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Characteristics of interaction of miRNA with mRNA of breast cancer candidate genes

Abstract: To establish associations of miRNAs with their target genes, the binding characteristics of miRNAs with mRNAs of candidate genes of various subtypes of breast cancer have been determined. The binding characteristics of miRNAs with mRNAs in 5'UTR, CDS and 3'UTR were found using the MirTarget program. Of the 600 genes involved in the development of breast cancer, 33 genes specific for the triple negative subtype, 16 genes specific for the luminal A, B subtype and 28 genes specific for the her2 subtype. For the triple negative subtype of breast cancer, *CBL, DRAM1, FGFR2, LAMC1, MMP2, NTRK2, PFN1, PTGS2, PRRT2, RAB5A* genes can be characterized as candidate target genes for miRNAs which binding sites are located in the 5'UTR. Candidate genes for binding miRNAs in the CDS are *JHDM1D, RUNX1* and in the 3'UTR – *RUNX1*. In the luminal A,B subtype of breast cancer, candidate genes for binding miRNAs in the 5'UTR are *FOXA1, GTF2IRD1, HMGA2, ITGA6, MAPT, SMAD3, TGFB1*. Candidate genes for binding miRNAs in the CDS are *FOXA1, ITGB1, SOX4* and in the 3'UTR – *SMAD3, TGFB1*. For the her2 subtype of breast cancer, candidate genes for binding miRNAs in the 5'UTR are *A4GALT, EPOR, MAZ, NISCH* and *RAD21*. Candidate genes for miRNA binding in the CDS are *EPOR, MAPK3, MAZ, NHS, RYR1* and in the 3'UTR – *H2AFX*. Based on the obtained characteristics of miRNA interaction with mRNA of candidate genes, associations of miRNA with mRNA have been proposed for use in the diagnosis of breast cancer subtypes.
Key words: miRNA, mRNA, genes, subtypes, breast cancer.

Introduction

Breast cancer (BC) is the leading cause of death in women and is the most common type of cancer. Although advances in the diagnosis and treatment of breast cancer have greatly reduced its incidence and mortality, there are still 500,000 breast cancer deaths per year worldwide [1; 2]. Annually more than 500 thousand cases of disease are registered in the world, as a rule, at the stage of expressed morphological signs. The disease proceeds in different forms, which differ in the degree of aggression, the rate of cell proliferation, invasiveness, the ability to metastasis, etc. The characteristics of each form of disease are the basis for the application of different treatment methods. The success of treatment depends on the correct diagnosis of subtype of the disease. The disturbance of the gene expression which determines the subtype of the disease lies on the basis of all subtypes of the disease. Therefore, in recent years, it is actively searched for genes involved in oncogenesis, taking into account the characteristics of subtypes. This task is extremely difficult, since even one or more genes causing disease interact with many other genes and molecular factors, from which it is difficult to identify the contribution of each participant of oncogenesis. Therefore, the primary interest in oncogenesis lies in the establishment of molecular bases for the initial stages of oncogenesis, when the participants in this process are still few. Currently, more than 600 genes involved in the development of breast cancer are known, of which we have chosen genes based on literature sources, which, according to the authors of the publications, are involved in the development of specific subtypes of breast cancer.

Among the low-molecular factors involved in the regulation of gene expression, miRNAs are actively studied. These molecules directly or indirectly regulate the expression of almost genes of the human genome. The miRNAs predominantly control the expression of transcription factor genes and protein genes involved in signaling systems. Many publications focus on the role of miRNAs in the development of breast cancer. However, the interaction of

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miRNAs with mRNA genes determining subtypes of breast cancer has been little studied. In this regard, the aim of our study was to establish associations of miRNAs and their target genes that can be used as molecular markers for the definition of subtypes of BC.

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 [3] 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 list of miRNAs interacting with candidate genes of breast cancer subtypes. Table 2 shows sources of information on candidate genes of breast cancer subtypes which were targeted for miRNAs from miRBase.

Results and discussion

Characteristics of miRNAs interaction with mRNAs of candidate genes of the triple negative subtype

The ANXA3 protein is a member of calcium-dependent phospholipid-binding proteins and is involved in proliferation, apoptosis, development, migration of metastasis, and invasion of breast cancer cells [4]. The mRNA gene of *ANXA3* gene has binding sites for two miRNAs in the 5'UTR (Table 3), which helps stop protein synthesis before the start of the translation. The mRNA of ASAH1 gene contains two miRNA binding sites in the 5'UTR. ATM and AXL genes are targets for one miRNA and their binding sites are located in the 5'UTR, respectively. The mRNA of BIRC5 gene contains binding sites for two miRNAs located in the 5'UTR starting at one position (Table 3). Therefore, identical nucleotide sequences of binding site could interact with different miRNAs.

Table 1 – List of miRNAs interacting with candidate genes of breast cancer subtypes

Triple-negative (basal-like) subtype: miR-1-1093-3p; miR-1-1101-3p; miR-1-155-3p; miR-1-163-3p; miR-1-1819-3p; miR-1-1922-3p; miR-1-2121-3p; miR-1-2180-3p; miR-1-2228-3p; miR-1-2558-3p; miR-1-3554-3p; miR-1-4241-5p; miR-1-875-3p; miR-2-3313-3p; miR-2-3962-5p; miR-2-4005-5p; miR-2-4119-3p; miR-2-4804-5p; miR-2-5355-3p; miR-2-5674-3p; miR-2-6166-5p; miR-2-6328-5p; miR-2-6862-5p; miR-2-7434-3p; miR-2-7838-5p; miR-3-10329-5p; miR-3-10870-3p; miR-3-7886-3p; miR-3-8100-5p; miR-3-8242-5p; miR-3-9317-3p; miR-4-11437-3p; miR-4-11565-3p; miR-4-11828-5p; miR-4-12861-5p; miR-5-14114-5p; miR-5-14202-5p; miR-5-14959-3p; miR-5-15432-3p; miR-5-15564-3p; miR-5-15733-3p; miR-5-15926-3p; miR-5-16438-3p; miR-5-17240-3p; miR-6-12155-5p; miR-6-16980-5p; miR-6-17815-3p; miR-6-17875-3p; miR-6-18496-3p; miR-7-18337-3p; miR-7-19239-3p; miR-7-20203-3p; miR-7-20411-3p; miR-7-20752-3p; miR-7-21068-3p; miR-7-21133-5p; miR-7-21139-3p; miR-7-22377-3p; miR-8-21445-5p; miR-8-21978-5p; miR-8-23953-5p; miR-9-20317-3p; miR-9-23270-3p; miR-9-23803-5p; miR-9-23969-3p; miR-9-24743-3p; miR-9-25082-3p; miR-9-25335-5p; miR-9-25681-5p; miR-9-25955-3p; miR-9-27797-5p; miR-9-28523-5p; miR-10-11641-3p; miR-10-12491-5p; miR-10-13655-3p; miR-10-16862-5p; miR-10-26483-5p; miR-11-29461-3p; miR-11-29831-3p; miR-11-29998-3p; miR-12-17092-3p; miR-13-32613-3p; miR-13-34600-3p; miR-13-35476-3p; miR-14-35161-5p; miR-14-35446-5p; miR-15-16874-3p; miR-16-35004-5p; miR-16-36024-3p; miR-16-36548-3p; miR-16-36971-3p; miR-16-37915-3p; miR-16-38416-3p; miR-16-38458-3p; miR-16-38712-3p; miR-16-40163-5p; miR-17-10097-3p; miR-17-12514-5p; miR-17-39143-3p; miR-17-39416-3p; miR-17-39440-3p; miR-17-39753-3p; miR-17-39859-5p; miR-17-40012-5p; miR-17-41183-5p; miR-18-39953-5p; miR-18-40163-3p; miR-18-41189-3p; miR-19-30988-5p; miR-19-33623-3p; miR-19-34067-3p; miR-19-41131-3p; miR-19-41746-3p; miR-19-41910-5p; miR-19-42189-5p; miR-19-42710-3p; miR-19-42772-5p; miR-19-43342-3p; miR-19-43662-5p; miR-19-43860-3p; miR-19-43963-5p; miR-19-44540-3p; miR-20-41939-3p; miR-20-43555-5p; miR-20-44079-5p; miR-20-44999-3p; miR-20-45152-5p; miR-20-45753-5p; miR-21-45132-5p; miR-21-45324-5p; miR-22-23987-3p; miR-22-46461-3p; miR-22-46522-5p; miR-X-13195-3p; miR-X-20136-3p; miR-X-25977-5p; miR-X-45905-3p; miR-X-46422-5p; miR-X-46723-3p; miR-X-47540-3p; miR-X-48174-3p

