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CHARACTERISTICS OF MIRNA INTERACTION WITH 5'UTR, CDS, 3'UTR MRNA CANDIDATE GENES OF BREAST CANCER SUBTYPES

Different subtypes of breast cancer are distinguished by a set of candidate genes involved in the development of this disease. The expression of many genes is regulated by binding of their mRNA with miRNA, therefore it is required to identify which candidate genes of oncogenesis and in what degree they can interact with miRNA. The purpose of this work was to establish the interaction characteristics of the known 3701 miRNAs with mRNA 92 candidate genes of breast cancer subtypes. 19 genes from 25 candidate genes of the her2 subtype were targets for miRNA. The binding sites of 67 miRNAs were located in 5'UTR, CDS, 3'UTR and the average free energy (ΔG) of miRNA binding with mRNA was equal to -120,2 ± -7,6 kJ/mole, -123,6 ± -9,8 kJ/mole, -110,4 ± -9,8 kJ/mole, respectively. 31 miRNA associations with mRNA, having the free binding energy more than -120 kJ/mole are recommended for the diagnosis of the her2 subtype. 33 genes from 47 candidate genes of the triple-negative subtype were targets for miRNA. The binding sites of 90 miRNAs were located in 5'UTR, CDS, 3'UTR and the average ΔG value of miRNA binding with mRNA was equal to -123,5 ± -7,0 kJ/mole, -114,1 ± −7,9 kJ/mole, -106,9 ± −4,9 kJ/mole, respectively. 36 miRNA associations with mRNA are recommended for the diagnosis of the triple-negative subtype. 14 genes from 20 candidate genes of luminal A and B subtypes were miRNA targets. The binding sites of 86 miRNAs were located in 5'UTR, CDS, 3'UTR and the average ΔG value of miRNA binding with mRNA was equal to -121.2 ± -9,5 kJ/mole, 120,4 ± -7,8 kJ/mole, -118,9 ± -8,1 kJ/mole, respectively. 51 miRNA associations with mRNA were recommended for diagnosis of luminal A and B subtypes. In the mRNA of many genes, sites containing two or more miRNA binding sites were identified with arranging of their nucleotide sequences, which reduces the proportion of binding sites in the nucleotide composition in 5'UTR, CDS, and 3'UTR several times. Based on the obtained results, the groups of miRNA and mRNA associations of candidate genes are recommended to develop methods for diagnosis subtypes of breast cancer.

Key words: miRNA, mRNA, subtypes of breast cancer, target genes.

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miRNA-дың сүт безі қатерлі ісігі субтиптерінің кандидатты гендерінің mRNA-ЛЫ 5'UTR, CDS, 3'UTR-мен өзара әрекетінің сипаттамалары

Сүт безі қатерлі ісігінің түрлі субтиптері осы аурудың дамуына қатысатын кандидатты гендер жиынтығымен ерекшеленеді. Көптеген гендердің экспрессиясы олардың mRNA-ның miRNA байланыстыруымен реттеледі, сондықтан онкогенездің кандидатты гендерін және олардың miRNA-мен әрекеттесу дәрежесін анықтау керек. Бұл жұмыстың мақсаты сүт безі қатерлі ісігінің субтиптерінің 92 кандидаты генінің mRNA-мен белгілі 3701 miRNA-ның өзара әрекеттесу сипаттамаларын анықтау болды. 25 кандидатты гендердің ішінде 19 ген miRNA нысаналары болып келді. 67 miRNA-дың байланыстыру сайттары 5'UTR, CDS, 3'UTR және mRNA мен miRNA орташа бос байланысу энергиясы (ΔG) -120,2 ± -7,6 kJ/mole, -123,6 ± -9,8 kJ/mole, -110,4 ± -9,8 kJ/mole тең болды, тиісінше. Her2 субтиптің диагностикасы үшін байланысудың бос энергиясы -120 kJ/mole жоғары, mRNA мен miRNA 31 ассоциациялары ұсынылады. Triple-negative субтиптің 47 кандидатты гендерінен 33 ген miRNA-ның нысаналары болды. 90 miRNA-дың байланыстыру сайттары mRNA-ның 5'UTR, CDS, 3'UTR орналасқан және miRNA мен mRNA байланысуының орташа ΔG мәні -123,5 ± -7,0 kJ/mole, -114,1 ± –7,9 kJ/mole, -106,9 ± –4,9 kJ/mole тең болды, тиісінше. Triple-negative субтипті диагностикалау үшін 36 mRNA мен miRNA ассоциациялары ұсынылады. A және B luminal субтиптерді диагностикалау үшін 20 геннің ішінде 14 ген miRNA нысандары болып келді. 86 miRNA байланыстыру сайттары 5'UTR, CDS, 3'UTR орналасқан және miRNA мен mRNA байланысуының орташа ΔG мәні -121.2 ± -9,5 kJ/mole, 120,4 ± -7,8 kJ/mole, -118,9 ± -8,1 kJ/mole тең болды, тиісінше. A және B luminal субтиптерді диагностикалау үшін mRNA мен miRNA 51 ассоциациялары ұсынылады. Көптеген гендердің mRNA-да екі немесе одан да көп miRNA байланыстыру сайттары бар учаскілер анықталған, олардың нуклеотидтік тізбектері қабаттасқан, бұл 5'UTR, CDS және 3'UTR нуклеотидтер құрамында байланыстыру сайттардың үлесін бірнеше ретке дейін азайтады. Алынған нәтижелердің негізінде сүт безі қатерлі ісігінің субтиптерін диагностикалау әдістерін өңдеу үшін кандидатты гендердің mRNA мен miRNA ассоциациялар топтары ұсынылады.

Түйін сөздер: miRNA, mRNA, сүт безінің қатерлі ісігінің субтиптері, нысана гендер.

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Характеристики взаимодействия miRNA С 5'UTR, CDS, 3'UTR mRNA кандидатных генов субтипов рака молочной железы

Различные субтипы рака молочной железы отличаются набором кандидатных генов, участвующих в развитии этого заболевания. Экспрессия многих генов регулируется связыванием их mRNA с miRNA, поэтому требуется выявить, какие кандидатные гены онкогенеза и в какой степени могут взаимодействовать с miRNA. Цель настоящей работы заключалась в установлении характеристик взаимодействия известных 3701 miRNA с mRNA 92 кандидатных генов субтипов рака молочной железы. Из 25 кандидатных генов субтипа her2 мишенями miRNA являлись 19 генов. Сайты связывания 67 miRNA располагались в 5'UTR, CDS, 3'UTR и средняя свободная энергия связывания (ΔG) miRNA с mRNA равнялась -120,2 ± -7,6 kJ/mole, -123,6 ± -9,8 kJ/mole, -110,4 ± -9,8 kJ/mole, соответственно. Для диагностики субтипа her2 рекомендованы 31

ассоциация miRNA с mRNA, имеющие свободную энергию взаимодействия более -120 kJ/mole. Из 47 кандидатных генов субтипа triple-negative мишенями miRNA являлись 33 гена. Сайты связывания 90 miRNA располагались в 5'UTR, CDS, 3'UTR и средняя величина ΔG связывания miRNA с mRNA равнялась -123,5 ± -7,0 kJ/mole, -114,1 ± –7,9 kJ/mole, -106,9 ± –4,9 kJ/mole, соответственно. Для диагностики субтипа triple-negative рекомендованы 36 ассоциаций miRNA с mRNA. Из 20 кандидатных генов субтипов luminal A и B мишенями miRNA являлись 14 генов. Сайты связывания 86 miRNA располагались в 5'UTR, CDS, 3'UTR и средняя величина ΔG связывания miRNA с mRNA равнялась -121.2 ± -9,5 kJ/mole, 120,4 ± -7,8 kJ/mole, -118,9 ± -8,1 kJ/mole, соответственно. Для диагностики субтипов luminal A и B рекомендованы 51 ассоциация miRNA с mRNA. В mRNA многих генов выявлены участки, содержащие два и более сайтов связывания miRNA с наложением их нуклеотидных последовательностей, что в несколько раз уменьшает долю сайтов связывания в составе нуклеотидов в 5'UTR, CDS и 3'UTR. На основе полученных результатов рекомендуются группы ассоциаций miRNA и mRNA кандидатных генов для разработки методов диагностики субтипов рака молочной железы.

