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CLUSTERS OF miRNAs BINDING SITES in 3′UTR mRNA OF BREAST CANCER CANDIDATE GENES

Abstract. Breast cancer is the most common disease among women and the development of methods for early diagnosis requires the identification of non-invasive molecular markers of oncogenesis. Associations of miRNAs and breast cancer candidate genes can serve as such markers. Characteristics of the interaction of miRNAs with mRNAs of several genes were predicted using the MirTarget program. It has been established that miRNA binding sites (BS) can be located in the mRNA with overlapping nucleotide sequences, forming clusters of BS. The studied genes contained BS of one or more miRNAs in the 3′UTR mRNA. When the nucleotide sequences of the BS in the cluster overlap, a multiple decrease in the occupied part of mRNA takes place. As a result of the compactization of BS between the miRNAs, there is competition for binding in the cluster. Identified ID00436.3p-miR and ID01030.3p-miR with BS with the overlap of nucleotide sequences in the 3′UTR mRNA containing multiple repeats of the GU and CA dinucleotides of *BACH1, CD19, CDK6, ETS1, FGFR3, FOXP1, IGF2R, FOXP1, IGF2R, IGF2R, IGF2R, SP1, ST8SIA1* and *WT1* genes. Established ID00470.5p-miR and ID02299.5p-miR which BS are also located with the overlap of nucleotide sequences in the mRNA of *CARNS1, CCND1, EFNB1, IGF2, SMAD4* and *ZEB1* genes. ID01727.5p-miR and ID02882.3p-miR have coinciding sites in the mRNA of *ELK4, FOXP1* and *SFN* genes. ID01382.3p-miR has BS in *TGFB1* and *SMAD3* genes consisting of four repeats of GCCCC. The associations of ID00436.3p-miR and ID01030.3p-miR with target genes are proposed for early molecular diagnosis of the disease. These associations include *BACH1, CDK6, ETS1, IGF2, SFN, SMAD4, SP1* and *ST8SIA1* genes. Associations of ID00470.5p-miR and ID02299.5p-miR with *CARNS1, CCND1, EFNB1, IGF2, SMAD4* and *ZEB1* genes are recommended to use for early molecular diagnosis of the disease.

Key words: miRNA, mRNA, cluster, target gene, breast cancer.

Introduction. The incidence of breast cancer (BC) has high rates in the world and Kazakhstan [1]. This is due to the insufficiency of preventive measures, such as early diagnosis. Recently, there have been many studies on the diagnosis of BC using miRNA (**m**RNA-inhibitory **RNA**), which play a key role in the post-transcriptional regulation of genes involved in proliferation, differentiation, angiogenesis, migration, apoptosis and carcinogenesis [2]. More than 600 genes are involved in the development of BC [3] and it seems important to identify which of them may be targets for miRNAs. Earlier, we studied the interactions of miRNAs from miRBase database with BC candidate genes [3-5] and it was shown that miRNAs can be strong regulators of the expression of many genes and serve as markers for developing methods for early diagnosis of BC [6]. According to the literary data, miRNAs are predominantly bind in the 3′UTR mRNA [7]. Thereby, in this work, we studied interaction characteristics of recently discovered and poorly studied 3707 miRNAs [8] with mRNA of BC candidate genes.

Materials and Methods. The nucleotide (nt) sequences of 19 BC candidate genes were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). The nucleotide sequences of 3707 miRNAs were taken from the publication [8]. RPKM values [9] are given in the Human Protein Atlas data (https://www.proteinatlas.org). Human Protein Atlas data were used as a quantitative measure of

transcript expression in mammary gland. Recently, computer approaches in biotechnology [10] and biology [11] have been actively used. The miRNAs binding sites (BS) in mRNAs of several genes were predicted using the MirTarget program [10]. This program defines the following features of miRNA binding to mRNA: a) the start of the initiation of miRNA binding to mRNAs; b) the localization of miRNA BS in 5′UTRs, CDSs and 3′UTRs of the mRNAs; c) the free energy of interaction miRNA and the mRNA (ΔG, kJ/mole); d) the schemes of nucleotide interactions between miRNAs and mRNAs. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of miRNA binding with its fully complementary nucleotide sequence). The miRNA BS located in mRNAs had ΔG/ΔGm ratios of 87% or more. ΔG/ΔGm ratios were taken on the assumption that the members of the miRNA of one family generally differ by no more than 1-3 nucleotides, that with a miRNA length of 22 nt, the ΔG/ΔGm value was 96% (21 nt/22 nt = 96%) - 87% (19 nt/22 nt = 87%). With a larger difference in the number of mismatched nucleotides, the probability of two or more miRNAs to bind in one site increases, which excludes the natural property of the miRNA to interact selectively with the mRNA of the target gene. The MirTarget program identifies the positions of the BSs on the mRNA, beginning from the first nucleotide of the mRNA's 5′UTR. 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 [12-14].