Luminal A,B subtypes: miR-1-1510-5p; miR-1-155-3p; miR-1-1714-3p; miR-1-1819-3p; miR-1-1904-5p; miR-1-1922-3p; miR-1-2121-3p; miR-1-265-3p; miR-1-275-3p; miR-1-3037-5p; miR-1-3554-3p; miR-1-356-5p; miR-2-2621-5p; miR-2-3313-3p; miR-2-4697-3p; miR-2-4782-5p; miR-2-4826-5p; miR-2-5674-3p; miR-2-7331-5p; miR-2-8239-5p; miR-2-8257-5p; miR-3-6515-3p; miR-3-7886-3p; miR-3-8100-5p; miR-3-9441-3p; miR-3-9461-3p; miR-4-11009-3p; miR-4-11421-3p; miR-4-11437-3p; miR-4-12154-5p; miR-4-13460-3p; miR-4-6496-3p; miR-5-14114-5p; miR-5-14202-5p; miR-5-14873-3p; miR-5-15432-3p; miR-5-15733-3p; miR-5-3563-5p; miR-5-6716-5p; miR-5-8853-5p; miR-6-12155-5p; miR-6-16980-5p; miR-7-15849-3p; miR-7-20142-5p; miR-7-20203-3p; miR-7-20411-3p; miR-7-21249-3p; miR-8-19447-3p; miR-8-21162-5p; miR-8-21445-5p; miR-8-23353-3p; miR-8-23953-5p; miR-8-24549-5p; miR-9-13610-3p; miR-9-20317-3p; miR-9-25846-3p; miR-9-26042-5p; miR-9-26255-5p; miR-9-27181-5p; miR-9-27797-5p; miR-9-5204-5p; miR-10-13655-3p; miR-10-26214-5p; miR-10-26423-3p; miR-10-26528-5p; miR-10-26815-5p; miR-10-28986-3p; miR-11-28041-3p; miR-11-29077-3p; miR-11-29827-3p; miR-11-31496-5p; miR-12-17704-3p; miR-12-26632-3p; miR-12-29625-3p; miR-12-30075-3p; miR-12-30416-5p; miR-12-31544-5p; miR-12-32764-3p; miR-12-32997-5p; miR-12-33279-5p; miR-12-33610-3p; miR-14-35670-5p; miR-15-11315-5p; miR-15-31763-5p; miR-15-32047-5p; miR-15-33256-3p; miR-15-36925-3p; miR-15-38620-5p; miR-16-13062-5p; miR-16-20199-5p; miR-16-36024-3p; miR-16-36745-3p; miR-16-40261-3p; miR-17-38733-3p; miR-17-39011-3p; miR-17-39023-3p; miR-17-39273-3p; miR-17-40081-5p; miR-17-40348-5p; miR-17-40711-5p; miR-17-40968-3p; miR-17-41168-3p; miR-18-39953-5p; miR-18-41332-3p; miR-18-41949-5p; miR-19-21199-3p; miR-19-30988-5p; miR-19-33623-3p; miR-19-41131-3p; miR-19-42772-5p; miR-19-42853-3p; miR-19-43315-5p; miR-19-43351-3p; miR-19-43373-3p; miR-19-43614-3p; miR-19-43966-3p; miR-19-44127-3p; miR-19-44540-3p; miR-19-9434-3p; miR-20-22562-3p; miR-20-43381-5p; miR-20-43873-3p; miR-20-45152-5p; miR-20-45753-5p; miR-22-16963-5p; miR-22-23987-3p; miR-22-46979-5p miR-X-13195-3p; miR-X-25977-5p; miR-X-48174-3p
Her2 subtype: miR-1-155-3p; miR-1-1630-3p; miR-1-163-3p; miR-1-1852-5p; miR-1-2121-3p; miR-1-2372-3p; miR-1-2597-5p; miR-1-2802-3p; miR-1-3554-3p; miR-1-356-5p; miR-1-3919-5p; miR-2-3313-3p; miR-2-4733-3p; miR-2-6809-5p; miR-2-7331-5p; miR-2-8257-5p; miR-3-4734-5p; miR-3-7886-3p; miR-3-8100-5p; miR-4-11421-3p; miR-4-11828-5p; miR-4-11923-3p; miR-4-12861-5p; miR-4-6496-3p; miR-5-12460-5p; miR-5-13733-5p; miR-5-14114-5p; miR-5-15026-5p; miR-5-15432-3p; miR-5-15733-3p; miR-5-16562-3p; miR-5-17008-3p; miR-5-17494-5p; miR-5-3563-5p; miR-5-6716-5p; miR-6-12155-5p; miR-6-17519-3p; miR-6-17811-3p; miR-7-12728-5p; miR-7-16350-5p; miR-7-21068-3p; miR-7-21142-5p; miR-7-21249-3p; miR-8-21445-5p; miR-8-21978-5p; miR-8-23986-3p; miR-9-20317-3p; miR-9-22187-3p; miR-9-23270-3p; miR-9-23969-3p; miR-9-25917-3p; miR-9-26042-5p; miR-9-26506-3p; miR-9-27797-5p; miR-10-13655-3p, miR-10-27682-5p; miR-10-28986-3p; miR-10-8412-5p miR-11-27076-3p; miR-11-28204-5p; miR-11-28656-5p; miR-11-29077-3p; miR-11-29324-3p; miR-11-29461-3p; miR-11-29998-3p; miR-12-17092-3p; miR-12-30578-5p; miR-12-31544-5p; miR-12-31979-3p; miR-12-32764-3p; miR-12-33610-3p; miR-13-32368-5p; miR-13-32613-3p; miR-13-33774-5p; miR-13-35476-3p; miR-14-15069-5p; miR-14-31624-3p; miR-14-35161-5p; miR-14-36092-3p; miR-15-32047-5p; miR-15-33256-3p; miR-15-36862-3p; miR-15-38560-5p; miR-16-20406-3p; miR-16-33136-3p; miR-16-36024-3p; miR-17-25894-5p; miR-17-39313-3p; miR-17-39440-3p; miR-17-39593-3p; miR-17-40081-5p; miR-17-40348-5p; miR-18-39953-5p; miR-18-41189-3p; miR-19-21199-3p; miR-19-25044-3p; miR-19-28028-5p; miR-19-30988-5p; miR-19-33623-3p; miR-19-36095-3p; miR-19-41131-3p; miR-19-42218-3p; miR-19-42224-5p; miR-19-43329-3p; miR-19-43644-3p; miR-19-43966-3p; miR-19-44540-3p; miR-20-22562-3p; miR-20-42898-3p; miR-20-43381-5p; miR-20-43646-5p; miR-20-43873-3p; miR-20-44980-3p; miR-20-45152-5p; miR-20-45753-5p; miR-22-16963-5p; miR-22-45335-5p; miR-22-45834-5p; miR-22-46522-5p; miR-22-46603-5p; miR-22-46979-5p; miR-X-13195-3p; miR-X-48174-3p; miR-X-48265-3p

Table 2 – List of candidate genes of subtypes of breast cancer

Triple-negative (basal-like) subtype: *ANXA3* (doi: 10.1016/j. clbc.2017.11.009)*;* *ASAH1 (doi:10.1158/1078-0432.CCR-06-1109); 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); *DRAM1 (doi: 10.1016/j.febslet.2012.12.027);* *ERBB3* (doi: 10.18632/oncotarget.13284); *FGFR2* (doi: 10.1007/s00428-016-1950-9); *FH* (doi: 10.2147/OTT.S101677)*; FIS1 (LINC01554)* (doi: 10.1186/bcr3588); *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); *MDK (doi: 10.1007/s13277-015-3710-x)*; *MMP2* (doi: 10.1038/srep28623); *MTCH2* (doi: 10.1016/j.ajpath.2013.02.046); *MTSS1 (doi: 10.1371/journal.pone.0074525)*; *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); *PTGS2* (doi: 10.1073/pnas.1709119114); *PRRT2 (PKC)* (doi: 10.1002/cmdc.201700640); *RAB5A (doi: 10.3390/ijms17040443)*; *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).

