4-бөлім

МОЛЕКУЛАЛЫҚ БИОЛОГИЯ ЖӘНЕ ГЕНЕТИКА

Раздел 4

МОЛЕКУЛЯРНАЯ БИОЛОГИЯ И ГЕНЕТИКА

Section 4

MOLECULAR BIOLOGY AND GENETICS

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FEATURES OF MIRNA BINDING WITH MRNA OF CANDIDATE GENES OF BREAST CANCER SUBTYPES

To determine associations of miRNA and mRNA of their target genes, binding characteristics of miRNA and mRNA of candidate genes of four subtypes of the breast cancer have been studied. Half of candidate genes of the triple-negative subtype had binding sites for several miRNAs. mRNA of ATM gene had seven binding sites for miR-5095, miR-619-5p, miR-5096, miR-5585-3p, miR-1273a, miR-1273g-3p, all of which bind in the 3′UTR. mRNA of AXL gene, the tyrosine kinase receptor, had binding sites for five miRNAs that are localized in 3′UTR, CDS, and 5′UTR. From five miRNA, miR-1908-3p may the most effective regulated the expression of protooncogene CBL. mRNA of CEACAM5 gene contained binding sites of miR-5095, miR-619-5p, miR-5585-3p with a high degree of complementarity. mRNA of F2RL1, IAPP genes have binding sites predominantly for miR-5095, miR-619-5p, miR-5585-3p, miR-5096. Based on the obtained data, it is necessary to control the expression of candidate genes of the triple-negative subtype with miR-5095, miR-619-5p, miR-5585-3p, miR-5096 and miR-1273a, miR-1273e, miR-1273g-3p. A high free binding energy was detected for pairs of miR-6089 and triple-negative subtype RUNX1 and SFN candidate genes mRNA. mRNA of IL11, MAGEA10 and STMN1 genes had binding sites of miR-619-5p and miR-1273a, miR-1273d, miR-1273e, miR-1273f.

mRNA of the subtype her2 candidate genes ADAM17, AURKA and BRCA2 strongly bind miR-619-5p. mRNA of BRIP1 gene has sites for miR-1285-5p, miR-5095, miR-619-5p, miR-5585-3p, miR-1273a, miR-1273g-3p. mRNA of CDK6 gene has binding sites for miR-548 family and multiple sites for miR-466. The presence of such binding sites in mRNA of CDK6 gene several times increases the probability of its interaction with these miRNAs. The key candidate gene ERBB3 of the her2 subtype interacts with miR-619-5p with high complementarity. 12 miRNAs can bind to mRNA of MAZ gene, binding sites are located in 5′UTR and CDS. mRNA of candidate genes of the subtype luminal A, B can bind: HMGA2 gene – five miRNA, MAPT gene – six miRNA, SMAD3 gene – four miRNA, TGFB1 gene – six miRNA. mRNA of TGFB1 and SMAD3 genes had four and three effective miR-6089 binding sites, respectively. A special feature of candidate genes of the subtype luminal A, B is the absence in their mRNA binding sites of the unique miRNA family miR-1273 and group miR-5095, miR-619-5p, miR-5585-3p, miR-5096, miR-1285-5p.

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

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Сүт безі қатерлі ісігі субтиптерінің кандидатты гендердің miRNA мен mRNAның байланысу ерекшеліктері

miRNA мен олардың нысана гендерінің mRNAның ассоциацияларын анықтау үшін сүт безі қатерлі ісігінің төрт субтиптерінің кандидатты гендерінің mRNA мен miRNAның әрекеттесу сипаттамалары зерттелді. Triple-negative субтипінің кандидатты гендердің жартысы бірнеше miRNA байланыстыратын сайттары болды. ATM генінің mRNA құрамында miR-5095, miR-619-5p, miR-5096, miR-5585-3p, miR-1273a, miR-1273g-3p үшін жеті байланысу сайттар бар, олардың барлығы 3'UTR-де байланысады. AXL генінің, тирозин киназаның рецепторының mRNAда бес miRNA байланыстыратын сайттары 3'UTR, CDS және 5'UTR-де локализацияланған болған. Бес miRNA-ның ішінен CBL протоонкогеннің экспрессиясының тиімді реттеуіне miR-1908-3p қатысуы мүмкін. CEACAM5 генінің mRNA құрамында miR-5095, miR-619-5p, miR-5585-3p жоғары дәрежелі комплементарлығымен байланыстыру сайттары бар. F2RL1, IAPP гендердің mRNAда miR-5095, miR-619-5p, miR-5585-3p, miR-5096 байланыстыру сайттары бар. Маркерлар ретінде алынған мәліметтер негізінде triple-negative субтипінің кандидатты гендердің экспрессиясын miR-5095, miR-619-5p, miR-5585-3p, miR-5096 және miR-1273a, miR-1273e, miR-1273g-3p қатысуымен реттеу қажет. miR-6089 және triple-negative субтиптің RUNX1 мен SFN кандидатты гендерінің mRNA жұптары үшін байланысудың жоғары бос энергиясы анықталды. IL11, MAGEA10 и STMN1 гендердің mRNAда miR-619-5p и miR-1273a, miR-1273d, miR-1273e, miR-1273f байланыстыру сайттар анықталды.

Her2 субтиптің ADAM17, AURKA және BRCA2 кандидаттық гендердің mRNAары miR-619-5p күшті байланыстырады. BRIP1 генінің mRNAнда miR-1285-5p, miR-5095, miR-619-5p, miR-5585-3p, miR-1273a, miR-1273g-3p байланыстыру сайттары бар. CDK6 генінің mRNAнда miR-548 отбасын байланыстыратын сайттары және miR-466 байланыстыратын көптік сайттары бар. CDK6 генінің mRNAнда мұндай байланыстыру сайттарының болуы оның осы miRNA-мен әрекеттесуін бірнеше есе арттырады. Her2 субтиптің ERBB3 кілтті кандидатты гені miR-619-5p-мен жоғары комплементарлықпен байланысады. MAZ генінің mRNA-мен 12 miRNA 5'UTR және CDS-де байланыса алады. A, B luminal субтиптің кандидатты гендерінің mRNAмен байланысады: HMGA2 геннің – бес miRNA, MAPT геннің – алты miRNA, SMAD3 геннің – төрт miRNA, TGFB1 геннің – алты miRNA. TGFB1 және SMAD3 гендерінің mRNAнда сәйкесінше төрт және үш эффективті miR-6089 байланыстыратын сайттары болды. A, B luminal субтиптің кандидатты гендерінің ерекшелігі miR-1273 отбасының және mR-5095, miR-619-5p, miR-5585-3p, miR-5096, miR-1285-5p тобының уникалды miRNA байланысу сайттарының болмауы болып табылады.