Ключевые слова: miRNA, mRNA, субтипы рака молочной железы, гены-мишени.

Introduction

In recent years, molecular markers have been actively searched for the diagnosis of breast cancer (Banin Hirata, 2014: 513158; Chistiakov, 2016: 107-121; Donepudi, 2014: 506-11; Healy, 2012: 2215-22; Hsieh, 2015: 494-509). Different subtypes of breast cancer are distinguished by a set of candidate genes participating in the development of the disease (Barba, 2017: 101; Ergün, 2015: 497-505; Fasching, 2018: JCO2D17772285; Feldinger, 2014: 6633-46; Jin, 2015: 1594-6D2; Leccia, 2014: 213; Ray, 2015: 224-34). These candidate genes may be targets for miRNA (mRNA-inhibiting RNA) that regulate their expression. In the process of oncogenesis, the concentration of miRNA is changed, which is usually interpreted as a cause, as a consequence of the disease (Chakraborty, 2016: 13039-13048; Hamam, 2016: 25997; Pastrello, 2010: 2124-6; Zhang, 2014: 950-8). However, the correlation between changes in the expression of gene and miRNA is not proof that these genes are targets of these miRNAs (Atambaeva, 2017: 428; Ivashchenko, 2014: e8; Ivashchenko, 2014: e11). It is impossible to experimentally identify how a well-known miRNA can interact with more than 20,000 genes and their isoforms. Therefore, it is required with the help of computational technologies to predict the target genes of certain miRNAs and then to test them experimentally. The purpose of this work is to establish the characteristics of the interaction of new miRNAs not included in the MirBase with mRNA of candidate genes involved in the development of different subtypes of breast cancer. Candidate genes are divided into groups that include candidate genes of only one subtype. This will reveal specific associations of miRNA and target genes on the basis of which it is possible to develop methods for early noninvasive diagnosis of selected subtypes of breast cancer.

Materials and methods

The nucleotide sequences of candidate genes of the BC subtypes were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). 3701 miRNA 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 breast cancer subtypes.

Table 1 – List of the candidate genes of breast cancer subtypes

Subtype Her2 *ADAM10* (doi: 10.1038/s41598-016-0013-4.); *ADAM17* (doi: 10.1016/j.acthis.2011.03.009); *AURKA* (doi: 10.1038/s41523-017-0049-z); *BRCA2* (doi: 10.1155/2016/5718104); *BRIP1(BRIP)* (doi: 10.18632/oncotarget. 7027); *CCNE2* (doi: 10.1371/journal.pone.0031422.); *CDK2* (doi: 10.1093/annonc/mdr340.); *CDK4* (doi: 10.2147/BCTT.S150540.); *CDK6* (doi: 10.2147/BCTT.S150540); *EPO* (doi: 10.5114/aoms.2016.62723.); *EPOR* (doi: 10.1007/s10549-012-2316-x); *ERBB3 (HER3)* (doi: 10.18632/oncotarget.22027); *FKBPL (FKBP4)* (doi: 10.1038/s41523-017-0049-z.); *H2AFX (H2AX)* (doi: 10.18632/oncotarget.2259); *LIN28B* (doi: 10.1089/ cbr.2014.1610.); *MAPK3 (ERK1)* (doi: 10.1016/j.bbrc.2017.06.001); *MAZ* (doi: 10.1371/journal.pone.0026122); *NISCH* (doi: 10.1016/j.artmed.2016.10.003); *PAEP (GD)* (doi: 10.1007/s10549-010-1065-y.); *PARP1 (PARP)* (doi: 10.1053/j.seminoncol.2017.06.006.); *PCNA* (doi: 10.1634/theoncologist.2013-0163.); *RAD21* (DOI: 10.1186/bcr3176); *RASSF1* (DOI: 10.18632/oncotarget.4062); *TIMP3* (doi: 10.1016/j.humpath. 2011.12.022); *TNF* (doi: 10.17219/acem/62120.).
Subtype Triple-negative (basal-like) *ANXA3 (doi: 10.1016/j.clbc.2017.11.009.); ARHGAP19 (*doi: 10.1186/bcr2867*.); ASAH1 (AC) (DOI:10.1158/1078-0432.CCR-06-1109); ATG4D (doi: 10.1038/emboj.2011.331.); ATM* (doi: 10.1007/s40262-017-0587-4); *AXL* (doi: 10.1155/2017/1686525); *BIRC5* (doi: 10.1186/1756-9966-31-58); *CBL* (doi: 10.1073/pnas.1300873110); *CD44* (doi: 10.1093/protein/gzx063); *CDC25C* (doi: 10.1038/onc.2010.510.); *CEACAM5 (CEA)* (doi: 10.1016/j.cca.2017.04.023); *CLDN1* (doi: 10.1186/1471-2407-13-268.); *CYP19A1* (DOI:10.1016/j.jsbmb.2005.04.028); *DRAM1* (doi: 10.1016/j.febslet.2012.12.027.); *ERBB3* (doi: 10.18632/oncotarget.13284); *F2RL1 (PAR2)* (doi: 10.1002/cmdc.201700640); *FGFR2* (doi: 10.1007/s00428-016-1950-9); *FH* (doi: 10.2147/OTT.S101677.); *FN1* (doi: 10.1016/j.jprot.2016.07.033;); *IAPP (IAP)* (doi: 10.18632/oncotarget.20227); *IL11* (doi: 10.1371/journal.pone.0037361); *JHDM1D(KDM7A)* (doi: 10.1002/ijc.27629); *LAMC1* (doi: 10.1016/j.molonc.2012.03.003*); LAMTOR5 (HBIXP)* (doi: 10.1002/ijc.28154.); *LASP1* (doi: 10.1186/1756-9966-31-58); *LEPR* (doi: 10.1016/j.stemcr.2017.11.010*.); LINC01554 (FIS) (doi: 10.1186/bcr3588.); MAGEA10 (A10)* (doi: 10.1016/j.acthis.2014.01.003); *MID1* (doi: 10.1016/j.ajpath.2013.02.046); *MMP2* (doi: 10.1038/srep28623); *MSN* (doi: 10.1186/bcr2867); *MTCH2* (doi: 10.1016/j.ajpath.2013.02.046.); *MYL9* (doi: 10.1002/ijc.27629); *NTRK2* (doi: 10.1186/bcr2867.); *PARP1* (doi: 10.1016/j.yexcr.2017.12.032.); *PFN1* (doi: 10.1080/15384101.2017.1346759); *PRKCE* (doi: 10.1038/onc. 2013.91); *PRRT2 (PKC)* (doi: 10.1002/cmdc.201700640); *PTGS2* (doi: 10.1073/pnas. 1709119114); *RAB5A* (doi: 10.3390/ijms17040443.); *RPSA (SA)* (doi: 10.1073/pnas.1005978107.); *RUNX1* (doi: 10.1016/j.ebiom. 2016.04.032); *SERPINE1 (PAI1)* (doi: 10.1186/1471-2407-13-268); *SFN* (doi: 10.1073/pnas.1315022110); *STMN1* (doi: 10.3892/ijo.2017.4085); *TBP* (doi: 10.1002/ijc.28154); *TLR-4* (doi: 10.1002/ijc.27629.).
Subtype Luminal A and B *AKT3 (doi: 10.1002/gcc.22279.); ANGPTL4* (doi: 10.1038/ncb2672.); *EZH1* (doi: 10.1371/journal.pgen. 1002751); *FOXA1* (doi: 10.1038/modpathol.2017.107); *GTF2IRD1* (doi: 10.2353/ajpath.2010.090837); *HMGA2* (doi: 10.1371/journal.pgen.1002751); *ITGA6* (doi: 10.1038/ncb2672.); *ITGB1* (doi: 10.1080/ 15548627.2016.1213928); *JAK1* (doi: 10.1371/journal.pgen.1002751); *LOX* (doi: 10.3390/ijms18122775.); *MAPT* (doi: 10.1007/s00428-012-1357-1); *MCM7* (doi: 10.1371/journal.pgen.1002751); *NAT1* (doi: 10.1007/ s10549-016-3741-z.); *PON1 (ESA)* (doi: 10.1186/1476-4598-13-213); *POSTN (PN)* (doi: 10.1186/1471-2407-12-216); *SMAD3* (doi: 10.1074/jbc.M113.506535); *SOX4* (doi: 10.1371/journal.pgen.1002751); *TGFB1 (TGFB)* (doi: 10.1038/ncb2672); *TNC (GP)* (doi: 10.2147/IJN.S56070.); *TP63* (doi: 10.1186/s13058-015-0607-y.).