Luminal A,B subtype: *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); *MAP3K14 (doi: 10.1186/bcr3683); 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); *TNC (doi: 10.2147/IJN.S56070)*.
Her2 subtype: *A4GALT (PMID: 24324107); ACSS2 (doi: 10.1186/ar4486.); 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* (doi: 10.18632/oncotarget.7027); *CDK2* (doi: 10.1093/annonc/mdr340); *CDK6* (doi: 10.2147/BCTT.S150540); *EPOR* (doi: 10.1007/s10549-012-2316-x); *EPO* (doi: 10.5114/aoms.2016.62723)*; ERBB3 (HER3)* (doi: 10.18632/oncotarget.22027); *FKBPL* (doi: 10.1038/s41523-017-0049-z)*; GTF2E1 (doi: 10.1186/1471-2407-11-140); H2AFX (H2AX)* (doi: 10.18632/oncotarget.2259); *KDM5D (doi: 10.1021/mp5007618); MAPK3 (ERK1)* (doi: 10.1016/j.bbrc.2017.06.001); *MAZ* (doi: 10.1371/journal.pone.0026122); *NHS (doi: 10.1002/jlcr.3287); NISCH* (doi: 10.1016/j.artmed.2016.10.003); *PARP1* (doi: 10.1053/j.seminoncol.2017.06.006)*; RAD21* (doi: 10.1186/bcr3176)*; RASSF1* (doi: 10.18632/oncotarget.4062)*; RPLP2 (doi: 10.1038/onc.2011.584.); RYR1 (HUON.2006.50.4.0349); STAR (doi: 10.1016/j.ajpath.2014.12.018); TIMP3* (doi: 10.1016/j.humpath.2011.12.022), *TNF* (doi: 10.17219/acem/62120).
Notes: In parentheses, sources of information of candidate genes of breast cancer subtypes

Table 3 – Characteristics of miRNAs binding with mRNAs of candidate genes of the triple negative subtype

ANXA3: miR-20-43555-5p, 56, -119, 88, 22; miR-16-38458-3p, 94, -121, 86, 24
ASAH1: miR-20-44079-5p, 17, -119, 89, 22; miR-19-42772-5p, 232, -121, 85, 23
ATM: miR-7-21133-5p, 9777**, -121, 89, 24
AXL: miR-17-39143-3p, 2690*, -119, 86, 24
BIRC5: miR-16-35004-5p, 110, -125, 89, 23; miR-16-36548-3p, 110, -125, 89, 23
CBL: miR-9-20317-3p (6), 12 ÷ 28, -127 ÷ -140, 86 ÷ 94, 24; miR-18-39953-5p (4), 15 ÷ 24, -123, 85, 23; miR-16-33136-3p (4), 16 ÷ 25, -117, 86, 22; miR-5-15733-3p (5), 16 ÷ 34, -127 ÷ -138, 86 ÷ 93, 24; miR-17-39416-3p (4), 17 ÷ 26, -121, 92, 22; miR-5-15564-3p, 27, -123, 89, 22; miR-X-48174-3p (2), 28 ÷ 31, -121, 85, 24; miR-1-1819-3p, 32, -125, 91, 23; miR-16-38712-3p, 174*, -123, 85, 24; miR-11-29461-3p, 176*, -125, 89, 23; miR-2-4804-5p, 7728**, -117, 93, 24
CD44: miR-16-40163-5p, 129, -121, 90, 23; miR-5-14959-3p, 352, -121, 85, 24; miR-1-2180-3p, 359, -121, 89, 22; miR-X-25977-5p, 362, -119, 87, 22
CEACAM5: miR-7-21133-5p, 3220**, -119, 87, 24; miR-17-39753-3p (2), 3223÷ 3267**, -115 ÷ -117, 87 ÷ 89, 21
DRAM1: miR-20-45753-5p, 57, -121, 89, 22; miR-19-30988-5p, 62, -123, 85, 23; miR-X-13195-3p, 73, -123, 87, 23; miR-14-35446-5p, 302, -121, 85, 24; miR-9-25335-5p, 410, -119, 89, 22
ERBB3: miR-3-8100-5p, 148, -125, 86, 24
FGFR2: miR-17-41183-5p, 37, -119, 86, 23; miR-4-11437-3p, 38, -121, 86, 23; miR-18-39953-5p, 42, -123, 85, 23; miR-19-41131-3p, 43, -123, 85, 23; miR-7-21139-3p, 48, -132, 89, 24; miR-11-29831-3p, 55, -129, 86, 24; miR-19-34067-3p, 60, -123, 92, 23; miR-1-2228-3p, 152, -125, 89, 24
FH: miR-14-35446-5p, 96*, -121, 85, 24
FIS1: miR-X-46422-5p, 529, -119, 86, 24; miR-X-47540-3p, 529, -119, 86, 24; miR-21-45132-5p, 535, -119, 89, 22
IL11: miR-7-21068-3p, 54, -125, 86, 24; miR-16-36971-3p, 630*, -123, 85, 24
JHDM1D: miR-9-20317-3p, 7*, -127, 86, 24; miR-5-15564-3p, 12*, -119, 86, 22; miR-7-19239-3p, 88*, -123, 88, 23; miR-1-155-3p, 92*, -119, 86, 22
LAMC1: miR-11-29998-3p, 28, -121, 86, 23; miR-20-44754-3p, 42, -119, 86, 23; miR-19-43342-3p, 51, -119, 90, 22; miR-19-44540-3p, 107, -121, 85, 23; miR-10-13655-3p (2), 111 ÷ 115, -117 ÷ -123, 86 ÷ 91, 22; miR-2-3313-3p, 112, -136, 85, 25; miR-19-42772-5p, 114, -121, 85, 23; miR-1-155-3p, 115, -119, 86, 22; miR-4-11437-3p, 244, -121, 86, 23; miR-3-7886-3p, 277*, -123, 85, 24; miR-6-18496-3p, 388*, -119, 90, 22
LASP1: miR-20-42659-3p, 47, -119, 89, 22; miR-16-36476-5p, 68, -119, 90, 22; miR-5-16438-3p, 206, -119, 90, 22; miR-1-2121-3p, 207, -134, 85, 25; miR-19-33623-3p, 207, -127, 86, 24
MAGEA10: miR-5-14114-5p, 599*, -119, 86, 23