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

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Особенности связывания miRNA с mRNA кандидатных генов субтипов рака молочной железы

Для выявления ассоциаций miRNA и mRNA их генов мишеней изучены характеристики взаимодействия miRNA и mRNA кандидатных генов четырех субтипов рака молочной железы. Половина кандидатных генов субтипа triple-negative имели сайты связывания для нескольких miRNA. mRNA гена ATM содержала семь сайтов связывания для miR-5095, miR-619-5p, miR-5096, miR-

5585-3p, miR-1273a, miR-1273g-3p, которые все связываются в 3′UTR. mRNA гена AXL, рецептора тирозин киназы, имела сайты связывания для пяти miRNA, которые локализованы в 3′UTR, CDS и 5′UTR. Из пяти miRNA наибольшей эффективностью регуляции экспрессии протоонкогена CBL может обладать miR-1908-3p. mRNA гена CEACAM5 содержала сайты связывания miR-5095, miR-619-5p, miR-5585-3p с высокой степенью комплементарности. mRNA генов F2RL1, IAPP имеют сайты связывания преимущественно для miR-5095, miR-619-5p, miR-5585-3p, miR-5096. На основании полученных данных в качестве маркеров необходимо контролировать экспрессию кандидатных генов субтипа triple-negative с miR-5095, miR-619-5p, miR-5585-3p, miR-5096 и miR-1273a, miR-1273e, miR-1273g-3p. Высокая величина свободной энергии связывания выявлена для пар miR-6089 и mRNA RUNX1 и SFN – кандидатных генов субтипа triple-negative. mRNA генов IL11, MAGEA10 и STMN1 имели сайты связывания miR-619-5p и miR-1273a, miR-1273d, miR-1273e, miR-1273f.

mRNA кандидатных генов субтипа her2 ADAM17, AURKA и BRCA2 сильно связывают miR-619-5p. mRNA гена BRIP1 имеет сайты miR-1285-5p, miR-5095, miR-619-5p, miR-5585-3p, miR-1273a, miR-1273g-3p. mRNA гена CDK6 имеет сайты связывания для семейства miR-548 и множественные сайты для miR-466. Наличие в mRNA гена CDK6 таких сайтов связывания в несколько раз увеличивает вероятность ее взаимодействия с этими miRNA. Ключевой кандидатный ген ERBB3 субтипа her2 взаимодействует с miR-619-5p с высокой комплементарностью. С mRNA гена MAZ могут связываться 12 miRNA, сайты связывания которых расположены в 5′UTR и CDS. С mRNA кандидатных генов субтипа luminal A,B связывались: гена HMGA2 – пять miRNA, гена MAPT – шесть miRNA, гена SMAD3 – четыре miRNA, гена TGFB1 – шесть miRNA. mRNA генов TGFB1 и SMAD3 имели соответственно четыре и три эффективных сайтов связывания miR-6089. Особенностью кандидатных генов субтипа luminal A,B является отсутствие в их mRNA сайтов связывания уникальных miRNA семейства miR-1273 и группы miR-5095, miR-619-5p, miR-5585-3p, miR-5096, miR-1285-5p.

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

Introduction

Due to the development of molecular genetic technologies for establishing the causes of oncological diseases (OD), tens and hundreds of genes participating in the development of specific types and subtypes of OD have been identified in recent years (Chistiakov, 2016: 107-121). Unfortunately, the established candidate genes responsible for the development of OD are not systematized and it is required to clarify the role of these genes in the development of various subtypes of the OD. Classification of subtypes of OD on the basis of molecular features is improved, but the lack of information on the expression of candidate genes in different subtypes is a barrier for using of these genes as targets for targeted therapy.

Breast cancer (BC) by molecular genetic traits is divided into several subtypes. At present, the opinion is being expressed that the subtypes of oncological diseases are formed due to the different expression of many genes that are expressed in different subtypes with a greater or lesser degree than in the tissue of a healthy person. Multiple differences in the expression of genes cause a variety of ways of the oncogenesis. Identification of these differences is necessary, since targeted therapy involves the identification of target genes that determine the subtype of oncogenesis in the greatest degree. Modern molecular classifications of subtypes of breast cancer are based on gene expression profiles according to the following markers: the estrogen receptor (ER), the progesterone receptor (PR), the androgen receptor (AR), the epidermal growth factor receptor (HER), the anti-apoptosis proteins (Bcl-2, p53), cell proliferation proteins, matrix metalloproteinases (MMP), integrins, transduction transfer proteins, cyclins, cyclin dependent kinases (CDK), epithelial-mesenchymal factors, cadherins, transcription factors, metastasis control factors, factors of angiogenesis, etc. (Yu, 2017: 142-152). The existing molecular classification of breast cancer includes subtypes: luminal A and luminal B, which we combined into a subtype of luminal A, B; subtype her2; subtype triple-negative, also known as basal like.