Results and Discussion. The *AKT1* gene is the target of three miRNAs (Table 1). The BSs of these miRNAs are located with the overlap of nucleotide sequences that form the mRNA region named by us as cluster of BSs. The total length of three miRNAs BSs is 64 nt and, due to compactization, they are located in a cluster of 35 nt length. The length of 3′UTR is 992 nt and there is no need to compactizied BSs. Probably, the compactization of BSs is intended to limit the dependence of gene expression on three miRNAs simultaneously, since only one miRNA can be bind at a 35 nt cluster. The binding characteristics of the three miRNAs are close (table 1) and miRNA present in greater concentration will have an advantage in interacting with mRNA.

Table 1 – Characteristics of miRNA binding in the 3′UTR mRNA of BC genes

Gene; RPKM	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
AKT1; 33.6	ID00403.3p-miR	2864	-117	93	21
	ID00722.5p-miR	2866	-113	93	20
	ID00843.5p-miR	2875	-119	90	23
BACH1; 5.6	ID00436.3p-miR (8)	4257÷4279	-104÷-106	89÷91	23
	ID01030.3p-miR (6)	4257÷4267	-108	89	23
BIK; 3.5	ID00700.5p-miR	546	-117	92	20
	ID02766.3p-miR	548	-123	92	22
	ID02108.3p-miR	553	-113	90	22
CARNS1; 0.8	ID00470.5p-miR (4)	3225÷3231	-108	89	23
CCND1; 27.6	ID00470.5p-miR (2)	2595÷2597	-108	89	23
CD19; 0.1	ID00436.3p-miR(2)	1862÷1864	-104÷-106	89÷91	23
	ID01030.3p-miR	1862	-108	89	23
CDK6; 2.2	ID00436.3p-miR (9)	1896÷1920	-104÷-106	89÷91	23
	ID01030.3p-miR (7)	1900÷1918	-108÷-115	89÷95	23
	ID02513.5p-miR	1901	-102	91	22
CREB1; 5.3	ID01332.3p-miR	2780	-113	91	22
	ID03006.5p-miR	2781	-121	89	24
	ID03149.5p-miR	2784	-115	92	22
*ELK4;*0.1	ID02868.3p-miR	8296	-113	90	23
	ID01727.5p-miR	8297	-104	89	23
	ID02882.3p-miR	8302	-110	93	21
EFNB1; 30.4	ID00470.5p-miR (3)	2526÷2530	-108	89	23
ETS1; 28.5	ID01030.3p-miR (12)	3875÷3908	-108	89	23
	ID00436.3p-miR (11)	3888÷3908	-104	89	23