MDK: miR-8-23986-3p, 16, -129, 90, 24; miR-5-14114-5p, 421*, -121, 88, 23; miR-7-20815-5p, 483*, -119, 86, 23
MMP2: miR-1-1819-3p, 110, -123, 89, 23; miR-9-25082-3p, 110, -121, 85, 24; miR-9-20317-3p, 112, -129, 87, 24; miR-X-48174-3p, 112, -121, 85, 24; miR-17-39416-3, 113, -121, 92, 22; miR-5-15733-3p, 115, -127, 86, 24; miR-7-20203-3p, 115, -121, 90, 22; miR-9-27797-5p (2), 118 ÷ 124, -121 ÷ -127, 85 ÷ 90, 24; miR-12-17092-3p, 124, -123, 89, 22; miR-2-6166-5p, 279, -119, 86, 23; miR-21-45324-5p, 379*, -125, 91, 23; miR-X-20136-3p, 380*, -121, 86, 24
MTCH2: miR-22-46522-5p, 26, -123, 89, 22; miR-5-15926-3p, 74, -123, 94, 22
MTSS1: miR-11-28201-3p, 26, -123, 85, 24; miR-20-42676-3p, 26, -121, 86, 23; miR-12-30578-5p, 1653*, -123, 85, 24
MYL9: miR-19-43662-5p, 639**, -121, 93, 23; miR-16-37915-3p, 873**, -119, 86, 24
NTRK2: miR-2-5674-3p, 57, -121, 88, 23; miR-17-39416-3p, 61, -125, 95, 22; miR-9-20317-3p, 63, -129, 87, 24; miR-5-15564-3p, 65, -127, 92, 22; miR-10-13940-3p, 171, -110, 96, 18; miR-9-23270-3p, 266, -129, 86, 24; miR-20-43555-5p, 285, -121, 89, 22
PARP1: miR-19-36095-3p, 1275*, -119, 90, 23
PFN1: miR-3-8242-5p, 78, -119, 89, 23; miR-9-23803-5p (3), 78 ÷ 90, -121 ÷ -129, 86 ÷ 92, 24; miR-19-44540-3p, 101, -121, 85, 23; miR-X-13268-5p, 103, -119, 90, 21; miR-19-42772-5p, 105, -125, 88, 23; miR-20-45753-5p, 108, -119, 88, 22; miR-17-41183-5p, 504, -119, 86, 23; miR-8-21978-5p, 508, -121, 85, 24; miR-19-41131-3p, 509, -123, 85, 23; miR-7-18337-3p, 512, -119, 87, 23; miR-11-30258-3p, 522, -119, 86, 24; miR-6-12155-5p, 606, -119, 86, 22; miR-20-41939-3p, 782*, -119, 87, 22; miR-3-10329-5p, 1130**, -119, 87, 24; miR-16-37915-3p, 1240**, -123, 89, 24
PTGS2: miR-5-15432-3p, 107, -121, 85, 23; miR-9-23969-3p, 108, -123, 92, 21; miR-X-13195-3p, 112, -121, 85, 23; miR-6-12155-5p, 113, -119, 86, 22
PRRT2: miR-11-28560-5p, 39, -119, 87, 23; miR-2-5674-3p, 44, -119, 86, 23; miR-9-20317-3p, 44, -127, 86, 24; miR-7-17529-3p, 48, -125, 86, 25; miR-X-48174-3p, 51, -125, 88, 24
RAB5A: miR-19-41910-5p, 37, -125, 86, 24; miR-X-48174-3p (4), 143 ÷ 192, -121 ÷ -127, 85 ÷ 90, 24; miR-18-40163-3p (2), 179 ÷ 185, -121, 86, 23; miR-18-39953-5p, 182, -125, 87, 23; miR-6-17815-3p, 184, -132, 89, 24; miR-8-23953-5p, 184, -125, 86, 24; miR-5-15733-3p (2), 186 ÷ 189, -127, 86, 24; miR-2-6862-5p (3), 187 ÷ 191, -117 ÷ -121, 86 ÷ 89, 23; miR-9-27797-5p, 189, -121, 85, 24; miR-1-1819-3p, 190, -121, 88, 23; miR-13-32613-3p, 190, -121, 85, 24; miR-13-32613-3p, 193, -121, 85, 24; miR-9-25082-3p, 193, -123, 87, 24; miR-19-42772-5p (2), 324 ÷ 327, -121, 85, 23; miR-2-3313-3p (2), 325 ÷ 329, -136 ÷ -140, 85 ÷ 88, 25; miR-10-13655-3p, 328, -119, 87, 22; miR-1-155-3p (3), 328 ÷ 334, -119 ÷ -127, 86 ÷ 92, 22; miR-9-28523-5p, 328, -121, 97, 20
RUNX1: miR-9-25955-3p, 12, -121, 90, 21; miR-5-14114-5p, 1417, -123, 89, 23; miR-5-14114-5p, 1434, -119, 86, 23; miR-10-13655-3p, 1605*, -121, 89, 22; miR-20-44999-3p, 1668*, -123, 88, 23; miR-10-16862-5p, 1684*, -119, 89, 21; miR-16-36024-3p, 2215*, -121, 85, 23; miR-8-21445-5p, 2807*, -119, 86, 22; miR-4-11828-5p, 2957**, -117, 87, 22; miR-16-38712-3p, 3038**, -123, 85, 24; miR-18-41189-3p, 3061**, -127, 87, 23; miR-2-4005-5p, 3119**, -129, 87, 24; miR-20-45152-5p, 3124**, -127, 86, 24
SERPINE1: miR-16-38458-3p, 30, -123, 88, 24; miR-2-3962-5p, 542*, -125, 88, 24
SFN: miR-6-16980-5p, 824**, -123, 88, 23; miR-6-16980-5p, 829**, -121, 86, 23; miR-19-30988-5p, 835**, -129, 90, 23
STMN1: miR-5-17240-3p, 1096**, -119, 89, 23; miR-2-5355-3p, 1987**, -119, 93, 22
Notes: miRNA; in brackets, the number of binding sites; the beginning of the binding site; without * – 5'UTR; * – CDS; ** – 3'UTR; binding energy, kJ / mole; the value ΔG / ΔGm,%; length, nt

The proto-oncogene *CBL* encodes ubiquitin ligase [5]. The *CBL* gene is highly expressed in the testes, and weakly in other organs and tissues. The *CBL* gene is the target for 11 miRNAs, several of which have multiple binding sites (Table 3). miR-9-20317-3p has six sites, miR-5-15733-3p – five sites, miR-17-39416-3p and miR-18-39953-5p – four sites, miR-X-48174-3p – two sites. The beginning of all these binding sites are located in the 5'UTR region from 12 nt to 34 nt, i.e., the nucleotide sequences of binding sites of these miRNAs are partially common. For miR-16-38712-3p and miR-11-29461-3p, the binding sites are also common and located in the CDS. One miRNA is bound in the 3'UTR. The *CBL* gene encodes oligopeptides GGGSGSGGSGSGG, HHHHHHH, DDDDDE and PPPPPP in the protein structure, the function of which is unknown.

CD44 protein is a glycoprotein involved in intercellular interactions, adhesion, cell migration, hematopoiesis, apoptosis and metastasis at the triple-negative subtype of the breast cancer [6]. The *CD44* gene is the target for three miRNAs which bind in the 5'UTR of mRNA with a free energy of -119 kJ/mole and -121 kJ/mole, which provides sufficient interaction of miRNA and mRNA. These miRNAs bind to mRNA in the 5'UTR region with the beginning at 352 nt, 359 nt and 362 nt, that is,

the binding sites overlap. This position of RISK interaction with mRNA prevents the initiation of translation.

The mRNA of *CEACAM5* gene links two miRNAs in the 3'UTR starting at positions of 3220 nt, 3223 nt and 3267 nt, that is, with a partial overlap of the nucleotide sequences of the binding sites.

The *DRAM1* gene is the target for five miRNAs, three of which bind with mRNA in the 5'UTR in one region from 57 nt to 73 nt.

The *ERBB3* gene encodes a member of the epidermal growth factor receptor family (EGFR) tyrosine kinase. Overexpression of this protein is detected in various organs, including the prostate, bladder, mammary gland [7]. The *ERBB3* gene is a target of a single miRNA that binds in the 5'UTR (Table 3). In mRNAs longer than 4000 nucleotides, there are no miRNA binding sites.

The protein encoded by *FGFR2* gene is a member of the epidermal growth factor receptor family (EGFR). It is highly conservative in the process of evolution [8]. In mRNA of *FGFR2* gene, there are eight miRNA binding sites in the 5'UTR (Table 3). Binding sites of seven miRNAs are located with a partial coincidence of nucleotide sequences in the region from 37 nt to 60 nt. Free binding energy changed from -119 kJ/mole to -132 kJ/mole. miR-7-21139-3p has the advantage in the binding with mRNA of *FGFR2* gene.

The *FIS1* gene is a target for three miRNAs that may bind in the 5'UTR in the region of 529 nt to 535 nt. The *FH* and *IL11* genes are targets for one and two miRNAs, respectively.

The mRNA of *JHDM1D* gene binds four miRNAs in the CDS. The starts of binding sites for two miRNAs are located at positions 7 nt and 12 nt, and for other two miRNAs at positions 88 nt and 92 nt. That is, CDS is characterized by the cluster location of miRNA binding sites. Typically, sites of miRNA binding in the protein coding region evolved long ago and they are conservative.

There are two sites for miRNA binding in the 5'UTR mRNA of *LAMC1* gene. In the first region from the position of 28 nt to 51 nt, binding sites for three miRNAs are located. In the second region from the position of 107 nt, the binding sites of five miRNAs are located.

In the 5'UTR mRNA of *LASP1* gene the binding site of the first miRNA immediately starts after the binding site of the second miRNA. Next three miRNAs have the beginning of binding sites at 206 nt and 207 nt. The genes *MAGEA10* and *MDK* are targets for miRNAs which bind in the CDS.