Results and Discussion

The interaction of miRNA with mRNA candidate genes subtype her2

Table 2 – Characteristics of miRNAs interaction in the 5'UTR mRNA of BC subtype her2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ADAM10	miR-19-43936-3p (ID01772)	134	-108	93	22
	miR-9-26506-3p (ID03238)	165	-117	95	22
	miR-5-15733-3p (ID02761)	416	-132	89	24
	miR-1-2047-5p (MIR12047)	420	-113	90	22
BRCA2	miR-19-42224-5p (ID01563)	25	-115	93	21
BRIP1	miR-18-39953-5p (ID01508)	7	-129	90	23
CDK6	miR-17-21769-5p (ID01415)	261	-106	91	21

Continuation of table 2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
EPOR	miR-19-39380-3p (ID01633)	77	-108	91	21
	miR-19-42218-3p (ID01599)	79	-119	89	23
	miR-19-41131-3p (ID01626)	80	-129	90	23
EPO	miR-12-31979-3p (ID00752)	12	-121	89	23
ERBB3	miR-1-163-3p (MIR1163)	114	-113	93	21
MAPK3	miR-17-36936-3p (ID01422)	84	-113	90	22
MAZ	miR-15-39164-3p (ID00968)	16	-117	93	20
	miR-18-41189-3p (ID01476)	16	-134	91	23
	miR-11-29998-3p (ID00620)	27	-127	91	23
	miR-14-31624-3p (ID00915)	112	-127	88	24
	miR-7-12728-5p (ID02979)	114	-121	92	22
NISCH	miR-X-48174-3p (ID03445)	31	-125	88	24
	miR-19-43644-3p (ID01560)	38	-123	89	23
	miR-8-21978-5p (ID03119)	41	-125	88	24
RAD21	miR-1-3919-5p (ID00264)	180	-121	88	24
TIMP3	miR-6-17519-3p (ID02903)	1102	-121	90	22

23 miRNAs were bound in the 5'UTR mRNAs of 12 target genes (Table 2). mRNA of *ADAM10, MAZ* and *NISCH* genes (Jin, 2013: 2884-96; Madoux, 2016: 11; Peurala, 2011: e26122) contained two miRNA binding sites, nucleotide sequences of which overlapped. Three binding sites of miR-19-39380-3p, miR-19-42218-3p and miR-19-41131-3p comprised a 26 nt cluster located from 77 nt to 103 nt in the 5'UTR mRNA of *EPOR* gene. Without overlapping sites, their length would be 67 nt, which is half of the 5'UTR site equal to 135 nt. Consequently, the compacting of miRNA binding sites is useful in reducing the proportion of binding sites in the 5'UTR mRNA of *EPOR* gene.

In mRNA of *MAZ* gene the binding sites of miR-15-39164-3p, miR-18-41189-3p, miR-11-29998-3p were located in a cluster with length of 34 nt from 16 nt to 50 nt. The total length of the three miRNA is equal to 66 nt, which would occupy an essential part of the 5'UTR equal to 168 nt.

The average free energy of binding miRNA to mRNA in the site of 5'UTR of all mRNA is equal to -120,2±-7,6 kJ/mole. Therefore, the number of associations of miRNA with mRNA, having free binding energy more than -120 kJ/mole are equal to 13. All of them can serve as biomarkers in the development of methods for early diagnosis of the subtype her2.

mRNA of eight genes were targeted by 26 miRNAs (Table 2). mRNA of *MAPK3* and *NISCH* genes had two binding sites of miRNA with overlaying of nucleotide sequences. The *MAZ* gene was targeted by 15 miRNAs, whose binding sites were located in four clusters. The first cluster included binding sites of miR-5-17008-3p and miR-4-12861-5p. The second cluster with the length of 74 nt is located from 457 nt to 530 nt. The total length of all binding sites of miRNAs of this cluster is 260 nt, which requires their compaction, since, as a rule, in the CDS, all nucleotides participate in the coding of functionally important amino acids.

Table 3 – Characteristics of miRNAs interaction in the CDS mRNA of BC subtype her2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
CDK2	miR-1-83-3p(MIR183)	906	-110	90	22
EPO	miR-3-8171-3p(ID02345)	741	-110	93	22
FKBPL	miR-3-4734-5p(ID02318)	769	-115	89	23