Continuation of table 1

FGFR3; 2.3	ID00436.3p-miR	2814	-108	93	23
	ID01030.3p-miR	2814	-113	93	23
	ID01727.5p-miR	2819	-104	89	23
FOXP1; 5.3	ID00436.3p-miR	5952	-104	89	23
	ID01727.5p-miR (2)	5953÷5955	-104÷-106	89÷91	23
	ID02882.3p-miR	5960	-110	93	21
	D00436.3p-miR	5970	-104	89	23
IGF2; 20.4	D00470.5p-miR (9)	2286÷2463	-108÷-113	89÷93	23
	ID02299.5p-miR (3)	2302÷2458	-100	94	21
	ID00470.5p-miR (3)	2520÷2539	-108	89	23
	ID00470.5p-miR (3)	2655÷2672	-108÷-110	89÷91	23
IGF2R; 13.5	ID00436.3p-miR(6)	8447÷8457	-104	89	23
	ID01030.3p-miR(5)	8447÷8455	-108	89	23
PAX2; 0.2	ID02062.3p-miR	3098	-121	90	22
	ID03306.3p-miR	3104	-123	94	21
	ID02781.3p-miR	3105	-117	93	20
	ID00329.3p-miR	3107	-127	92	22
SFN; 9.4	ID00790.3p-miR	1179	-104	89	23
	ID02868.3p-miR	1188	-113	90	23
	ID00436.3p-miR (7)	1190÷1202	-104	89	23
	ID01030.3p-miR (6)	1190÷1200	-108	89	23
	ID01727.5p-miR	1203	-106	91	23
	ID02882.3p-miR	1210	-108	91	21
SMAD3; 14.0	ID02822.5p-miR	2070	-127	91	23
	ID00978.5p-miR	2072	-119	90	22
	ID01382.3p-miR	2075	-113	93	20
SMAD4; 9.8	ID00470.5p-miR (5)	7744÷7752	-108	89	23
	ID02299.5p-miR (6)	7743÷7753	-96	90	21
SOX4; 13.2	ID01839.3p-miR	2994	-123	89	23
	ID01282.3p-miR	3000	-125	95	23
	ID00101.3p-miR	3001	-115	92	22
	ID03445.3p-miR	3001	-121	89	23
SP1; 15.5	ID00436.3p-miR (8)	4147÷4161	-104÷-106	89÷91	23
	ID01030.3p-miR (7)	4147÷4159	-108	89	23
ST8SIA1; 0.5	ID00790.3p-miR	4531	-104	89	23
	ID00436.3p-miR (14)	4537÷4563	-104÷-106	89÷91	23
	ID01030.3p-miR (12)	4537÷4559	-108	89	23
	ID01727.5p-miR	4562	-106	91	23
TGFB1; 19.5	ID03306.3p-miR	2060	-123	94	21
	ID01382.3p-miR	2062	-113	93	20
	ID03208.5p-miR	2066	-125	88	24
WT1; 0.0	ID02513,5p-miR	2698	-104	92	22
	ID00436.3p-miR (8)	2705÷2719	-108	89	23
	ID01030.3p-miR (12)	2705÷2715	-104	89	23
ZEB1; 11.3	ID00470.5p-miR (10)	3587÷3605	-108	89	23
ZIC1; 0.1	ID03324.3p-miR	2547	-115	90	22
	ID00849.3p-miR (2)	2551÷2558	-119÷-121	92÷93	22
	ID01545.3p-miR (2)	2552÷2559	-110÷-113	91÷93	21
	ID01911.5p-miR	2553	-127	92	23

In the mRNA of *BACH1* gene, the BSs of two miRNAs are located, which have eight and six arranged sites. The nucleotide sequence of cluster is represented by 17 repeats of GU dinucleotide. With a length of 3′UTR equal to 3315 nt, there is no point in compactizing BSs. By overlapping the miRNA BSs, as in the *AKT1* gene, competition is created between two miRNAs. To date, there is no explanation for the biological function of nucleotide repeats in the 3′UTR. In our work, the value of dinucleotide repeats as miRNA BSs was established for the first time. The mRNA of *CDK6* gene contains BSs of ID00436.3p-miR and ID01030.3p-miR, which have nine and seven arranged BSs, respectively. The 46 nt cluster consists of 23 dinucleotide GU repeats. The length of 3′UTR is equal to 10219 nt, that is, it is much longer than the length of the cluster and there is no need for superposition of these miRNAs BSs. However, there is a competition between these miRNAs for binding in the cluster.

The cluster of ID00436.3p-miR and ID01030.3p-miR BSs in the mRNA of *ETS1* gene contains 11 and 12 BSs of these miRNAs 57 nt long, consisting of 28 repeats of GU dinucleotide. The length of 3′UTR is equal to 3601 nt and the superposition of two miRNAs BSs slightly reduces its length. That is, in this case, there is competition between two miRNAs. The mRNA of *FGFR3* gene has only one BS for ID00436.3p-miR and ID01030.3p-miR, but the nucleotide sequences of their BSs are overlap. The cluster of six miRNAs BSs in the mRNA of *SFN* gene contains seven and six BSs of ID00436.3p-miR and ID01030.3p-miR, respectively. The cluster 67 nt long consists of 33 repeats of GU dinucleotide. 3′UTR includes 498 nt so clustering does not significantly affect the length of 3′UTR, but competition between miRNAs arises. The mRNA of *SP1* gene has eight and seven BSs for ID00436.3p-miR and ID01030.3p-miR. The cluster of BSs 38 nt long consists of 19 GU dinucleotides with a length of 3′UTR equal to 5207 nt.