On the basis of clinical data, these subtypes differ in frequency of occurrence, rate of growth, invasiveness, ability to metastasis, etc. The reason for these differences in the subtypes of breast cancer is different sets of genes involved in the development of these subtypes of oncogenesis. In connection with this, it is required to develop methods for early diagnosis of subtypes of OD, based on the identification of candidate genes and other molecular genetic factors involved in the regulation of expression of these genes. With the correct diagnosis, it will be possible to develop methods of targeted therapy on the base of identified

molecular genetic causes of the disease. Among the molecules involved in the regulation of the expression of most genes of the human genome are miRNAs that effectively affect on the expression of protein-coding target genes. (Ergün, 2015: 497-505; Hannafon, 2016: 90; Krishnan, 2015: 735; Li, 2017: 133; MacFarlane, 2010: 537-561; Wang, 2017: 72) A number of studies have shown a change in the concentration of miRNA in the development of BC, however, there are no publications showing the specific role of miRNA in the disease. This is due to a number of reasons that will be discussed in this paper. The aim of this study is to establish associations of miRNAs and their target genes that can serve as markers of subtypes of breast cancer. It is shown that one miRNA can bind to several hundred mRNA target genes (Atambayeva, 2017: 428; Ivashchenko, 2014: e8; Ivashchenko, 2014: e11). In this study, 47 genes have been studied, the expression of which varies with different 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). The miRNA nucleotide sequences were downloaded from miRBase database (http://www.mirbase.org). The MirTarget program (Ivashchenko, 2014: 423-427) was used to search for binding sites, free energy of binding (ΔG), and interaction schemes. The value of ΔG/ΔGm was used as a comparative quantitative criterion of the interaction strength of miRNA with mRNA, where ΔGm is equal to the free energy of miRNA binding with a completely complementary nucleotide sequence. The MirTarget program calculates the ratio ΔG/ΔGm, determines the location of microRNA site in the 5'-untranslated region (5'UTR), in the protein-coding region (CDS) or in the 3'-untranslated region (3'UTR). Table 1 shows sources of information on candidate genes of breast cancer subtypes which were targets for miRNAs from miRBase.

Table 1 – Candidate genes of subtypes of BC, indicating sources of information on their participation in oncogenesis of breast cancer

Subtype her2 *ADAM17* (doi: 10.1016/j.acthis.2011.03.009); *AURKA* (doi: 10.1038/s41523-017-0049-z); *BRCA2* (doi: 10.1155/2016/5718104); *BRIP1* (doi: 10.18632/oncotarget.7027); *CDK6* (doi: 10.2147/BCTT.S150540); *EPOR* (doi: 10.1007/s10549-012-2316-x); *ERBB3(HER3)* (doi: 10.18632/oncotarget.22027); *H2AFX(H2AX)* (doi: 10.18632/oncotarget.2259); *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); *TIMP3* (doi: 10.1016/j.humpath.2011.12.022).
Subtype triple negative (basal like) *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); *CEACAM5 (CEA)* (doi: 10.1016/j.cca.2017.04.023); *ERBB3* (doi: 10.18632/oncotarget.13284);*F2RL1 (PAR2)* (doi: 10.1002/cmdc. 201700640);*FGFR2* (doi: 10.1007/s00428-016-1950-9); *FIS1 (LINC01554)* (doi: 10.1186/bcr3588); *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); *LASP1* (doi: 10.1186/1756-9966-31-58);*MAGEA10*(doi: 10.1016/j.acthis.2014.01.003); *MID1* (doi: 10.1016/j.ajpath.2013.02.046); *MMP2* (doi: 10.1038/srep28623);*PFN1* (doi: 10.1080/15384101.2017.1346759);*PRKCE*(doi: 10.1038/onc.2013.91); *PRRT2 (PKC)* (doi: 10.1002/cmdc.201700640); *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).
Subtype luminal A,B *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); *ITGB1* (doi: 10.1080/15548627. 2016.1213928); *MAPT* (doi: 10.1007/s00428-012-1357-1); *MCM7* (doi: 10.1371/journal.pgen.1002751); *SMAD3* (doi: 10.1074/jbc.M113.506535); *SOX4* (doi: 10.1371/journal.pgen.1002751); *TGFB1 (TGFB)* (doi: 10.1038/ ncb2672).

Results and Discussion

To diagnose subtypes of breast cancer, it is necessary to use a number of associations combining the reliability and economy of using these associations. We selected candidate genes (Table 1) from more than 600 genes involved in the development of BC, which can serve as a basis for selective diagnosis of subtypes of BC. For the candidate genes listed in Table 1, the change in their expression in subtypes of breast cancer was experimentally established.

Characteristics of the interaction of miRNA with mRNA of candidate genes of the triple- negative subtype

25 candidate genes of the triple-negative subtype were targets for miRNA (Table 2). Many of them had binding sites for several miRNAs. At equal concentrations of these miRNA and mRNA gene, there will be a significant suppression of protein synthesis by miRNAs that bind more strongly to mRNA. The *ATM* gene encodes a kinase that is highly expressed in the lymph nodes and by phosphorylation of a wide range of proteins, including tumor suppressors p53 and BRCA1, kinase CHK2, proteins RAD17 and RAD9, DNA repair protein NBS1, involved in the regulation of the cell cycle. mRNA of *ATM* gene had seven binding sites for unique miRNAs: miR-5095, miR-619-5p, miR-5096, miR-5585-3p, miR-1273a, miR-1273g-3p, which all bind in the 3'UTR (Table 2).