Continuation of table 3

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
MAPK3	miR-1-2802-3p(ID00149)	1144	-117	93	22
	miR-19-42375-3p(ID01748)	1144	-110	91	21
MAZ	miR-5-17008-3p(ID02623)	363	-125	89	23
	miR-4-12861-5p(ID02460)	372	-119	92	22
	miR-3-8100-5p(ID02294) (3)	457 ÷ 469	-134 ÷ -138	91 ÷ 94	24
	miR-7-16350-5p(ID02986)	459	-119	93	21
	miR-2-6809-5p(ID01819)	461	-125	87	25
	miR-2-3313-3p(ID01804) (2)	464 ÷ 467	-140	88	25
	miR-20-43381-5p(ID02064)	489	-121	92	21
	miR-4-11923-3p(ID02538)	489	-125	94	22
	mir-1-2121-3p(ID00296)	500	-138	88	25
	miR-19-33623-3p(ID01641)	506	-132	89	24
	miR-19-33623-3p(ID01641)	608	-134	90	24
	miR-19-41914-3p(ID01705)	608	-117	92	21
	miR-3-7886-3p(ID02344)	671	-129	90	24
	miR-19-43065-3p(ID01768)	893	-113	90	22
	miR-2-7331-5p(ID01911)	900	-123	89	23
	miR-13-35476-3p(ID00849)	901	-125	97	22
NISCH	miR-3-7979-3p(ID02290)	1474	-93	92	21
	miR-12-32603-3p(ID00777)	2045	-117	93	23
	miR-9-26506-3p(ID03238)	2055	-113	91	22
	miR-17-36936-3p(ID01422)	2120	-115	92	22
PARP1	miR-19-36095-3p(ID01616)	1275	-119	90	23
TNF	miR-20-42898-3p(ID02050)	230	-121	92	23

The third cluster consisted of miR-19-33623-3p, miR-19-41914-3p binding sites with the length of 24 nt. The fourth cluster with the length of 31 nt included binding sites for three miRNAs from 893 nt to 923 nt. All binding sites for miRNAs interacted with mRNA gene have a total length of 440 nt, which is about 30% of the total CDS length. Clustered binding sites for miRNA occupy only 12% of the CDS length. The *MAZ* gene is the most vulnerable target for miRNA, so its expression should be monitored as a matter of priority.

The average free energy of miRNA binding in the CDS mRNA of all target genes was equal to -123,6 ± -9,8 kJ/mole. 13 from 26 miRNAs had free binding energy more than -120 kJ/mole, which gives the reason to use their associations with the corresponding target genes as biomarkers for the development of the subtype her2.

Table 4 – Characteristics of miRNAs interaction in the 3'UTR mRNA of BC subtype her2

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ADAM17	miR-7-20036-5p(ID02997)	3449	-113	93	22
BRCA2	miR-1-356-5p(MIR1356)	10722	-102	91	21
	miR-5-18072-3p(ID02744)	10738	-104	92	22
	miR-22-45335-5p(ID02199)	10821	-113	90	23

Continuation of table 4

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
BRIP1	miR-6-19858-3p(ID02880)	4481	-110	93	22
	miR-1-735-3p(ID00162)	6417	-102	92	22
	miR-X-45975-5p(ID03437)	6689	-96	92	22
	miR-2-4826-5p(ID01836)	6811	-113	90	23
CDK6	miR-9-24961-3p(ID03264)	1678	-98	90	22
	miR-10-29282-3p(ID00436) (9)	1896 ÷ 1920	-104 ÷ -106	89 ÷ 91	23
	miR-15-36862-3p(ID01030) (7)	1900 ÷ 1918	-108 ÷ -115	89 ÷ 95	23
	miR-4-13015-5p(ID02513)	1901	-102	91	22
	miR-9-9900-3p(ID03284)	3041	-102	94	20
	miR-8-23986-3p(ID03163)	7773	-127	88	24
H2AFX	miR-20-45152-5p(ID02052)	506	-136	91	24
	miR-1-3919-5p(ID00264)	632	-123	89	24
	miR-16-33136-3p(ID01069)	827	-123	91	22
	miR-17-37169-3p(ID01440)	834	-110	96	18
	miR-7-20437-5p(ID03031)	970	-115	89	23
LIN28B	miR-19-38260-3p(ID01654)	3909	-113	90	22
MAPK3	miR-5-14412-5p(ID02712)	1789	-115	90	23
MAZ	miR-X-48174-3p(ID03445)	2072	-127	90	24

In the 3'UTR mRNA of *BRCA2* and *H2AFX* genes, two miRNA binding sites were identified with overlapping of nucleotide sequences (Table 4). The gene *CDK6* (Johnson, 2016: 4829-35) was the target for six miRNAs. Nine binding sites of miR-10-29282-3p, seven binding sites of miR-15-36862-3p and one miR-4-13015-5p binding site formed a cluster from 1896 nt to 1943nt with the length of 47 nt. The total length of all 17 binding sites was equal to 390 nt, which is 3.8% of the total length of the 3'UTR mRNA of *CDK6* gene of 10219 nt. Compacting the sites of miRNA binding is difficult to explain only by saving the length of 3'UTR. Apparently, there are other reasons for compacting the binding sites. For example, the binding of a one RISC to miRNA does not allow other miRNAs to communicate with their site, and if this miRNA is a signal of the host gene, the *CDK6* gene does not perceive this signal. That is, there is competition between different miRNAs for the binding site and for the ability to regulate the expression of the target gene.

The average value of the free energy of binding of all miRNAs with mRNA in 3'UTR was equalto -110.4 ± -9.8 kJ / mole. Five miRNAs from 22 miRNAs were bound with the mRNA of the corresponding target genes with free binding energy more than -120 kJ/mole (Table 3). Associations of these miRNAs with their target genes are recommended to be used as markers for diagnosis of the subtype her2. miR-10-29282-3p and miR-15-36862-3p, having nine and seven binding sites, respectively, in the mRNA of *CDK6* target gene, are also recommended as markers for early diagnosis because an increase in the number of miRNAs binding sites contributes to an increase in the likelihood of their detection. Only miR-20-45152-5p interacted with mRNA of *H2AFX* gene with ΔG more than -130 kJ/mole.

Table 5 shows the interaction patterns of some miRNAs with different mRNA regions (5'UTR, CDS, 3'UTR) of the candidate genes of subtype her2 of breast cancer. These data demonstrate the advantages of our MirTarget program before the programs used in many studies. The disadvantage of these programs is the search for binding sites only over the site of the miRNA called the "seed" of 7-9 nucleotides located at the 5'-end of the miR. Therefore, these programs predict many false positive binding sites. The MirTarget program calculates the complementarity of the miRNA nucleotides with mRNA over the entire length of their interaction and such errors are excluded.

Table 5 – Schemes of miRNA binding sites with mRNA of candidate gene's of BC subtype her2

BRCA2; miR-5-18072-3p; 3'UTR; 10738; -104; 92 5'–AGCUCGGUGGCUCAUGCCUGUA–3' 3'–UUGAGUCACCGAGUAUGGAUAU–5'	*BRCA2*; miR-1-356-5p; 3'UTR; 10723; -102; 91 5'–AAACAUCUUUGGCUGAGCUCG–3' 3'–UUUGUAAAAACCGGCCCGAGC–5'
CDK6; miR-10-29282-3p; 3'UTR; 1920; -106; 91 5'–GUGUGUGUGUGUGUGUGUGUGUA–3' 3'–CACACACGCAUAUAUACACACAU–5'	*CDK6*; miR-17-21769-5p; 5'UTR; 261; -106; 91 5'–GGCGGCGGCGGCGGCGACUCU–3' 3'–CUGUCGUCGCCGUCGUUGAGA–5'
ERBB3; miR-1-163-3p; 5'UTR; 115; -110; 91 5'–CCCGGACUCCGGCUCCGGCUC–3' 3'–GAGUCCGAGGCCGAGGCUGAG–5'	*MAZ*; miR-7-12728-5p; 5'UTR; 114; -121; 92 5'–CGGCCCGCGCCCCCGGCCCCCG–3' 3'–GCUGGACGCGGGGGUCGGGGGA–5'
EPOR; miR-5-16562-3p; CDS; 173; -119; 88 5'–GCUCCCUUUGUCUCCUGCUCGCUG–3' 3'–CGAGGGAGAGAGAGGACGACCGAC–5'	*MAZ*; miR-1-2121-3p; CDS; 615; -134; 85 5'–CGGCCCGCGCCCCCGGCCCCCG–3' 3'–GCUGGACGCGGGGGUCGGGGGA–5'
Note: Gene; miRNA; the beginning of binding site; the miRNA region; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt). In bold type highlighted the "seed" nucleotides.	