The mRNA of *ST8SIA1* gene contains 14 and 12 BSs of ID00436.3p-miR and ID01030.3p-miR, which are included in the cluster of BSs of four miRNAs. The cluster consists of 23 GU dinucleotide repeats, 46 nt long, which is significantly less than the length of 3′UTR equal to 8162 nt. Six and five BSs of ID00436.3p-miR and ID01030.3p-miR, respectively, were identified in the mRNA of *IGF2R* gene. Eight and twelve BSs of ID00436.3p-miR and ID01030.3p-miR, respectively, were identified in the mRNA of *WT1* gene.

In addition to ID00436.3p-miR and ID01030.3p-miR, which have BSs with overlapping nucleotide sequences in mRNA of several genes, we have identified ID00470.5p-miR and ID02299.5p-miR BSs, which are also located with overlapping nucleotide sequences in mRNA of *IGF2* gene. The first cluster is located from 2286 nt to 2486 nt, the second from 2520 nt to 2562 nt and the third from 2655 nt to 2695 nt. All three clusters consist of CA dinucleotide repeats with irregular inserts of C or A nucleotides. The length of 3′UTR is 3871 nt. ID00470.5p-miR and ID02299.5p-miR have five and six sites in *SMAD4* gene, respectively. ID00470.5p-miR has four sites in the mRNA of *CARNS1* and *CCND1* genes, three sites in *EFNB1* mRNA and ten BSs in *ZEB1* mRNA.

The results show that a single gene can be targeted by several alternative miRNAs. One miRNA can interact with several alternative target genes. On the one hand, such links between miRNA and target genes significantly complicate the use of associations of miRNA and genes in the diagnosis of the disease. On the other hand, such associations of miRNA and target genes make diagnosis more reliable, since such associations are not random and more accurately reflect the contribution of each component to the development of the disease.

A feature of the miRNA BSs in the 3′UTR mRNA BC candidate genes is their organization into clusters with the number of sites from two to six. However, in the 3′UTR mRNA of many genes there are clusters consisting of multiple BSs of one or two miRNAs. These miRNAs can significantly affect the translation of mRNA of many BC candidate genes, which increases the probability of their influence on oncogenesis. The associations of ID00436.3p-miR and ID01030.3p-miR with target genes must be used for early molecular diagnosis of the disease. These genes include *BACH1, CDK6, ETS1, IGF2, SFN, SMAD4, SP1* and *ST8SIA1*, characterized by the RPKM value from 0.5 to 28.5. Consequently, these miRNAs can suppress translation regardless of the rate of target genes expression.

Schemes of interaction of nucleotide sequences of BS of miRNA with mRNA candidate genes of breast cancer in table 2 show how miRNA interacts with mRNA by all miRNA nucleotides. The miRNA and mRNA interaction schemes clearly demonstrate advantages of MirTarget program over the existing programs for predicting miRNA BSs with mRNA target genes.

Table 2 – Schemes of interaction miRNA nucleotide sequences with mRNA BSs of breast cancer candidate genes

IGF2R; ID00436.3p-miR; 8447; -104; 89; 23 5'-GUGUGUGUGUGUGUGUGUGUGUG-3' 3'-CACACACGCAUAUAUACACACAU-5'	*IGF2R*; ID01030.3p-miR; 8443; -106; 88; 23 5'-GUUUGUGUGUGUGUGUGUGUGU-3' 3'-CGUACACGUACA-ACACACACACA-5'
SMAD4; ID02299.5p-miR; 7743; -96; 90; 21 5'-CACACACACACACACACACACA-3' 3'-GUAUAUGUGUGUACGU-UGUGU-5'	*SMAD4;* ID00470.5p-miR; 7744; -108; 90; 23 5'-ACACACACACACACACACACACA-3' 3'-UGUGUGUGCGUAUGUGUGCAUGU-5'
WT1; ID00436.3p-miR; 2705; -104; 89; 23 5'-GUGUGUGUGUGUGUGUGUGUGUG-3' 3'-CACACACGCAUAUAUACACACAU-5'	*WT1*; ID01030.3p-miR; 2705; -108; 89; 23 5'-GUGUGUGUGUGUGUGUGUGUGUGU-3' 3'-CGUACACGUACA-ACACACACACA-5'
FOXP1; ID01727.5p-miR; 5953; -106; 91; 23 5'-UGUGUGUGUGUGUGUGUGUGUGC-3' 3'-ACACACAAACAAACAUACACACG-5'	*FOXP1;* ID02882.3p-miR; 5960; -110; 93; 21 5'-GUGUGUGUGUGUGUGCGCGCGU-3' 3'-CACUCACACGCACACGCGC-CA-5'
SFN; ID01727.5p-miR; 1203; -106; 91; 23 5'-UGUGUGUGUGUGUGUGUGUGUGC-3' 3'-ACACACAAACAAACAUACACACG-5'	*SFN*; ID02882.3p-miR; 1210; -108; 91; 21 5'-GUGUGUGUGUGUGUGCGCGCGC-3' 3'-CACUCACACGCACACGCGC-CA-5'
SMAD3; ID01382.3p-miR; 2075; -113; 93; 20 5'-GCCCCGCCCCGCCCCGCCCC-3' 3'-CGGGGCGGGGAAGAGCGGGG-5'	*TGFB1*; ID01382.3p-miR; 2062; -113; 93; 20 5'-GCCCCGCCCCGCCCCGCCCC-3' 3'-CGGGGCGGGGAAGAGCGGGG-5'
Note: Gene; miRNA; start of binding site (nt); the free energy, ΔG (kJ/mole); length of miRNA (nt). The upper and lower nucleotide sequences of mRNA and miRNA, respectively.	