Table 2 – Schemes of interaction of miRNA with mRNA of candidate genes of Triple-negative (Basal-like) subtype

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm, %	Lenght, nt
1	2	3	4	5	6
ATM	miR-5095(SCP2)	9787	-108	93	21
ATM	miR-619-5p(SSH1)	9793	-119	98	22
ATM	miR-5096(BMP2K)	9882	-104	92	21
ATM	miR-5585-3p(TMEM39B)	9950	-110	95	22
ATM	miR-1273a(RGS22	11054	-119	90	25
ATM	miR-1273g-3p(SCP2	11076	-113	96	21
ATM	miR-1273e(x)	11119	-108	93	22
AXL	miR-6743-5p(ig)	124**	-117	90	22
AXL	miR-7152-3p(ig)	2620*	-106	94	20
AXL	miR-6086(EGFL6)	2792*	-106	94	20
AXL	miR-1273g-3p(SCP2)	3323	-115	98	21
AXL	miR-3929(ig)	3518	-110	90	23
BIRC5	miR-5095(SCP2)	352*	-106	91	21
CBL	miR-1908-3p(0-0)	30**	-121	92	21
CBL	miR-1273a(RGS22)	7727	-117	89	25
CBL	miR-1273g-3p(SCP2)	7749	-115	98	21
CBL	miR-1470(AC020911)	9246	-115	90	21
CBL	miR-4743-5p(KIAA0427)	9822	-113	87	23
CD44	miR-4763-3p(RP4)	354**	-121	85	24
CEACAM5	miR-1291(C12orf41)	2159*	-113	85	24
CEACAM5	miR-5585-3p(TMEM39B)	2441	-108	93	22
CEACAM5	miR-5095(SCP2)	3229	-115	98	21
CEACAM5	miR-619-5p(SSH1)	3235	-119	98	22
CEACAM5	miR-5585-3p(TMEM39B)	3378	-113	96	22
ERBB3	miR-619-5p(SSH1)	4950	-117	96	22
ERBB3	miR-619-5p(SSH1)	5104	-121	100	22
ERBB3	miR-1322(PINX1)	5632	-87	85	19
F2RL1	miR-619-5p(SSH1)	1943	-110	91	22
F2RL1	miR-5096(BMP2K)	2016	-104	92	21
FGFR2	miR-6749-5p(ATG2A)	405**	-119	92	22
FGFR2	miR-1322(PINX1)	504**	-87	85	19

Continuation of table 2

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm, %	Lenght, nt
1	2	3	4	5	6
FIS1	miR-1273g-3p(SCP2)	14**	-110	95	21
FIS1	miR-6892-3p(ig)	17**	-110	93	21
FIS1	miR-1914-3p(UCKL1)	230**	-117	90	22
FIS1	miR-933(ATF2)	506**	-117	93	22
FIS1	miR-6756-5p(MCAM)	1156*	-123	92	23
IAPP	miR-619-5p(SSH1)	804	-117	96	22
IAPP	miR-5096(BMP2K)	876	-113	100	21
IAPP	miR-5585-3p(TMEM39B)	944	-108	93	22
IL11	miR-328-5p(0-0)	216**	-125	91	23
IL11	miR-4436b-5p(MALL)	1253	-113	90	22
IL11	miR-1273f(SCP2-5UTR)	1466	-102	98	19
IL11	miR-1273d(KIF1B)	1467	-121	89	25
IL11	miR-1273e(x)	1476	-113	96	22
IL11	miR-619-5p(SSH1)	1988	-113	93	22
JHDM1D	miR-7158-5p(ig)	28*	-115	86	24
JHDM1D	miR-6729-5p(MIIP)	94*	-117	89	22
LAMC1	miR-3187-5p(LPPR3)	652*	-115	87	23
LASP1	miR-149-5p(GPC1)	734*	-115	90	23
MAGEA10	miR-1273g-3p(SCP2)	2145	-108	93	21
MAGEA10	miR-1273d(KIF1B)	2179	-117	86	25
MAGEA10	miR-1273e(x)	2188	-110	95	22
MID1	miR-6735-5p(SZT2)	2115*	-119	86	25
MMP2	miR-1285-5p(AC000120)	1376*	-104	92	21
MMP2	miR-328-5p(0-0)	3009	-119	86	23
PFN1	miR-6867-5p(ig)	1160	-110	91	23
PRKCE	miR-328-5p(0-0)	2558	-119	86	23
PRKCE	miR-6831-5p(ig)	2563	-110	85	24
PRRT2	miR-6743-5p(ig)	1379*	-115	89	22
RUNX1	miR-6089(ig)	1431**	-127	86	24
RUNX1	miR-466(ig)	5456	-106	91	23
RUNX1	miR-466(ig)	5460	-110	95	23
SERPINE1	miR-4758-3p(LAMA5)	277*	-119	90	23
SFN	miR-638(DNM2)	40**	-127	86	25
SFN	miR-6089(igc)	826	-129	87	24
SFN	miR-6846-5p(ig)	839	-113	91	22
SFN	miR-466(ig)	1190 ÷ 1200	-106	91	23
STMN1	miR-1273a(RGS22)	1729	-115	87	25
STMN1	miR-1273g-3p(SCP2)	1751	-108	93	21
STMN1	miR-1268a(ig)	1855	-102	94	18
STMN1	miR-1972(PDXDC1)	1991	-117	95	22
Note. Without an asterisk – 3'UTR, * – CDS, ** – 5'UTR. The host gene or the intergenic origin of ig miRNA is in parentheses.					