In mRNA of *MMP2* gene, the starts of binding sites of nine miRNAs are located from 110 nt to 124 nt in the 5'UTR. In the protein coding region of mRNA, the beginning of binding sites of two miRNAs is coincided. Therefore, the location of miRNA binding sites in mRNA of *MMP2* gene also has a clear tendency to form clusters of miRNA binding sites. This number of binding sites suggests a strong dependence of *MMP2* gene expression on miRNAs, which compete with each other for mRNA binding.

There are few sites for miRNAs binding in mRNA of *MTCH2, MYL9* and *MTSS1* genes, and only two miRNA binding sites in the 5'UTR mRNA of *MTSS1* gene are coincided.

The *NTRK2* gene is a target for seven miRNAs whose binding sites are located in the 5'UTR. The first four miRNAs have very close positions at the beginning of binding sites.

The mRNA of *PFN1* gene has binding sites for 15 miRNAs. The first six miRNAs has the beginning of binding sites from 78 nt to 108 nt. The next five miRNAs have the beginning of binding sites from 508 nt to 522 nt in the 5'UTR. Therefore, the expression of *PFN1* gene is strongly controlled by miRNA.

All binding sites of the four miRNAs in mRNA of *PTGS2* gene are located in the 5'UTR and constituted one cluster. Similarly, all binding sites of the five miRNAs in mRNA of *PRRT2* gene are located in the 5'UTR and constitute one cluster.

The beginning of binding sites for 14 miRNAs are located in the 5'UTR mRNA of *RAB5A* gene in a region from 143 to 193 nt. Moreover, miR-X-48174-3p has four and miR-2-6862-5p three binding sites. miR-13-32613-3p, miR-18-40163-3p, miR-5-15733-3p have two binding sites. From 324 nt to 334 nt, there are binding sites for five miRNAs, three of which have two binding sites. The *RAB5A* gene is unique among 17,000 genes we studied for the ability to bind miRNA in the 5'UTR of mRNA. The free binding energy of these miRNAs varies from -115 kJ/mole to – 140 kJ/mole, which indicates a strong interaction of miRNA with mRNA of *RAB5A* gene. The need to suppress gene expression with miRNA has been experimentally confirmed. It was found that increasing the expression of *RAB5A* gene increases the mobility of tumor cells and increases the lymph node metastases [9]. Suppressing the expression of *RAB5A* gene leads to a decrease in the mobility of cancer cells and a decrease in invasiveness [10].

The *RUNX1* gene is the target for nine miRNAs that have been bind in the 5'UTR, CDS and 3'UTR (Table 3). In each of these mRNA regions, two miRNAs form clusters of common binding sites.

The mRNA of *SERPINE1* and *STMN1* genes has two miRNA binding sites. The mRNA of *SFN* gene has binding sites for three miRNAs in the 3'UTR with beginning at positions 824 nt, 829 nt and 835 nt.

Characteristics of the interaction of miRNAs with mRNAs of candidate genes of the luminal subtype

The *ANGPTL4* gene in breast cancer is expressed higher than in the control [11] and the gene is associated with the regulation of invasion in breast cancer [12]. The mRNA of *ANGPTL4* gene has binding sites for two miRNAs, one of which is fully complementarily bound in the CDS (Table 4). The mRNA of *EZH1* gene has binding sites for one miRNA in the 3'UTR.

Table 4 – Characteristics of miRNAs binding with mRNA of candidate genes of the luminal subtype

ANGPTL4: miR-19-44540-3p, 227*, -125, 88, 23; miR-19-43315-5p, 259*, -134, 100, 23
EZH1: miR-17-39273-3p, 3085**, -115, 89, 23; miR-19-43614-3p, 3832**, -125, 91, 23
FOXA1: miR-8-19447-3p, 55, -125, 87, 24; miR-11-31496-5p, 56, -125, 88, 23; miR-11-28041-3p, 62, -119, 86, 23; miR-1-1904-5p, 99, -123, 89, 24; miR-22-16963-5p, 100, -123, 88, 22; miR-17-40081-5p, 101, -129, 88, 23; miR-20-22562-3p, 101, -129, 86, 24; miR-20-22562-3p, 102, -129, 86, 24; miR-1-155-3p (3), 109 ÷ 130, -119 ÷ -129, 86; ÷ 94, 22miR-20-43873-3p, 110, -123, 89, 23; miR-1-1510-5p, 111, -140, 94, 24; miR-1-2121-3p (5), 112 ÷ 118, -134 ÷ -140, 85 ÷ 89, 25; miR-5-3563-5p, 112, -127, 92, 22; miR-X-13195-3p, 112, -121, 85, 23; miR-10-26423-3p, 113, -129, 87, 24; miR-19-30988-5p, 113, -127, 88, 23; miR-20-45152-5p, 115, -129, 87, 24; miR-19-33623-3p (3), 115 ÷ 122, -129 ÷ -134, 87 ÷ 90, 24; miR-19-44127-3p, 117 ÷ 126, -127 ÷ -129, 86 ÷ 87, 24; miR-3-8100-5p (2), 117 ÷ 121, -127, 87, 24; miR-1-1714-3p, 118, -121, 97, 20; miR-1-1922-3p, 118, -119, 89, 22; miR-15-32047-5p, 118, -125, 86, 24; miR-X-13195-3p, 118, -123, 87, 23; miR-19-21199-3p (3), 118 ÷ 121, -134 ÷ -136, 85 ÷ 86, 25; miR-19-44540-3p, 118 ÷ 137, -121 ÷ -123, 85 ÷ 87, 23; miR-17-40348-5p, 120, -123, 91, 23; miR-19-21199-3p, 120, -140, 89, 25; miR-4-11421-3p, 120, -121, 86, 23; miR-5-6716-5p, 121, -125, 88, 23; miR-2-3313-3p, 122, -136, 85, 25; miR-19-42772-5p, 123, -121, 85, 23; miR-20-22562-3p, 123, -129, 86, 24; miR-10-13655-3p, 124, -123, 91, 22; miR-19-43966-3p, 125, -121, 86, 23; miR-4-6496-3p (2), 127 ÷ 130, -119 ÷ -121, 92 ÷ 93, 21; miR-8-21445-5p, 133, -121, 88, 22; miR-2-8257-5p, 135, -123, 87, 23; miR-6-12155-5p, 231, -119, 86, 22; miR-2-2621-5p, 238, -121, 86, 22; miR-19-9434-3p, 756*, -119, 87, 23; miR-8-19447-3p, 766*, -125, 87, 24; miR-5-15733-3p, 768*, -132, 89, 24; miR-12-33610-3p, 1128*, -127, 86, 24; miR-19-43351-3p, 1128*, -119, 87, 23; miR-4-12154-5p, 1129*, -125, 87, 24; miR-8-23953-5p, 1130*, 125, 86, 24; miR-9-27797-5p, 1135*, -121, 85, 24; miR-9-20317-3p, 1150*, -134, 90, 24; miR-7-20203-3p, 1159*, -119, 89, 22; miR-X-25977-5p, 1279*, -119, 87, 22; miR-15-33256-3p, 1280*, -127, 86, 24; miR-17-40081-5p, 1287*, -129, 88, 23; miR-1-1819-3p, 1325*, -119, 86, 23; miR-5-14202-5p, 1325*, -119, 88, 22; miR-12-31544-5p, 1396*, -119, 87, 23
GTF2IRD1: miR-2-8257-5p, 124, -121, 85, 23; miR-10-26423-3p, 127, -129, 87, 24; miR-4-11421-3p, 133, -121, 86, 23; miR-1-2121-3p, 135, -136, 86, 25; miR-1-3037-5p, 160, -119, 86, 23; miR-12-32997-5p, 208, -125, 89, 23; miR-16-36024-3p, 210, -121, 85, 23; miR-6-12155-5p, 234, -123, 89, 22; miR-8-23353-3p, 340, -123, 92, 22; miR-8-21162-5p, 959*, -121, 92, 23
HMGA2: miR-2-3313-3p, 99, -138, 87, 25; miR-1-155-3p (4), 515 ÷556, -119 ÷-132, 86 ÷95, 22; miR-19-43373-3p, 539, -119, 93, 21; miR-X-13195-3p, 541, -123, 87, 23; miR-15-32047-5p (3), 541÷ 545, -125 ÷-134, 86 ÷91, 24; miR-1-265-3p, 542, -125, 91, 22; miR-17-41168-3p, 542, -117, 95, 20; miR-17-40348-5p (2), 543÷ 547, -119, 87, 23; miR-19-21199-3p (3), 543 ÷549, -134 ÷-136, 85 ÷86, 25; mir-1-2121-3p (4), 544÷ 548, -134 ÷-146, 85 ÷93, 25; miR-19-33623-3p, 544÷ 548, -129 ÷-142, 87 ÷96, 24; miR-1-275-3p, 547, -121, 85, 23; miR-1-1922-3p, 550, -119, 89, 22; miR-22-23987-3p, 553, -119, 90, 21; miR-10-26815-5p, 575, -121, 88, 24; miR-1-1819-3p, 788, -123, 89, 23; miR-18-41949-5p, 822*, -119, 89, 22; miR-2-7331-5p, 1270**, -119, 86, 23
ITGA6: miR-4-11009-3p, 71, -125, 88, 23; miR-9-26042-5p, 73, -119, 87, 22; miR-1-3554-3p, 180, -117, 86, 23; miR-9-25846-3p, 200, -117, 86, 23; miR-5-15432-3p, 248*, -121, 85, 23
ITGB1: miR-10-26815-5p, 61*, -127, 92, 24; miR-22-46979-5p, 91*, -127, 92, 23; miR-9-5204-5p, 91*, -119, 89, 22; miR-10-13655-3p, 95*, -123, 91, 22; miR-5-8853-5p, 98*, -117, 93, 20; miR-16-40261-3p, 101*, -117, 93, 20; miR-3-9441-3p, 101*, -121, 86, 23
JAK1: miR-11-29827-3p, 66, -129, 90, 24; miR-7-21249-3p, 66, -123, 87, 23; miR-17-40968-3p, 75, -123, 85, 24
LOX: miR-12-32764-3p, 711*, -123, 87, 23; miR-17-40081-5p, 723*, -125, 86, 23
MAP3K14: miR-15-31763-5p, 30, -123, 85, 24; miR-19-42853-3p, 854*, -119, 86, 23; miR-12-33279-5p, 2342*, -127, 91, 24; miR-2-8239-5p, 3041**, -121, 89, 22; miR-2-4697-3p, 3333**, -132, 87, 24
MAPT: miR-19-44540-3p, 108, -121, 85, 23; miR-12-26632-3p, 147, -123, 88, 23; miR-3-6515-3p, 174, -119, 86, 24; miR-17-40348-5p, 224, -119, 87, 23; miR-19-33623-3p, 225, -129, 87, 24; miR-9-26042-5p, 3168**, -119, 87, 22; miR-7-20411-3p, 3743**, -119, 87, 23
MCM7: miR-17-39023-3p, 23, -123, 87, 24; miR-7-20142-5p, 26, -119, 89, 23; miR-8-23353-3p, 111, -121, 90, 22