The obtained results in this work show that several miRNAs can affect the expression of several genes and one gene can be target of several miRNAs. For example, ID00436.3p-miR and ID01030.3p-miR have the BSs of the mRNA of *FGFR3, FOXP1, SFN* and *ST8SIA1* genes. Therefore, the use of individual miRNA and individual genes for the diagnosis does not give a reliable result. Thus, it is necessary to control several miRNA associations and the corresponding target genes to identify the contribution of each association to the development of the disease.

Conclusion. In the mRNA of BC candidate genes, both single BSs of miRNAs and two or more miRNA BSs organized into cluster were identified. The formation of clusters of BSs leads to competition between the miRNAs for binding to one mRNA. The identified associations of miRNA and target genes can be used to develop molecular methods for the BC diagnosis.

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Conflicts of Interest. The authors declare that there is no conflict of interest regarding the publication of this paper.

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СҮТ БЕЗІ ҚАТЕРЛІ ІСІГІНІҢ mRNA КАНДИДАТТЫ ГЕНДЕРІНІҢ 3′UTR-де miRNA КЛАСТЕРЛЕРІНІҢ БАЙЛАНЫСУ САЙТТАРЫ

Аннотация. Сүт безінің қатерлі ісігі әйелдердің арасында ең көп кездесетін ауру болып табылады және алдын ала диагностикалау әдістерін дамыту үшін онкогенездің инвазивті емес молекулярлық маркерлерін

анықтау керек. миРНҚ-лар және сүт безінің қатерлі ісік кандидатты гендерінің ассоциациялары мұндай маркерлер ретінде болуы мүмкін. MirTarget бағдарламасы арқылы miRNA-дың және бірнеше гендердің мРНК-мен өзара әрекеттесуінің сипаттамалары анықталды. МиРНҚ-ның байланысу сайттары мРНҚ-да нуклеотидтік тізбектердің бүркелуі арқылы кластерлерді құрайтыны анықталды. Зерттелген гендердің мРНК-ның 3'UTR-де бір немесе бірнеше миРНҚ-дың көптеген байланысу сайттары болды. Кластердегі байланысу сайттардың нуклеотидтік тізбектері қабаттасқанда, миРНҚ-дың бөлігінің бірнеше есе төмендеуі болады. Байланысу сайттары тығыздалуы нәтижесінде кластерде миРНҚ-дың арасындағы байланыстыру үшін бәсекелестік пайда болады, өйткені бірнеше миРНҚ-лар мРНҚ-ның ұзындығы 25-50 нуклеотидтер кластерінде бір уақытта өзара әрекеттесе алмайды. GU және CA динуклеотидтер бар *CD19, CDK6, ETS1, FGFR3, FOXP1, IGF2R, SFN, BACH1* гендерінің мРНҚ-ның 3'UTR-де ID00436.3p-miR және ID01030.3p-miR миРНҚ-дың нуклеотидтік тізбектердің бүркеліп орналасқан көптеген байланысу сайттары бар екені белгілі болды. *CARNS1, CCND1, EFNB1, IGF2, SMAD4, ZEB1* гендерінің мРНҚ-сында ID00470.5p-miR және ID02299.5p-miR-дың байланысу сайттары қабаттасып орналасқан. Кластердің барлық байланысу сайттары CA динуклеотидті қайтамалардан тұрады. *ELK4, FOXP1, SFN* гендерінің мРНҚ-сында ID01727.5p-miR және ID02882.3p-miR байланысу сайттары сәйкес келеді. *TGFB1, SMAD3* гендерінде ID01382.3p-miR-ның төрт рет қайталанатын GCCCC пентануклеотидтен туратын байланысу сайттары бар. Нәтижелер бір ген бірнеше альтернативті миРНҚ-дың нысанысы болуы мүмкін екендігін көрсетеді. ID00436.3p-miR және ID01030.3p-miR нысана гендерімен ассоциацияларды аурудың алдын ала молекулалық диагностикасы үшін ұсынуға болады. Бұл ассоциацияларға *BACH1, CDK6, ETS1, IGF2, SFN, SMAD4, SP1, ST8SIA1* гендер кіреді. *CARNS1, CCND1, EFNB1, IGF2, SMAD4, ZEB1* нысана гендердің және ID00470.5p-miR мен ID02299.5p-miR асоциацияларды аурудың алдын ала молекулалық диагностика үшін пайдалануға ұсынылады.