Control of the expression of *ATM* gene and the detection of the level of miR-5095, miR-619-5p, miR-5096, miR-5585-3p, miR-1273a, miR-1273g-3p and miR-1273e with high probability characterize the development of breast cancer on the triple-negative subtype and the appearance of metastases in the lymph nodes in which *ATM* gene is highly expressed. That is, suppression of its synthesis will increase the probability of metastasis in the lymph nodes. The data in Table 2 shows strong control of the expression of *ATM* gene by unique miRNAs mentioned above. The specificity of the *ATM* gene is that all miRNA binding sites are located in the 3'UTR of its mRNA. mRNA of *AXL* gene (Bonora, 2015: 1608), the tyrosine kinase receptor, had five binding sites for five miRNAs that are located in 3'UTR, CDS, and 5'UTR. miR-1273g-3p was almost completely complementary to mRNA (Table 2). Based on the presented data, the expression of the *AXL* gene can be substantially controlled by miR-6743-5p, miR-1273g-3p, miR-3929, miR-6086 and miR-7152-3p, three of which are encoded in intergenic regions, i.e. expressed independently. Unique miR-1273a, miR-1273g-3p had binding sites in the mRNA of the protooncogene *CBL* (Lee, 2013: 11121-6). Of the five miRNAs, the most effective regulation effect on the *CBL* gene expression may have miR-1908-3p which bind to mRNA with free energy of -121 kJ/mole. Note that binding miRNA in the 5'UTR mRNA allows to stop protein synthesis at the beginning of this process in order not to waste energy on the synthesis of a polypeptide that can be interrupted by the strong binding of miRNA. The mRNA of *CBL* gene had miRNA binding sites encoded the oligopetides HHHHHHH, DDDDD and PPPPPP in CBL protein. The characteristics of miRNA binding sites encoded these oligopeptides are not given in Table 2, since they bind with a lower free energy, but at a concentration greater than the concentration of mRNA, they can significantly inhibit translation. The mRNA of *CEACAM5* (Wang, 2017: 51-55) gene had binding sites of miR-5095, miR-619-5p, miR-5585-3p with a high degree of complementarity: ΔG/ΔGm value is varied from 85% to 98%. Binding sites for these miRNAs were located in a restricted region of mRNA. The mRNA of *F2RL1* (Zhang, 2017: 59086-59102) and *IAPP* (Jo, 2017: 78781-78795) genes had binding sites predominantly for miR-5095, miR-619-5p, miR-5585-3p, miR-5096. Moreover, miR-5096 was completely complementary to the binding site of mRNA of IAPP gene (Table 2). miR-6089 with a high free binding energy interacts with mRNA of *RUNX1* and *SFN* (Boudreau, 2013: e3937-44) target genes. For this reason, miR-6089 can be used as a marker for the diagnosis of the triple negative subtype. The mRNA of *RUNX1* and *SFN* genes, in addition to binding miR-6089, have binding sites for miR-466, which is also recommended as a marker because it has multiple binding sites in mRNA of *RUNX1* gene. The mRNA of *IL11* gene had binding sites for six miRNAs (Table 2), of which miR-619-5p and miR-1273a, miR-1273d, miR-1273e, miR-1273f have been recommended above as participant of associations for markers. Some of these miRNAs bind to mRNA of *MAGEA10* and *STMN1* genes (Table 2), which confirms the necessity of control their concentration to establish the development of the disease by the triple negative subtype. The mRNA of *ERBB3* gene (Hayes, 2017: e0177919; Mota, 2017: 89284-89306) effectively bind miR-619-5p in two sites, in one site even with full complementarity. In addition, miR-1322 had multiple sites in mRNA, which puts the expression of *ERBB3* gene in a strong dependence on these miRNAs. Based on the obtained data (Table 2), it is necessary to control the expression of candidate genes of the triple-negative subtype with the following miRNAs as markers: miR-5095, miR-619-5p, miR-5585-3p, miR-5096 and miR-1273a, miR-1273e , miR-1273g-3p.

Table 3 shows the schemes and characteristics of binding of some miRNAs to mRNA of candidate genes of the triple-negative subtype of BC.

These data show that in all cases, miRNA binds to mRNA without disrupting the double-stranded structure, since the interaction between non-canonical pairs of nucleotides A-C and G-U does not change the distance between RNA chains. These schemes demonstrate the advantage of the MirTarget program among the commonly used programs in determining the free energy of miRNA interaction with mRNA, which is calculated taking into account the formation of non-canonical pairs of nucleotides A and C, G and U.

Characteristics of the interaction of miRNA with mRNA of candidate genes of subtype her2

The twelve candidate genes of subtype her2 shown in Table 1 were targets for miRNAs (Table 4). The *ADAM17* gene belongs to the family of disintegrins and metalloproteases. It is involved in the processing of tumor necrosis factor α on the cell surface and in intracellular membranes of the trans-network of Golgi apparatus (Pham, 2017: 5507-5513). The mRNA of *ADAM17* gene fully complementary bind miR-619-5p.

Table 3 – Schemes of the interaction of miRNA with mRNA of candidate genes of the triple negative subtype of BC

ATM; miR-619-5p; 3'UTR; 9793; -119; 98 5'- GGCUCACGCCUGUAAUCCCAGC - 3' 3'- CCGAGUACGGACAUUAGGGUCG - 5'	*AXL*; miR-1273g-3p; 3'UTR; 3323;-115; 98 5' - CCCAGGCUGGAGUGCAGUGGU - 3' 3' - GAGUCCGACCUCACGUCACCA - 5'
CBL; miR-1273g-3p; 3'UTR;7749; -115; 98 5' - CCCAGGCUGGAGUGCAGUGGU - 3' 3' - GAGUCCGACCUCACGUCACCA - 5'	*CEACAM5*; miR-5095; 3'UTR;3229; -115; 98 5' - CGCGGUGGCUCACGCCUGUAA - 3' 3' - GCGCCACCAAGUGCGGACAUU - 5'
CEACAM5; miR-619-5p; 3'UTR;3235; -115; 98 5' - CGCGGUGGCUCACGCCUGUAA - 3' 3' - GCGCCACCAAGUGCGGACAUU - 5'	*F2RL1*; miR-619-5p;3'UTR;1943; -110; 91 5' - GCCUCAUGCCUGUAAUCCUAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'
IAPP; miR-5096; 3'UTR; 876; -113; 100 5' - GCCUGACCAACAUGGUGAAAC - 3' 3' - CGGACUGGUUGUACCACUUUG - 5'	*ATM*; miR-1273e; 3'UTR; 11119; -108; 93 5' - UCUGCCUCCUGGGUUCAAGCAA - 3' 3' - AGGUGAAGGACCCAAGUUCGUU - 5'
ERBB3; miR-619-5p; 5104; 3'UTR; -121; 100 5'- GGCUCAUGCCUGUAAUCCCAGC - 3' 3'- CCGAGUACGGACAUUAGGGUCG - 5'	*IL11*; miR-1273e; 3'UTR; -113; 96 5' - UCCACCUCCCGGGUUCAAGCAA - 3' 3' - AGGUGAAGGACCCAAGUUCGUU - 5'
IL11; miR-1273f; 1466; 3'UTR; -102; 98 5' - CACUGCAACCUCCACCUCC - 3' 3' - GUGACGUUGGAGGUAGAGG - 5'	*ERBB3*; miR-619-5p; 4950; 3UTR; -117; 96 5' - GGCUCAUGCCUGUAAUCUCAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'
MAGEA10; miR-1273e; 2188; 3'UTR; -110; 95 5'- UCCGCCUCCUGGGUUCAAGCGA - 3' 3'- AGGUGAAGGACCCAAGUUCGUU - 5'	*MAGEA10*;miR-1273f; 2178; 3'UTR; -96; 92 5' - GCCUCAUGCCUGUAAUCCUAGC - 3' 3' - CCGAGUACGGACAUUAGGGUCG - 5'
Note. Here and in Tables 5 and 7, the first line shows: the name of the gene; miRNA; mRNA site; beginning of the miRNA binding site, nt; the value of ΔG, kJ / mole; the value of ΔG / ΔGm,%.	