The interaction of miRNA with mRNA candidate genes subtype triple-negative

Table 6 – Characteristics of miRNA interaction in the 5'UTR mRNA of BC subtype triple-negative

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ANXA3	miR-6-17875-3p(ID02846)	132	-117	90	23
AXL	miR-17-40012-5p(ID01321)	131	-115	93	21
BIRC5	miR-16-35004-5p(ID01145)	110	-125	89	23
	miR-16-36548-3p(ID01145)	110	-125	89	23
CBL	miR-9-20317-3p(ID03332) (4)	16 ÷ 25	-134 ÷ -140	90 ÷ 94	24
	miR-17-39416-3p(ID01310) (4)	17 ÷ 26	-121	92	22
	miR-5-15733-3p(ID02761)	28	-138	93	24
	miR-1-1819-3p(ID00278)	32	-125	91	23
	miR-3-9439-3p(ID02430)	34	-110	98	18
CD44	miR-16-40163-5p(ID01213)	129	-121	90	23
	miR-6-7754-5p(ID02860)	376	-113	91	21
ERBB3	miR-1-163-3p(MIR1163)	114	-113	93	21
FGFR2	miR-7-21139-3p(ID03047)	48	-132	89	24
	miR-19-34067-3p(ID01542)	60	-123	92	23
	miR-1-2228-3p(MIR12228)	152	-125	89	24
FN1	miR-19-43437-5p(ID01723)	109	-115	90	23
IL11	miR-2-4531-3p(ID01869)	384	-106	91	21
LAMC1	miR-19-43342-3p(ID01667)	51	-119	90	22
	miR-10-13655-3p(ID00457)	115	-123	91	22
LASP1	miR-16-36476-5p(ID01071)	68	-119	90	22
	miR-5-16438-3p(ID02653)	206	-119	90	22

Continuation of table 4

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
MMP2	miR-1-1819-3p(ID00278)	110	-123	89	23
	miR-17-39416-3p(ID01310)	113	-121	92	22
	miR-7-20203-3p(ID03037)	115	-121	90	22
	miR-9-27797-5p(ID03345)	124	-127	90	24
	miR-9-24743-3p(ID03368)	125	-117	89	23
	miR-2-6328-5p(ID02014)	264	-117	89	23
MTCH2	miR-5-15926-3p(ID02660)	74	-123	94	22
NTRK2	miR-17-39416-3p(ID01310)	61	-125	95	22
	miR-5-15564-3p(ID02611)	65	-127	92	22
	miR-10-13940-3p(ID00332)	171	-110	96	18
PFN1	miR-3-8242-5p(ID02292)	78	-119	89	23
	miR-9-23803-5p(ID03396)	78 ÷ 84	-123 ÷ -129	88 ÷ 92	24
	miR-19-42375-3p(ID01748)	436	-113	93	21
	miR-3-10870-3p(ID02450)	507	-115	92	21
	miR-3-9317-3p(ID02428)	511	-115	93	22
PRRT2	miR-X-48174-3p(ID03445)	51	-125	88	24
PTGS2	miR-9-23969-3p(ID03397)	108	-123	92	21
RAB5A	miR-12-32603-3p(ID00777)	137	-113	90	23
	miR-6-17815-3p(ID02930)	184	-132	89	24
	miR-X-48174-3p(ID03445)	189	-127	90	24
	miR-2-6862-5p(ID01859)	191	-121	89	23
	miR-2-3313-3p(ID01804)	325	-140	88	25
	miR-9-28523-5p(ID03367)	328	-121	97	20
	miR-1-155-3p(MIR1155)	334	-127	92	22
RUNX1	miR-5-14114-5p(ID02592)	1417	-123	89	23
SERPINE1	miR-16-38458-3p(ID01098)	30	-123	88	24

Twenty candidate genes of the triple-negative subtype of breast cancer were targets for 47 miRNAs (Table 6). There are two miRNA binding sites in the 5'UTR of *BIRC5, FGFR2* and *NTRK2* (Burstein, 2015: 1688-98; Howe, 2011: R45; Wang, 2012: 58) genes and two clusters of two miRNAs were located in the mRNA of *PFN1* gene.

The *CBL* gene was a target for five miRNAs, two of which had four binding sites. A cluster of binding sites for five miRNAs is located from 16 nt to 45 nt. All binding sites for miRNAs had a total length of 247 nt. The cluster size was 29 nt. With a length of 5'UTR mRNA of *CBL* gene 142 nt, the need for cluster organization of miRNA binding sites is obvious.

The five miRNAs were bound in the 5'UTR mRNA of *MMP2* gene with a length of 311 nt with overlapping binding sites. As a result, with a total length of 114 nt, they occupied a section of mRNA with a length of 38 nt, that is, three times less than the total length of binding sites.

The *RAB5A* gene was a target for seven miRNAs, three of them were formed into two clusters. As a result, six binding sites of length 138 nt were compacted into a section of 61 nt of length, which is considerably smaller than the length of the 5'UTR mRNA of *RAB5A* gene.

The average free energy of all miRNAs binding was equal to -123,5 ± -7,0 kJ/mole. 16 miRNAs had the free binding energy more than -123 kJ/mole. Associations of these miRNAs with relevant target genes are recommended as markers for the diagnosis of subtype triple-negative breast cancer.

Table 7 – Characteristics of miRNAs interaction in the CDS mRNA of BC subtype triple-negative

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ATG4D	miR-10-12491-5p(ID00455)	239	-115	89	23
AXL	miR-1-875-3p(ID00166)	2838	-115	90	22
CBL	miR-11-29461-3p(ID00478)	176	-125	89	23
FH	miR-13-33973-3p(ID00844)	939	-104	91	22
FN1	miR-6-16740-5p(ID02776)	1392	-110	90	22
JHDM1D	miR-3-8153-3p(ID02337)	95	-113	91	21
LAMC1	miR-6-18496-3p(ID02883)	388	-119	90	22
MMP2	miR-21-45324-5p(ID02146)	379	-125	91	23
	miR-19-43421-5p(ID01636)	1681	-108	91	21
	miR-17-39037-3p(ID01456)	1691	-113	90	22
MSN	miR-1-1585-3p(ID00272)	821	-96	92	21
PARP1	miR-19-36095-3p(ID01616)	1275	-119	90	23
PRRT2	miR-12-31369-5p(ID00657)	343	-108	89	23
	miR-19-41746-3p(ID01631)	1081	-117	90	23
SERPINE1	miR-2-3962-5p(ID01786)	542	-125	88	24

The 12 mRNA of genes from 47 candidate genes of the triple-negative subtype had binding sites in the CDS (Table 7). miR-19-43421-5p and miR-17-39037-3p had overlapping binding sites only in mRNA of *MMP2* gene. The average value of the free energy of binding of all miRNAs with mRNAs was equal to -114,1 ± –7,9 kJ/mole.