SMAD3: miR-16-13062-5p, 3, -129, 87, 24; miR-7-15849-3p, 4, -115, 100, 18; miR-20-45753-5p, 54, -119, 88, 22; miR-10-26214-5p, 89, -123, 88, 23; miR-9-26255-5p, 139, -123, 87, 24; miR-15-11315-5p, 194, -117, 100, 19; miR-16-20199-5p, 201, -121, 88, 22; miR-12-29625-3p, 243, -125, 92, 23; miR-6-16980-5p, 2070*, -127, 91, 23; miR-12-17704-3p, 2071**, -123, 88, 23; miR-15-38620-5p, 2072*, -119, 90, 22; miR-14-35670-5p, 4330*, -119, 89, 23
SOX4: miR-16-36745-3p, 739, -123, 87, 24; miR-10-28986-3p, 766, -121, 86, 23; miR-4-13460-3p, 1291*, -123, 91, 22; miR-5-14873-3p, 1293*, -121, 90, 22; miR-18-39953-5p, 1295*, -125, 87, 23; miR-4-11437-3p, 1402*, -125, 89, 23; miR-X-48174-3p, 1454*, -125, 88, 24; miR-3-8100-5p, 1482*, -125, 86, 24; miR-2-3313-3p, 1483*, -136, 85, 25; miR-1-155-3p, 1486*, -121, 88, 22; miR-3-7886-3p, 1624*, -123, 85, 24; miR-12-30075-3p, 1721*, -127, 88, 24; miR-9-27181-5p, 1723*, -127, 92, 22; miR-X-13195-3p, 1799*, -123, 87, 23; miR-1-356-5p, 1837*, -127, 87, 23; miR-15-36925-p, 1838*, -127, 87, 24; miR-11-31496-5p , 1884*, -123, 87, 23; miR-9-13610-3p, 1900*, -121, 92, 21; miR-16-36024-3p, 2405**, -121, 85, 23; miR-11-29077-3p, 2428**, -123, 88, 24; miR-2-5674-3p, 2994**, -123, 89, 23; miR-17-39011-3p, 3000**, -125, 95, 23; miR-X-48174-3p, 3000**, -127, 90, 24
TGFB1: miR-19-43966-3p, 1, -121, 86, 23; miR-20-43381-5p, 1, -121, 92, 21; miR-1-155-3p, 3, -121, 88, 22; miR-9-13610-3p, 6, -121, 92, 21; miR-2-4782-5p, 78, -119, 86, 22; miR-12-30416-5p, 186, -117, 92, 22; miR-10-13655-3p, 209, -129, 95, 22; miR-1-155-3p, 212, -119, 86, 22; miR-20-43381-5p, 213, -119, 90, 21; miR-19-41131-3p, 235, -123, 85, 23; miR-17-38733-3p, 241, -119, 89, 24; miR-3-8100-5p (2), 900 ÷ 903*, -127, 87, 24; miR-3-9461-3p, 910*, -119, 87, 23; miR-17-40711-5p, 1053*, -121, 88, 23; miR-6-16980-5p (3), 2057 ÷ 2087**, -121 ÷ -123, 86 ÷ 88, 23; miR-19-30988-5p (2), 2058 ÷ 2073**, -123, 85, 23; miR-1-356-5p, 2059**, -127, 87, 23; miR-9-13610-3p, 2060**, -123, 94, 21; miR-8-24549-5p, 2066**, -125, 88, 24; miR-12-17704-3p, 2088**, -123, 88, 23; miR-15-38620-5p, 2089**, -119, 90, 22; miR-5-14114-5p, 2091**, -119, 86, 23; miR-1-2121-3p, 2093**, -140, 89, 25; miR-19-33623-3p, 2093**, -129, 87, 24; miR-18-41332-3p, 2095**, -121, 88, 23
TNC: miR-10-26528-5p, 1165*, -119, 86, 24; miR-2-4826-5p, 8073**, -115, 92, 23
Notes: miRNA; in brackets, the number of binding sites; the beginning of the binding site; without * – 5'UTR; * – CDS; ** – 3'UTR; binding energy, kJ / mole; the value ΔG / ΔGm,%; length, nt

FOXA1 transcription factor involves in the regulation of differentiation of breast cancer cells and other processes of oncogenesis [13-17]. Suppressing the expression of *FOXA1* gene significantly reduces the mobility of BC cells [18]. The mRNA of *FOXA1* gene has 50 binding sites in the 5'UTR including three miRNAs in the region from 55 nt to 62 nt. 33 miRNAs have binding sites in the region from 99 nt to 137 nt.

miR-1-2121-3p has five binding sites and miR-19-21199-3p has three binding sites with a free binding energy equal to -134 kJ/mole ÷ -140 kJ/mole for both miRNAs. miR-19-33623-3p has three binding sites with a free binding energy equal to -129 kJ/mole ÷ -134 kJ/mole. miR-1-155-3p also has three binding sites in this region. miR-2-3313-3p has binding site with a free binding energy equal to -136 kJ/mole. The mRNA of *FOXA1* gene has nine binding sites in the CDS of which seven are located from 1128 to 1150 nt. Three miRNAs are bound in the CDS at positions 1279 nt, 1280 nt, and 1287 nt. Two miRNAs have binding sites in the CDS starting at 1325 nt and three miRNAs have the beginning of binding sites at 756 nt, 766 nt, and 768 nt. Therefore, clusters of miRNA binding sites are detected in both 5'UTR and CDS. The established characteristics of miRNA binding with mRNA of *FOXA1* gene indicate that this gene is under the strong control of more than 50 miRNAs. In addition, five miRNAs have free binding energy more than -130 kJ/mole. According to these data, *FOXA1* gene expression will not occur if all these miRNAs are present in the medium at concentrations comparable to the mRNA concentration. Therefore, it is necessary to consider which miRNA and in what concentration are present during the expression of *FOXA1* gene. According to the gene bank, the *FOXA1* gene is expressed only in the prostate.