Table 4 – Characteristics of the interaction of miRNA with mRNA candidate genes of the subtype her2

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm,%	Lenght, nt
1	2	3	4	5	6
ADAM17	miR-619-5p(SSH1)	3466	-121	100	22
ADAM17	miR-1285-5p(AC000120)	3524	-104	92	21
AURKA	miR-5095(SCP2)	420**	-108	93	21
AURKA	miR-619-5p(SSH1)	426**	-119	98	22
BRCA2	miR-619-5p(SSH1)	10746	-117	96	22
BRIP1	miR-1273a(RGS22)	4222	-113	85	25
BRIP1	miR-1273g-3p(SCP2)	4244	-110	95	21
BRIP1	miR-5095(SCP2)	6581	-115	98	21
BRIP1	miR-619-5p(SSH1)	6587	-119	98	22
BRIP1	miR-5585-3p(TMEM39B)	6728	-113	96	22

Continuation of table 4

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm,%	Lenght, nt
1	2	3	4	5	6
BRIP1	miR-1285-5p(AC000120)	6827	-104	92	21
BRIP1	miR-1972(PDXDC1)	7273	-117	95	22
CDK6	miR-548h-3p(ig)	1677	-104	91	23
CDK6	miR-548z(RASSF3)	1677	-104	91	23
CDK6	miR-548aq-3p(IGF2BP2)	1678	-102	94	22
CDK6	miR-548az-3p(ig)	1678	-98	94	21
CDK6	miR-466(ig)	1892 ÷ 1926	-100	85	23
CDK6	miR-466(ig)	1908	-108	93	23
CDK6	miR-466(ig)	1920	-108	93	23
EPOR	miR-328-5p(0-0)	1461*	-121	88	23
ERBB3	miR-619-5p(SSH1)	4950	-117	96	22
ERBB3	miR-619-5p(SSH1)	5104	-121	100	22
H2AFX	miR-328-5p(0-0)	672	-119	86	23
MAPK3	miR-1181(CDC37)	114*	-115	90	21
MAPK3	miR-6884-3p(ig)	175*	-113	88	23
MAPK3	miR-6805-3p(ig)	1145*	-117	87	23
MAPK3	miR-6887-5p(ig)	1528	-113	88	23
MAZ	miR-1470(AC020911)	19**	-123	97	21
MAZ	miR-6850-5p(ig)	92**	-115	87	22
MAZ	miR-4466(ARID1B)	107**	-110	98	18
MAZ	miR-762(RP11)	111**	-123	91	22
MAZ	miR-6729-5p(MIIP)	361*	-115	87	22
MAZ	miR-2861(CDK9)	376*	-110	95	19
MAZ	miR-762(RP11)	499*	-117	86	22
MAZ	miR-3960(0-0)	505*	-119	95	20
MAZ	miR-4706(FNTB)	605*	-123	87	25
MAZ	miR-3960(0-0)	614*	-117	93	20
MAZ	miR-1247-3p(DIO3OS)	664*	-119	86	24
MAZ	miR-1343-5p(0-0)	1609	-115	86	22
MAZ	miR-6805-3p(ig)	2552	-115	86	23
NISCH	miR-762(RP11)	3282*	-117	86	22
NISCH	miR-6756-5p(MCAM)	3419*	-115	86	23
TIMP3	miR-4449(KIAA0114)	1072*	-115	87	22
TIMP3	miR-197-5p(MIR197)	1838	-115	87	23
TIMP3	miR-1224-5p(VWA5B2)	3268	-104	96	19
Note. Without an asterisk – 3'UTR, * – CDS, ** – 5'UTR. The host gene or the intergenic origin of ig miRNA is in parentheses.					

Considering the free binding energy of -121 kJ/mole, the association of miR-619-5p with mRNA of *ADAM17* is a good marker of the disease. miR-619-5p, in combination with the genes *AURKA* (Golmohammadi, 2017: e7933*)* and *BRCA2* (Couch, 2007: 1416-21; Pan, 2014: 1-8), on the same bases can serve as markers of the subtype her2. The mRNA of *BRIP1* gene can bind to unique miRNAs of group miR-1285-5p, miR-5095, miR-619-5p, miR-5585-3p and family miR-1273a, miR-1273g-3p. These

miRNAs, with a high level of complementarity, bind to mRNA of *BRIP1* gene (ΔG/ΔGm reached 98%) and with a high free interaction energy, so they can be used as markers for the subtype her2.

The mRNA of *CDK6* gene (Johnson, 2016: 4829-35) had binding sites for miR-548 family and multiple binding sites for miR-466. The presence in mRNA of *CDK6* gene of multiple binding sites several times increases the probability of its interaction with these miRNAs.

The key member of candidate genes of the subtype her2 is the *ERBB3* gene (Grabinski, 2014: 1021-9; Koutras, 2010: 73-8; Wu, 2013: 427-37). Two binding sites in mRNA of *ERBB3* gene for the unique miR-619-5p are characterized by high complementarity and high free energy, which is the basis for suggesting this association as a marker for identifying the development of the subtype her2 (Table 4).