Only miR-11-29461-3p, miR-21-45324-5p and miR-2-3962-5p interacted with free energy more than -120 kJ/mole with mRNA of *CBL, MMP2* and *SERPINE1* genes, respectively. These three associations of miRNA and genes can be used as markers for the diagnosis of the triple-negative BC subtype.

Table 8 – Characteristics of miRNAs interaction in the 3'UTR mRNA of BC subtype triple-negative

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ARHGAP19	miR-3-8671-3p(ID02367)	2305	-96	90	22
ATM	miR-7-21133-5p(ID03006)	9778	-121	89	24
	miR-10-26483-5p(ID00367)	11069	-110	90	22
AXL	miR-12-31899-3p(ID00677)	3071	-102	91	22
	miR-17-39935-3p(ID01360)	3313	-104	91	21
CBL	miR-4-13015-5p(ID02513)	3219	-102	91	22
	miR-2-4804-5p(ID01838)	7728	-117	93	24
	miR-2-5355-3p(ID02017)	7984	-115	90	22
DRAM1	miR-2-5411-3p(ID01933)	2045	-102	89	23
	miR-22-45335-5p(ID02199)	2984	-113	90	23
IAPP	miR-14-35161-5p(ID00913)	824	-117	89	24
	miR-22-45902-3p(ID02175)	992	-110	91	22
IL11	miR-17-34996-5p(ID1404)	1470	-113	91	23
JHDM1D	miR-9-27051-5p(ID03228)	5526	-102	92	23

Continuation of table 8

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
LASP1	miR-10-27287-3p(ID00318)	1287	-108	93	20
	miR-15-36862-3p(ID01030)	3899	-108	89	23
MID1	miR-22-45438-5p(ID02185)	5669	-106	93	22
MYL9	miR-19-43662-5p(ID01657)	639	-121	93	23
	miR-X-46723-3p(ID03478)	1083	-115	93	21
NTRK2	miR-11-26830-5p(ID00623)	7962	-106	91	22
PFN1	miR-16-37915-3p(ID01151)	1240	-123	89	24
RUNX1	miR-4-11239-3p(ID02574)	3123	-115	93	20
	miR-1-2558-3p(MIR12558)	3368	-117	93	22
	miR-15-36862-3p(ID01030) (2)	5454 ÷ 5464	-108 ÷ -113	89 ÷ 93	23
	miR-10-29282-3p(ID00436)	5464	-108	93	23
SFN	miR-19-30988-5p(ID01774)	835	-129	90	23
	miR-20-44122-5p(ID02037)	945	-108	91	22
	miR-12-31413-3p(ID00790)	1179	-104	89	23
	miR-6-17487-3p(ID02868)	1188	-113	90	23
	miR-15-36862-3p(ID01030) (6)	1190 ÷ 1200	-108	89	23
	miR-10-29282-3p(ID00436) (6)	1190÷ 1202	-104	89	23
	miR-19-42814-5p(ID01727) (2)	1203 ÷ 1205	-104 ÷ -106	89 ÷ 91	23
	miR-6-17605-3p(ID02882)	1210	-108	91	21
STMN1	miR-5-17240-3p(ID02697)	1096	-119	89	23
	miR-7-13347-5p(ID03011)	1730	-106	91	22
	miR-10-26483-5p(ID00367)	1744	-113	91	22
	miR-2-5355-3p(ID02017)	1987	-119	93	22

The 16 candidate genes had binding sites of 38 miRNAs in the 3'UTR mRNA (Table 8). The clusters of binding sites for two miRNAs were identified in the 3'UTR mRNA of *RUNX1* and *STMN1* genes. There is a cluster of binding sites for six miRNAs in the region with the length 53 nt, from 1179 nt to 1231 nt in the 3'UTR mRNA of *SFN* gene. The sum of the lengths of all miRNAs binding sites is equal to 366 nt. Due to the clustering of the binding sites of six miRNAs, the site occupied by them is only 10% of the length of the 3'UTR mRNA of *SFN* gene equal to 498 nt.

The free energy of miRNA binding in the 3'UTR mRNA of target genes was low, equal to -106,9 ± –4,9 kJ/mole. Only with the interaction of miR-19-43662-5p, miR-16-37915-3p and miR-19-30988-5p with the mRNA of *MYL9, PFN1* and *SFN* genes, respectively, the free energy value was larger than -120 kJ/mole. Associations of these miRNAs with relevant target genes are recommended as markers for the diagnosis of subtype triple-negative breast cancer.

Table 9 shows the schemes of miRNA binding with mRNA of candidate genes of the triple-negative subtype. In all cases, the interaction of nucleotides occurs over the entire length, with the exception of the absence of hydrogen bonding between purines (A, G) or pyrimidines (C, U).

The 20 binding sites of miRNAs with overlapping of nucleotide sequences were identified in the 5'UTR mRNA of *FOXA1* (Chaudhary, 2017: 1247-1264) gene (Table 10). All 18 miRNAs formed a cluster from 99 nt to 130 nt with the length of 31 nt. The total length of all 20 sites was 427 nt. The formation of a cluster of binding sites for *FOXA1* gene in the 5'UTR indicates a greater ability of this gene for compaction, which causes the competition of miRNAs data for the binding site. The average binding energy for 20 miRNAs was -116,8 kJ/mole.

Table 9 – Schemes of miRNAs binding sites with candidate gene's of BC subtype triple-negative

ATM; miR-7-21133-5p;3'UTR; 9778; -121; 89 5'–CGGGCUGGGCGCAGCGGCUCACGC–3' 3'–ACCCGACCCGUGUCCCCGAGUGUA–5'	*CEACAM5*; miR-7-21133-5p; 3'UTR; 3220; -119; 87 5'–UGGGCCGGGCGCGGUGGCUCACGC–3' 3'–ACCCGACCCGUGUCCCCGAGUGUA–5'
ERBB3; miR-1-163-3p; 5'UTR; 115; -110; 91 5'–CCCGGACUCCGGCUCCGGCUC–3' 3'–GAGUCCGAGGCCGAGGCUGAG–5'	*ERBB3*; miR-14-35161-5p; 3'UTR; 4970; -113; 86 5'–GCACUUUGGGAGGCUGAGGCAGAA–3' 3'–UGUGAAACCCUCUCGCUCCGUCCU–5'
FH; miR-13-33973-3p; CDS; 939; -104; 91 5'–GGUUGCUGCAAAAGUGGCUGCA–3' 3'–CCAACAACGUUUUCAUUGACGC–5'	*IL11*; miR-17-34996-5p; 3'UTR; 1470; -113; 91 5'–GCAACCUCCACCUCCCGGGUUCA–3' 3'–CGUUAGAGAAGGAGAGCCCAAGU–5'
NTRK2; miR-9-20317-3p; 5'UTR; 63; -129; 87 5'–AGCAGAGGCGGCGGCGGCGGCUCC–3' 3'–CCGCCUCCGCCUCCGCCGCCGCGG–5'	*PFN1*; miR-9-23803-5p; 5'UTR; 84; -129; 92 5'–GGCGCAGGCGCAGGCGCGGGCACA–3' 3'–CCGCGUCCGCGUCCGCGUCUGCAU–5'
Note: Gene; miRNA; the beginning of binding site; the miRNA region; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt). In bold type highlighted the "seed" nucleotides.	