Expression of the transcription factor GTF2IRD1 varies with BC [19]. The mRNA of *GTF2IRD1* gene binds with four miRNAs which binding sites are located in the 5'UTR in the region from 124 nt to 135 nt. Out of ten miRNAs, only miR-8-21162-5p is bound in the CDS. GTF2IRD1 is expressed in half of the tissues.

HMGA2 factor modulates transcription, is involved in the regulation of growth and invasion of breast cancer [20-22]. 14 miRNAs bind with mRNA of *HMGA2* gene in the 5'UTR in a region from 515 nt to 575 nt. Of these, miR-1-2121-3p has four binding sites with a binding energy equal to -134 kJ/mole ÷ -146 kJ/mole. miR-19-21199-3p has three binding sites with a free binding energy equal to -134 kJ/mole ÷ -136 kJ/mole. miR-1-155-3p has four binding sites with a free binding energy equal to -119 kJ/mole ÷ -132 kJ/mole. miR-19-33623-3p has three binding sites with a free binding energy equal to -129 kJ/mole ÷ -142 kJ/mole. miR-15-32047-5p has two binding sites with a free binding energy equal to -125 kJ/mole ÷ -129 kJ/mole. miR-2-3313-3p has a binding site with a free binding energy equal to -138 kJ/

mole. All binding sites for all miRNAs are located in the 5'UTR. The genes are similar in the number of miRNAs that bind with mRNA of *HMGA2* and *FOXA1* genes. The gene encoding the HMGA protein is actively expressed at the embryonic stage of development, while in adult cells its expression is at the background level [23]. However, with the development of malignant tumors of epithelial origin, the expression level of this gene significantly increases again. The increase in the level of expression of HMGA2 was noted in colon cancer [24], bladder [25], thyroid gland [26], skin [27], ovaries [28], etc.

The mRNA of *ITGA6* gene has five miRNA binding sites, of which only one is located in the CDS, and others in the 5'UTR.

Integrin ITGB1 is a member of the family of membrane receptors including cell adhesion, embryogenesis, immune and metastasis [29; 30]. The mRNA of *ITGB1* gene is associated with seven miRNAs in the CDS with the beginning of binding sites of six of them in a region from 91 nt to 101 nt, which is a rare case of a cluster of miRNA binding sites in the CDS.

The three sites for binding miRNAs with mRNA of *JAK1* gene are a cluster located in the 5'UTR. The mRNA of *LOX* gene has binding sites for two miRNAs located in the CDS with partial common nucleotide sequences. The *MAP3K14* gene is a target for five miRNAs. The binding sites of two of them are located in the CDS, two in the 3'UTR and one in the 5'UTR. In mRNA of *MAPT* gene, there are five miRNA binding sites in the 5'UTR and two in the 3'UTR. In mRNA of *MCM7* gene, three miRNA binding sites in the 5'UTR were identified. The *SMAD3* gene is a target for 12 miRNAs, the binding sites of eight miRNAs are located in the 5'UTR and four miRNAs in the 3'UTR. miR-15-11315-5p is fully complementary to its binding site.

In mRNA of *SOX4* gene, 23 miRNA binding sites were identified. Only two miRNAs are bind in the 5'UTR and five miRNAs in the 3'UTR. Three miRNAs are included in the cluster in the CDS with the beginning of miRNA binding sites at position 1291 nt. Three miRNAs are in clusters with a start at position 1482 nt and at position 1837 nt. Two miRNAs are bind at positions 1721 nt and 1723 nt.

In mRNA of *TGFB1* gene, 25 miRNA binding sites were identified. The first cluster constitutes the binding sites of four miRNAs beginning with 1 nt of 5'UTR. The next cluster consists of binding sites for five miRNAs in the 5'UTR starting at 209 nt. In 3'UTR, a cluster for 11 miRNAs with binding sites starting at position 2057 nt was identified. One miRNA has three binding sites and two miRNAs have two binding sites in this cluster. Two miRNAs are bound in the CDS with a nearest location of beginning of binding sites. The TGFB1 polymorphism has been associated with breast cancer risk inducing an increase in TGF-β1 cellular expression and elevating plasma TGF-β1 levels, which might suppress the immune regulatory activities of macrophages and increase the risk of breast cancer [31], although other authors suggest that lower levels of circulating TGF-β1 are associated with a higher metastatic risk and poor disease prognosis [32]. The *TNC* gene is a target for two miRNAs binding in CDS and 3'UTR.

Characteristics of the interaction of miRNAs with mRNAs of candidate genes of her2 subtype

The mRNA of *A4GALT* gene binds 15 miRNAs, two out of which have binding sites in the CDS and others in the 5'UTR (Table 5).

The free binding energy value varies from -119 kJ/mole to -129 kJ/mole. The starts of the binding sites of 13 miRNAs in the 5'UTR are located at a region from 9 nt to 41 nt, that is, the nucleotide sequences for miRNA binding are partially common. The presence of a such strong dependence of gene expression on miRNA both on the free interaction energy value and on the number of miRNA is difficult to explain. It seems that the mRNA of gene is unlikely to be translated with such control by miRNA. However, one must take into account that not all miRNAs can be synthesized simultaneously and their total concentration in the cell may be less than the concentration of mRNA. As a result, the protein can be synthesized depending on the ratio of concentrations of miRNA and mRNA. Another factor limiting the effect of miRNA is the presence in the cell of miRNA-free RISC complexes. It should be noted that about half of miRNAs are synthesized when the host gene is expressed from introns during the splicing process and this gene may not be expressed permanently or temporarily in the tissue cell.

mRNA of *ACSS2, ADAM17* and *AURKA* genes have binding sites for only one miRNA. However, if the concentrations of the respective miRNAs are comparable or exceed the concentration of mRNA, the effect of inhibition of mRNA translation will be significant.

Table 5 – Characteristics of miRNAs binding with mRNAs of candidate genes of the her2 subtype