Four miRNAs that bind to mRNA of *MAPK3* gene can strongly affect on its expression. Binding sites of three miRNAs are located in the CDS, which indicates their early occurrence. 12 miRNAs can bind to mRNA of *MAZ* gene, binding sites are located in 5'UTR and CDS, with the ΔG value of -110 kJ/mole to -123 kJ/mole, and ΔG/ΔGm value of 86% to 97%. Since miR-1470, miR-762, and miR-4706 bind with mRNA of *MAZ* gene with ΔG value of -123 kJ/mole, first of all it is necessary to use these miRNAs as markers for subtype her2 subtype diagnostics. Several arranged located miR-3960 binding sites encode the polyAla oligopeptide. This number of miR-3960 binding sites significantly increases the efficiency of controlling the expression of *MAZ* gene by this miRNA. MAZ is a Myc associated transcription factor and therefore it can influence on the transcription of several genes involved in oncogenesis. The mRNA of *NISCH* gene can bind two miRNA with a ΔG value varying from -115 kJ/mole to -117 kJ/mol, what is the basis for characterising the *NISCH* gene with miR-762 and miR-6756-5p as markers for the subtype her2. The mRNA of *TIMP3* gene had three binding sites for three miRNAs, which gives reason to control the level of these miRNAs during the development of the subtype her2. Table 5 shows examples of the interaction of some miRNA with mRNA of their target genes, offered as associations for use as markers of the subtype her2 of breast cancer.

Table 5 – Schemes and characteristics of the interaction of miRNA with mRNA of candidate genes subtype her2 BC

ERBB3; miR-619-5p; 3'UTR; 4950; -117; 96
5'- GGCUCAUGCCUGUAAUCUCAGC - 3'
  ||||||||||||||||||||
3'- CCGAGUACGGACAUUAGGGUCG - 5'
	MAZ; miR-3960; CDS; 614; -117; 93
	5' - CCCCCGCCUCCGCCGCCACU - 3'
	  ||||||||||||||||||||
	3' - GGGGGCGGAGGCGGCGGCGG - 5'

ADAM17; miR-619-5p;3'UTR;3466; -121; 100
5'- CCCAGGCUGGAGUGCAGUGGU - 3'
  ||||||||||||||||||||
3'- GAGUCCGACCUCACGUCACCA - 5'
	AURKA; miR-619-5p;5UTR;426;-119; 98
	5'- GGCUCAUGCCCGUAAUCCCAGC - 3'
	  |||||||||||||||||||||||
	3'- CCGAGUACGGACAUUAGGGUCG - 5'

BRIP1; miR-5095;3'UTR;6851; -115; 98
5' - CGCGGUGGCUCACGCCUGUAA - 3'
  ||||||||||||||||||||
3' - GCGCCACCAAGUGCGGACAUU - 5'
	BRIP1; miR-1273g-3p; 3'UTR; 4244; -110; 95
	5' - CCCAGGCUGGAAUGCAGUGGU - 3'
	  ||||||||||||||||||||
	3' - GAGUCCGACCUCACGUCACCA - 5'

ERBB3; miR-619-5p;3'UTR;5104; -121; 100
5'- GGCUCAUGCCUGUAAUCCCAGC - 3'
  ||||||||||||||||||||
3'- CCGAGUACGGACAUUAGGGUCG - 5'
	AURKA; miR-5095; 5UTR;420;-108; 93
	5' - CGCGGUGGCUCAUGCCCGUAA - 3'
	  ||||||||||||||||||||
	3' - GCGCCACCAAGUGCGGACAUU - 5'

BRCA2; miR-619-5p; 3'UTR;10746; -117; 96
5'- GGCUCAUGCCUGUAAUCCCAAC - 3'
  ||||||||||||||||||||
3'- CCGAGUACGGACAUUAGGGUCG - 5'
	BRIP1; miR-5585-3p; 3'UTR; 6728; -113; 96
	5'- GCCUGUAGUCCCAGCUACUCAG - 3'
	  ||||||||||||||||||||
	3'- UGGACAUCAGGGUCGAUAAGUC - 5'

CDK6; miR-548av; 3'UTR; 1677; -98; 85
5'- UGCAAGAGUGAUUGCAGCUUUA - 3'
  ||||||||||||||||||||
3'- ACGUUCUCAUUAGCGCCAAAAC - 5'
	MAZ; miR-877-3p; 3'UTR; 2273; -106; 91
	5' - CCAGGGGGAGGGAGGAGAGGA - 3'
	  ||||||||||||||||||||
	3' - GACCCUCCUCCCUCUUCUCCU - 5'

The given data demonstrate the important role of non-canonical pairs of nucleotides in the interaction of miRNA with mRNA of candidate genes involved in the development of the subtype her2 BC. For example, in the interaction of miR-548av with mRNA of *CDK6* gene, three pairs of A-C and two pairs of G-U are formed. miR-877-3p binding to mRNA of *MAZ* gene forms two pairs of A-C and two pairs of G-U.

Characteristics of the interaction of miRNA with mRNA of candidate genes of subtype luminal A, B

Ten candidate genes of the subtype luminal A, B were determined as targets for miRNAs. Five miRNAs were associated with mRNA of *HMGA2* gene, six mRNAs with mRNA of *MAPT* gene, four miRNAs with mRNA of *SMAD3* gene, six miRNAs with mRNA of *TGFB1* gene (Table 6).

Consequently, these genes strongly depend on miRNAs. The mRNA of *TGFB1* gene had four binding sites of miR-6089 and mRNA of *SMAD3* gene three miR-6089 binding sites with a ΔG value varying from -127 kJ/mole to -136 kJ/mole. Such large free energy of miRNA interaction with mRNA is very rare characteristic for binding sites located in the 3'UTR, which determines their high functional significance.

The feature of candidate genes of the subtype luminal A, B is the absence in their mRNA binding sites of unique miRNA of family miR-1273 and group of miR-5095, miR-619-5p, miR-5585-3p, miR-5096, miR-1285-5p. This feature will be taken into account in the analysis of miRNA expression in the subtype luminal A, B.