The interaction of miRNA with mRNA candidate genes subtype luminal A and B

Table 10 – Characteristics of miRNA binding in the 5'UTR mRNA of BC subtype luminal A and B

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
FOXA1	miR-1-1904-5p(ID00297)	99	-123	89	24
	miR-20-43873-3p(ID02106)	110	-123	89	23
	miR-1-1510-5p(ID00252)	111	-140	94	24
	miR-5-3563-5p(ID02769)	112	-127	92	22
	mir-1-2121-3p(ID00296)	115	-140	89	25
	miR-16-16153-5p(ID01099)	116	-108	100	17
	miR-1-1714-3p(MIR11714) (2)	118 ÷ 121	-117 ÷ -121	93 ÷ 97	20
	miR-16-29933-5p(ID01190)	118	-108	100	17
	miR-4-9774-3p(ID02457)	118	-108	100	17
	miR-5-16727-5p(ID02595)	118	-115	92	20
	miR-17-40348-5p(ID01403)	120	-123	91	23
	miR-19-21199-3p(ID01702)	120	-140	89	25
	miR-9-28523-5p(ID03367) (2)	121 ÷ 122	-117	93	20
	miR-19-33623-3p(ID01641)	122	-134	90	24
	miR-10-13655-3p(ID00457)	124	-123	91	22
	miR-1-155-3p(MIR1155)	127	-129	94	22
	miR-4-6496-3p(ID02499) (2)	127 ÷ 130	-119 ÷ -121	92 ÷ 93	21
GTF2IRD1	miR-14-35532-3p(ID00962)	206	-117	89	23
	miR-12-32997-5p(ID00663)	208	-125	89	23
	miR-8-23353-3p(ID03172)	340	-123	92	22

Continuation of table 10

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
HMGA2	miR-2-3313-3p(ID01804)	99	-138	87	25
	miR-17-38391-3p(ID01352)	312	-115	90	23
	miR-3-9317-3p(ID02428)	314	-110	90	22
	miR-19-43373-3p(ID01737)	539	-119	93	21
	miR-15-32047-5p(ID01041) (2)	541 ÷ 544	-129 ÷ -134	88 ÷ 91	24
	miR-1-265-3p(MIR1265)	542	-125	91	22
	miR-17-41168-3p(ID01323)	542	-117	95	20
	miR-3-9301-5p(ID02296)	542	-115	93	20
	mir-1-2121-3p(ID00296)	544	-146	93	25
	miR-19-33623-3p(ID01641)	544	-142	96	24
	miR-1-155-3p(MIR1155)	550	-132	95	22
	miR-9-28523-5p(ID03367)	550	-115	92	20
	miR-10-26815-5p(ID00425)	575	-121	88	24
	miR-11-18690-5p(ID00564)	585	-110	90	22
	miR-1-1819-3p(ID00278)	788	-123	89	23
ITGA6	miR-2-4035-3p(ID01810)	111	-115	89	23
	miR-7-20589-3p(ID02982)	161	-113	91	21
JAK1	miR-11-29827-3p(ID00580)	66	-129	90	24
MAPT	miR-5-13986-5p(ID02608)	107	-113	90	22
	miR-17-40141-3p(ID01315)	120	-115	92	20
MCM7	miR-7-20142-5p(ID03055)	26	-119	89	23
	miR-8-23353-3p(ID03172)	111	-121	90	22
	miR-16-39014-5p(ID01191)	846	-106	91	21
SMAD3	miR-7-15849-3p(ID03064)	4	-115	100	18
	miR-4-12789-5p(ID02547)	31	-115	93	21
	miR-15-11315-5p(ID01020)	194	-117	100	19
	miR-12-29625-3p(ID00659)	243	-125	92	23
TGFB1	miR-20-43381-5p(ID02064)	1	-121	92	21
	miR-5-8853-5p(ID02770)	6	-115	92	20
	miR-9-13610-3p(ID03306)	6	-121	92	21
	miR-12-30416-5p(ID00795)	186	-117	92	22
	miR-10-13655-3p(ID00457)	209	-129	95	22
	miR-11-29785-3p(ID00529)	232	-108	91	21
	miR-9-26506-3p(ID03238)	237	-113	91	22
	miR-17-38733-3p(ID01344)	241	-119	89	24

The mRNA of *GTF2IRD1* gene (Cicatiello, 2010: 2113-30) had three binding sites, of which two sites formed a cluster with overlapping of nucleotide sequences. The *HMGA2* gene had 16 binding sites for 15 miRNAs, miR-17-38391-3p and miR-3-9317-3p formed a cluster from 312 nt to 214 nt, 11 miRNAs had a cluster from 539 nt to 585 nt, with a total length of 268 nt. The *ITGA6, MAPT* genes had two binding sites, the *JAK1* gene had one binding site for miR-11-29827-3p. The *MCM7* gene had three binding sites for different miRNAs, the *SMAD3* gene had four binding sites for miRNAs from 4nt to 243 nt without overlapping of nucleotide sequences. The *TGFB1* gene had nine binding sites

for different miRNAs, two clusters of them for miR-9-26506-3p, miR-17-38733-3p, there was a cluster of binding sites for miR-20-43381-5p, miR-5-8853-5p , miR-9-13610-3p.

The average free energy of binding of all miRNAs with mRNAs in the 5'UTR was equal to -119.2 ± 9.0 kJ/mole. The 29 miRNAs from all miRNAs, were bound with mRNAs of the corresponding target genes with a free binding energy more than -120 kJ/mole. The associations of these miRNAs with their target genes are recommended to be used as markers for diagnosis of the subtype luminal A and B.

Table 11 – Characteristics of miRNAs interaction in the CDS mRNA of BC subtype luminal A and B

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
ANGPTL4	miR-19-43315-5p(ID01593)	259	-134	100	23
	miR-9-26025-3p(ID03320)	567	-113	90	22
FOXA1	miR-X-44972-5p(ID03416)	762	-117	92	20
	miR-5-15733-3p(ID02761)	768	-132	89	24
	miR-9-20317-3p(ID03332)	1150	-134	90	24
GTF2IRD1	miR-8-21162-5p(ID03096)	959	-121	92	23
ITGB1	miR-10-26815-5p(ID00425)	61	-127	92	24
	miR-22-46979-5p(ID02187)	91	-127	92	23
	miR-10-13655-3p(ID00457)	95	-123	91	22
	miR-5-8853-5p(ID02770)	98	-117	93	20
	miR-16-40261-3p(ID01184)	101	-117	93	20
JAK1	miR-3-10699-5p(ID02371)	2532	-108	96	21
SOX4	miR-2-6184-3p(ID01787)	883	-117	90	23
	miR-4-13460-3p(ID02568)	1291	-123	91	22
	miR-5-14873-3p(ID02692)	1293	-121	90	22
	miR-6-16793-3p(ID02813)	1323	-113	93	20
	miR-4-11437-3p(ID02477)	1402	-125	89	23
	miR-X-48174-3p(ID03445)	1454	-125	88	24
	miR-9-28523-5p(ID03367)	1544	-115	92	20
	miR-12-30075-3p(ID00695)	1721	-127	88	24
	miR-9-27181-5p(ID03354)	1723	-127	92	22
	miR-8-24013-5p(Id03222)	1826	-113	91	21
	miR-9-13610-3p(ID03306)	1900	-121	92	21
TNC	miR-19-42814-5p(ID01727)	1199	-104	89	23
	miR-19-41413-3p(ID01524)	3739	-110	90	22

Two binding sites were found in the CDS mRNA of *ANGPTL4, TNC* genes, for miR-19-43315-5p with 100% complementarity and binding energy -134 kJ/mole. The *FOXA1* gene had a cluster of binding sites for two miRNAs. The *GTF2IRD1, JAK1* genes had one binding site for miR-8-21162-5p with the binding energy -121 kJ/mole and a degree of complementarity of 92%, and for miR-3-10699-5p, respectively. The *ITGB1* gene had a cluster for four binding sites from 91 nt to 101 nt each. The *SOX4* gene had 11 binding sites, two clusters of them had two binding sites each.