Түйін сөздер: miRNA, mRNA, кластер, нысана ген, сүт безінің қатерлі ісігі.

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КЛАСТЕРЫ САЙТОВ СВЯЗЫВАНИЯ miRNA В 3′UTR mRNA КАНДИДАТНЫХ ГЕНОВ РАКА МОЛОЧНОЙ ЖЕЛЕЗЫ

Аннотация. Рак молочной железы является самым распространенным заболеванием среди женщин и для разработки методов ранней диагностики необходимо выявление неинвазивных молекулярных маркеров онкогенеза. Такими маркерами могут служить ассоциации miRNA и кандидатных генов РМЖ. Характеристики взаимодействия miRNA с mRNA нескольких генов были предсказаны с помощью программы MirTarget. Установлено, что сайты связывания miRNA могут располагаться в mRNA с наложением нуклеотидных последовательностей, образуя кластеры сайтов связывания. Изученные гены содержали в 3′UTR mRNA множественные сайты связывания одной и более miRNA. При наложении нуклеотидных последовательностей сайтов связывания в кластере происходит многократное уменьшение занимаемой ими доли mRNA. В результате компактизации сайтов связывания между miRNA возникает конкуренция за связывание в кластере поскольку несколько miRNA не могут одновременно взаимодействовать с mRNA в кластере длиной 25-50 нуклеотидов. Выявлены ID00436.3p-miR и ID01030.3p-miR, имеющие множественные сайты связывания с наложением нуклеотидных последовательностей в участках 3′UTR mRNA, содержащих множественные повторы динуклеотидов GU и CA генов *BACH1, CD19*, *CDK6, ETS1, FGFR3, FOXP1, IGF2R*, *SFN, SP1, ST8SIA1, WT1*. Установлены ID00470.5p-miR и ID02299.5p-miR, сайты связывания которых тоже расположены с наложением нуклеотидных последовательностей в mRNA генов *CARNS1, CCND1, EFNB1, IGF2, SMAD4, ZEB1*. Все сайты связывания кластера состоят из повторов CA динуклеотида. ID01727.5p-miR и ID02882.3p-miR имеют совпадающие сайты связывания в mRNA генов *ELK4, FOXP1, SFN*. ID01382.3p-miR имеет сайты связывания в генах *TGFB1, SMAD3*, состоящие из четырех повторов пентануклеотида GCCCC. Полученные результаты показывают, что один ген может быть мишенью нескольких альтернативных miRNA. А одна miRNA может взаимодействовать с несколькими альтернативными генами мишенями. Ассоциации ID00436.3p-miR и ID01030.3p-miR с генами мишенями предлагается использовать для ранней молекулярной диагностики заболевания. В эти ассоциации входят гены *BACH1, CDK6, ETS1, IGF2, SFN, SMAD4, SP1, ST8SIA1*, Ассоциации ID00470.5p-miR и ID02299.5p-miR с генами мишенями *CARNS1, CCND1, EFNB1, IGF2, SMAD4, ZEB1* рекомендуется использовать для ранней молекулярной диагностики заболевания.

Ключевые слова: miRNA, mRNA, кластер, ген-мишень, рак молочной железы.

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