The results of the interaction of some miRNAs with mRNA of candidate genes are shown in Table 7. Note that even with a ΔG/ΔGm of 86%, the structure of the double-stranded RNA is preserved, despite the presence of one pair of A-C and three G-U pairs in the interaction of miR-670-3p with mRNA of *MCM7* gene. When miR-4433b-5p binds to mRNA of *MCM7* gene, two pairs of A-C and three pairs of G-U are formed.

Table 6 – Characteristics of the interaction of miRNA with mRNA candidate genes of the subtype luminal

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm, %	Lenght, nt
1	2	3	4	5	6
EZH1	miR-6127(ig)	2497	-102	94	19
FOXA1	miR-3960(0-0)	120**	-115	92	20
FOXA1	miR-6848-5p(ig)	1287*	-115	87	23
GTF2IRD1	miR-4734(ig)	138**	-115	87	22
GTF2IRD1	miR-6729-5p(MIIP)	245**	-115	87	22
HMGA2	miR-6894-5p(ig)	189**	-115	86	24
HMGA2	miR-3960(0-0)	512**	-108	86	20
HMGA2	miR-6756-5p(MCAM)	529**	-117	87	23
HMGA2	miR-3960(0-0)	549**	-117	93	20
HMGA2	miR-4739(ig)	573**	-123	85	25
ITGB1	miR-4787-5p(ig)	92*	-123	92	22
MAPT	miR-4665-5p(RP11)	112**	-117	86	23
MAPT	miR-7106-5p(ig)	1008*	-106	94	20
MAPT	miR-5088-5p(0-0)	1586*	-115	86	24
MAPT	miR-762(RP11)	2725	-119	87	22
MAPT	miR-6756-3p(MCAM)	3207	-98	85	20
MAPT	miR-650(IGLV2)	3495	-110	93	21
MCM7	miR-4433b-5p(0-0)	248**	-100	85	21
MCM7	miR-670-3p(AC023085)	2679	-89	86	21
SMAD3	miR-6848-5p(ig)	138	-115	87	23
SMAD3	miR-4690-5p(PCNXL3)	2066	-115	92	22
SMAD3	miR-3620-5p(ARF10)	2069	-117	89	22

Continuation of table 6

Gene	miRNA	Beginning of sites, nt	ΔG,kJ/mole	ΔG/ΔGm, %	Lenght, nt
1	2	3	4	5	6
SMAD3	miR-6089(ig)	2072	-127	86	24
SMAD3	miR-6089(ig)	2073	-132	89	24
SMAD3	miR-3620-5p(ARF1)	2074	-115	87	22
SMAD3	miR-6089(ig)	2078	-136	91	24
SOX4	miR-935(CACNG8)	1303*	-115	89	23
SOX4	miR-6765-5p(JAG2)	1924*	-125	87	25
TGFB1	miR-4787-5p(ig)	205**	-117	87	22
TGFB1	miR-877-3p(ABCF1)	233**	-108	93	21
TGFB1	miR-4632-5p(TNFRSF1B)	871**	-115	86	23
TGFB1	miR-6089(ig)	2060	-132	89	24
TGFB1	miR-6089(ig)	2065	-136	91	24
TGFB1	miR-3620-5p(ARF10)	2086	-115	87	22
TGFB1	miR-6089(ig)	2089	-127	86	24
TGFB1	miR-6089(ig)	2095	-127	86	24

Note. Without an asterisk – 3'UTR, * – CDS, ** – 5'UTR. The host gene or the intergenic origin of ig miRNA is in parantheses.

Table 7 – Schemes and characteristics of the interaction of miRNA with mRNA of candidate genes of the subtype luminal A, B of BC

HMGA2; miR-3960; 5'UTR; 512; -108; 86 5' – CCUCCACCUCCACCGCCACC – 3' 3' – GGGGGCGGAGGCGGCGGCGG – 5'	*MAPT*; miR-6756-3p; 3'UTR; 3207; -98; 85 5' – CUGGGCAGAGGGGAGAGGAA – 3' 3' – GACCCGUCCCUCCUUCCCCU – 5'
MCM7; miR-4433b-5p; 5'UTR;248; -100; 85 5' – GCGGGAGCGGGGGUGGGGUGC – 3' 3' – UGUCCUCACCCCCACCCUGUA – 5'	*MCM7*; miR-670-3p; CDS; 2769;-89; 86 5' – CUCUGGAUGAAUAUGAGGAGC – 3' 3' – AGGACUUACUUAUACUCCUUU – 5'
EZNH; miR-4290; 3'UTR;3705; -89; 86 5' – GGGGGAAGAAGAGAGGGUG – 3' 3' – CUCCCUUCUUUCCUCCCGU – 5'	*GTF21RD1*; miR-4271; 5'UTR; 268; -91; 86 5' – CUCUGCCUCUCCUUCCCCC – 3' 3' – GGGGUGGAAAAGAAGGGGG – 5'
HMGA2; miR-329-5p; 5'UTR; 11; -102; 86 5'- GGGGCAGGAACUCAGAAAACUUC- 3' 3'- CUUUGUCUUUGGGUCUUUUGGAG- 5'	*SMAD3*; miR-7977; 3'UTR; 2601; -85; 85 5' – UGGCACAUUGACUGGGAA – 3' 3' – ACCACGCAACCGACCCUU – 5'

Conclusion

Establishing associations of miRNAs with mRNAs suggests using them to develop methods for early detection of subtypes of breast cancer. The material for analysis can be the blood of patients in which miRNA circulates in the free state and in the composition of exosomes. Using all associations of miRNAs with mRNAs of candidate genes requires relatively large material costs, therefore in the Tables 2, 4 and 6 are shown those associations which include the most probable candidate genes and miRNAs that interact strongly with their mRNAs. We note that the associations we have identified can be used to establish subtypes on the biopsy material and in postoperative tumor samples. Such analysis is necessary for the application of specific therapy of the disease, which should be targeted to genes that

are the main cause of the development of subtypes of the disease.

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