster length of all binding sites for the *SSX2* gene is 496 nt, and the cluster length is 25 nt, which is 19.8 times smaller than the total length.

The binding sites of the mRNA of these two genes are identical by the site of localization, the value of free energy and the percentage equivalent of the bond (Score).

Conclusion

Of the 243 genes participating in the development of the NSCLC subtype of lung cancer, 85 genes are targets of 260 miRNAs whose binding sites are localized in the 5'UTR, 79 genes are targets of 261 miRNAs whose binding sites are localized in the CDS and 32 genes are targets of 49 miRNAs whose binding sites are localized in the 3'UTR. The location of mRNA binding sites in clusters containing two or more binding sites with overlapping their nucleotide sequences has been found. The mRNA of *CHKA, E2F1, HMGA2, PTEN, HTRA2, ING1, MTA3, SMARCA4, NFATC2* genes contain clusters of miRNA binding sites in the 5'UTR. Several clusters are available in the CDS mRNA of *CEBPA, NOTCH3, TRIO, LATS2, RB1* and *ZBTB7A* genes. The *CDK6, IRS1, AKT1, EMP, HMGA2, BCAR1, ITGA11, MYLK, PTK6, PXN, SOX2* and *SSX2* genes have clusters of miRNA binding sites in the 3'UTR.

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