The average free energy of binding of all miRNAs with mRNAs in the CDS was equal to 120.4 ± 7.8 kJ/mole. The 14 miRNAs from 25 miRNAs were bound with mRNAs of the corresponding target genes with a free interaction energy more than -120 kJ/mole (Table 11), which allows us to recommend miRNAs as markers for the diagnosis of breast cancer of the subtype luminal A and B.

Table 12 – Characteristics of miRNAs interaction in the 3'UTR mRNA of BC subtype luminal A and B

Gene	miRNA	Position, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
EZH1	miR-17-39273-3p(ID01256)	3085	-115	89	23
	miR-9-26506-3p(ID03238)	3699	-110	90	22
	miR-19-43614-3p(ID01533)	3832	-125	91	23
HMGA2	miR-2-6081-3p(ID01970)	1255	-113	90	23
	miR-13-35476-3p(ID00849) (2)	1261 ÷ 1268	-117	90	22
	miR-19-43804-3p(ID01545)	1275	-115	95	21
ITGA6	miR-4-13401-5p(ID02524)	5720	-96	92	22
SMAD3	miR-6-16980-5p(ID02822)	2070	-127	91	23
	miR-15-38620-5p(ID00978)	2072	-119	90	22
	miR-17-12804-3p(ID01382)	2075	-113	93	20
	miR-14-35670-5p(ID00956)	4330	-119	89	23
SOX4	miR-11-29077-3p(ID00568)	2428	-123	88	24
	miR-2-5674-3p(ID01839)	2994	-123	89	23
	miR-17-39011-3p(ID01282)	3000	-125	95	23
	miR-X-48174-3p(ID03445)	3000	-127	90	24
	miR-1-2558-3p(MIR12558)	3001	-115	92	22
TGFB1	miR-9-13610-3p(ID03306)	2060	-123	94	21
	miR-17-12804-3p(ID01382)	2062	-113	93	20
	miR-8-24549-5p(ID03208)	2066	-125	88	24
	miR-15-38620-5p(ID00978)	2089	-119	90	22
	mir-1-2121-3p(ID00296)	2093	-140	89	25
TNC	miR-2-4826-5p(ID01836)	8073	-115	92	23

Table 13 – Schemes of miRNAs binding sites with candidate gene's of BC subtype luminal A and B

ANGPTL4; miR-19-43315-5p; CDS; 259; -134; 100 5'-AGCGCUCAGGGCGGACCCGUGCA-3' 3'-UCGCGAGUCCCGCCUGGGCACGU-5'	*FOXA1*; miR-4-12154-5p; CDS; 1129; -125; 87 5'-GGGGCCGGCGGCGGGGGCGGGAGC-3' 3'-CCUCGGCCGCCGCCUCGGCUCCCA-5'
FOXA1; miR-9-20317-3p; CDS; 1150; -134; 90 5'-AGCGGAAGCGGGGGCAGCGGCGCC-3' 3'-CCGCCUCCGCCUCCGCCGCCGCGG-5'	*LOX*; miR-3-11226-3p; CDS; 533; -113; 88 5'-AGGUGUUCAGCUUGCUGAGCCUG-3' 3'-UUCACAGGUCGAUCGACCCGGAC-5'
SMAD3; miR-15-11315-5p; 5'UTR; 194; -117; 100 5'-GCGACCGCGGCAGGCCCCG-3' 3'-CGCUGGCGCCGUCCGGGGC-5'	*SMAD3*; miR-17-12804-3p; 3'UTR; 2075; -113; 93 5'-GCCCCGCCCCGCCCCGCCCC-3' 3'-CGGGGCGGGGAAGAGCGGGG-5'
SOX4; miR-2-6184-3p; CDS; 883; -118; 90 5'-UCGCCUCCUCCCCCACGCCCGGC-3' 3'-AGUGGAGGAGGAGGUGAGGGACG-5'	*TNC*; miR-14-35161-5p; 3'UTR; 7856; -113; 85 5'-ACACUUUGGGAGGCCAAGGUGGGA-3' 3'-UGUGAAACCCUCUCGCUCCGUCCU-5'
Note: Gene; miRNA; the beginning of binding site; the miRNA region; the free energy change (ΔG, kJ/mole); the ΔG/ΔGm (%); length of miRNA (nt). In bold type highlighted the "seed" nucleotides.	

There were three binding sites for different miRNAs in the 3'UTR mRNA of *EZH1* gene (table 12). For *HMGA2* gene there was a cluster for three binding sites from 1261 nt to 1275 nt. *ITGA6, TNC* genes had one binding site. There were four binding sites for *SMAD3* gene, of which three formed a

cluster for three miRNAs. The mRNA of *SOX4, TGFB1* genes had five binding sites for miRNAs, where was a cluster of binding sites for five miRNAs with mRNA of *TGFB1* gene and a cluster for four binding sites for *SOX4* gene.

The average free energy of binding of all miRNAs with mRNAs in the 3'UTR was equal to -118.9 ± 8.1 kJ/mole. The nine miRNAs from 22 miRNAs were bound with mRNAs of the corresponding target genes with a free binding energy more than -120 kJ/mole (Table 6). The associations of these miRNAs with their target genes are recommended to be used as markers for the diagnosis of the triple-negative subtype.

Table 13 shows diagrams of binding of miRNAs with mRNAs of candidate genes of the subtype luminal A and B. The interaction of nucleotides occurs along the entire length, except for the absence of hydrogen bonding between purines (A, G) or pyrimidines (C, U). Interactions of non-canonical pairs of nucleotides A-C and G-U are accounted for by the MirTarget program.

Conclusion

1. The associations of miRNA and their target genes have been identified in each group of candidate genes, they have an increased degree of interaction and can serve as markers for the development of methods for early diagnosis of her2, triple negative and luminal A and B subtypes.

2. The average free energy of miRNA binding with mRNA genes of all subtypes is greater in 5'UTR and CDS as compared to the 3'UTR, which suggests preferential binding of miRNA to 5'UTR and CDS.

3. The location of mRNA binding sites in clusters containing two or more binding sites with overlapping their nucleotide sequences has been found. Such a compact arrangement of binding sites in mRNA allows a multiple reduction in the proportion of binding sites in the nucleotide sequence of mRNA. The overlap of miRNA binding sites creates competition between miRNAs for binding sites, since the RISC complex with miRNA having a large amount of free interaction energy will not allow binding to another RISC complex with miRNA having a weaker interaction with mRNA.

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