A4GALT: miR-8-21445-5p, 9, -123, 89, 22; miR-1-356-5p, 12, -127, 87, 23; miR-9-23969-3p, 13, -127, 95, 21; miR-12-32764-3p, 19, -121, 85, 23; miR-19-30988-5p, 19, -123, 85, 23; miR-11-29077-3p, 28, -123, 88, 24; miR-4-11828-5p, 30, -119, 89, 22; miR-11-28656-5p, 32, -121, 86, 23; miR-1-356-5p, 33, -125, 86, 23; miR-2-8257-5p, 37, -125, 88, 23; miR-10-28986-3p, 39, -121, 86, 23; miR-20-22562-3p, 39, -129, 86, 24; miR-5-3563-5p, 39, -119, 86, 22; miR-17-40348-5p, 41, -121, 89, 23; miR-5-12460-5p, 457*, -123, 85, 24; miR-11-29324-3p, 995*, -121, 86, 23
ACSS2: miR-2-8257-5p, 231*, -123, 87, 23
ADAM10: miR-9-26506-3p, 165, -117, 95, 22; miR-5-15733-3p, 416, -132, 89, 24; miR-9-20317-3p, 416, -129, 87, 24
ADAM17: miR-20-43873-3p, 2378*, -121, 88, 23
AURKA: miR-9-22187-3p, 197, -115, 86, 23
BRCA2: miR-19-42224-5p, 25, -115, 93, 21; miR-22-45335-5p, 10821**, -113, 90, 23
BRIP1: miR-18-39953-5p, 7, -129, 90, 23; miR-16-20406-3p, 14, -125, 86, 23; miR-17-39440-3p, 4342**, -117, 86, 24; miR-20-43646-5p, 6567**, -119, 87, 24; miR-14-35161-5p, 6607**, -115, 87, 24
CDK2: miR-12-31544-5p, 116, -117, 86, 23
CDK6: miR-5-15733-3p, 258, -129, 87, 24; miR-12-30578-5p, 466, -123, 85, 24; miR-6-17811-3p, 483, -127, 86, 24; miR-15-36862-3p (2); 1900 ÷ 1906**, -115, 95, 23; miR-8-23986-3p, 7773**, -127, 88, 24
EPOR: miR-19-42218-3p, 79, -119, 89, 23; miR-16-20406-3p, 80, -125, 86, 23; miR-19-41131-3p, 80, -129, 90, 23; miR-22-46522-5p, 83, -121, 88, 22; miR-5-16562-3p, 173*, -119, 88, 24; miR-17-39313-3p, 340*, -125, 86, 24; miR-2-6809-5p, 922*, -123, 85, 25
EPO: miR-12-31979-3p, 12, -121, 89, 23; miR-2-8257-5p, 18, -123, 87, 23
ERBB3: miR-1-3554-3p, 105, -117, 86, 23; miR-1-163-3p, 114, -113, 93, 21; miR-3-8100-5p, 148, -125, 86, 24
FKBPL: miR-13-33774-5p, 35, -121, 86, 24; miR-20-45753-5p, 42, -119, 87, 22; miR-2-8257-5p, 49, -121, 85, 23; miR-3-4734-5p, 769*, -115, 89, 23
GTF2E1: miR-19-25044-3p, 1750**, -117, 87, 24
H2AFX: miR-20-45152-5p, 506**, -136, 91, 24; miR-5-3563-5p, 509**, -123, 89, 22; miR-1-1630-3p, 511**, -119, 89, 22; miR-10-27682-5p, 604**, -123, 85, 24; miR-1-3919-5p, 632**, -123, 89, 24; miR-6-12155-5p, 641**, -121, 88, 22; miR-1-2121-3p, 643**, -134, 85, 25; miR-17-40081-5p (2), 647÷ 724**, -125, 86, 23; miR-22-16963-5p, 683**, -121, 86, 22; miR-2-8257-5p, 688**, -123, 87, 23; miR-5-15432-3p, 688**, -121, 85, 23; miR-16-33136-3p, 827**, -123, 91, 22
KDM5D: miR-22-46603-5p, 2489*, -119, 87, 24; miR-9-22187-3p, 3065*, -119, 89, 23
MAPK3: miR-11-29461-3p, 103*, -123, 88, 23; miR-13-32613-3p, 110*, -121, 85, 24; miR-7-21142-5p, 113*, -121, 86, 23; miR-1-2802-3p, 1144*, -117, 93, 22; miR-2-8257-5p, 1381**, -123, 87, 23; miR-5-14114-5p, 1527**, -119, 86, 23
MAZ: miR-12-17092-3p, 16, -123, 89, 22; miR-18-41189-3p, 16, -134, 91, 23; miR-9-25917-3p, 26, -125, 88, 23; miR-11-29998-3p, 27, -127, 91, 23; miR-12-33610-3p, 27, -129, 87, 24; miR-13-32368-5p, 29, -121, 85, 23; miR-1-2372-3p, 43, -121, 85, 24; miR-14-36092-3p, 79, -121, 85, 23; miR-11-28204-5p, 107, -121, 90, 21; miR-14-31624-3p, 112, -127, 88, 24; miR-7-12728-5p, 114, -121, 92, 22; miR-16-36024-3p, 118, -125, 88, 23; miR-5-17008-3p, 363*, -125, 89, 23; miR-4-12861-5p, 372*, -119, 92, 22; miR-22-16963-5p, 373*, -121, 86, 22; miR-7-21249-3p, 377*, -121, 85, 23; miR-7-21068-3p, 433*, -125, 86, 24; miR-2-4733-3p, 439*, -121, 88, 22; miR-3-8100-5p (5), 457 ÷ 472*, -125 ÷ -138, 86 ÷ 94, 24; miR-7-16350-5p, 459*, -119, 93, 21; miR-2-6809-5p, 461*, -125, 87, 25; miR-19-44540-3p, 462*, -121, 85, 23; miR-2-3313-3p (3), 464 ÷ 467*, -136 ÷ -140, 85 ÷ 88, 25; miR-11-28656-5p, 470*, -121, 86, 23; miR-1-155-3p, 470*, -123, 89, 22; miR-3-7886-3p, 671*, -129, 90, 24; miR-19-21199-3p, 473*, -134, 85, 25; miR-20-43381-5p, 489*, -121, 92, 21; miR-4-11923-3p, 489*, -125, 94, 22; miR-14-31624-3p, 495*, -123, 85, 24; miR-15-33256-3p, 499*, -129, 87, 24; mir-1-2121-3p (4), 500 ÷ 615*, -134 ÷ -138, 85 ÷ 88, 25; miR-16-36024-3p, 500*, -125, 88, 23; miR-19-33623-3p (3), 500 ÷ 608*, -127 ÷ -134, 86 ÷ 90, 24; miR-X-13195-3p, 503*, -125, 88, 23; miR-9-27797-5p, 898*, -123, 87, 24; miR-2-7331-5p, 900*, -123, 89, 23; miR-13-35476-3p, 901*, -125, 97, 22; miR-8-23986-3p, 905*, -123, 85, 24; miR-X-48174-3p, 2072**, -127, 90, 24; miR-8-23986-3p, 2340**, -123, 85, 24
NHS: miR-19-44540-3p, 426*, -121, 85, 23; miR-1-2597-5p, 431*, -129, 87, 24; miR-2-3313-3p, 532*, -146, 92, 25; miR-10-13655-3p (2), 534 ÷ 537, -121 ÷ -123, 89 ÷ 91, 22; miR-15-32047-5p, 534*, -132, 90, 24; miR-22-46979-5p, 535*, -123, 89, 23; miR-3-8100-5p, 536*, -125, 86, 24; miR-19-21199-3p (3), 539 ÷ 543*, -134 ÷ -138, 85 ÷ 88, 25; miR-1-155-3p (2), 540 ÷ 546*, -127 ÷ -134, 92 ÷ 97, 22; miR-4-11421-3p, 541*, -121, 86, 23; miR-22-16963-5p, 543*, -121, 86, 22; miR-4-6496-3p, 543*, -121, 93, 21; miR-19-43966-3p, 544*, -123, 88, 23; miR-19-43329-3p, 608*, -123, 91, 24; miR-9-23270-3p, 630*, -129, 86, 24; miR-5-15733-3p, 674*, -127, 86, 24; miR-22-45834-5p, 676*, -121, 86, 23

NISCH: miR-9-20317-3p, 31, -127, 86, 24; miR-X-48174-3p, 31, -125, 88, 24; miR-7-21142-5p, 35, -121, 86, 23; miR-1-1852-5p, 38, -121, 86, 23; miR-19-43644-3p, 38, -123, 89, 23; miR-8-21978-5p, 41, -125, 88, 24; miR-16-20406-3p, 42, -127, 87, 23; miR-18-39953-5p, 43, -123, 85, 23; miR-22-46522-5p, 47, -123, 89, 22; miR-17-39313-3p, 2198*, -125, 86, 24; miR-1-2121-3p, 3421*, -134, 85, 25; miR-10-8412-5p, 3432*, -123, 85, 23
PARP1: miR-19-36095-3p, 1275*, -119, 90, 23
RAD21: miR-9-26042-5p, 57, -121, 89, 22; miR-18-39953-5p, 120, -125, 87, 23; miR-1-3919-5p, 180,-121, 88, 24; miR-12-31979-3p, 214, -119, 87, 23
RASSF1: miR-5-15432-3p, 63, -121, 85, 23; miR-14-36092-3p, 83, -121, 85, 23
RPLP2: miR-15-32047-5p, 68, -125, 86, 24; miR-19-43966-3p, 81, -121, 86, 23; miR-17-25894-5p, 328*, -125, 87, 24
RYR1: miR-19-28028-5p, 51, -136, 91, 24; miR-5-12460-5p, 56, -123, 85, 24; miR-5-6716-5p, 5291*, -121, 85, 23; miR-19-41131-3p (2), 12919 ÷ 12934*, -125 ÷ -127, 87 ÷ 88, 23; miR-13-32613-3p, 12929*, -123, 87, 24; miR-9-20317-3p, 12931 ÷ 12994*, -127 ÷ -132, 86 ÷ 89, 24; miR-12-33610-3p, 12934*, -127, 86, 24; miR-17-39593-3p, 12958*, -136, 89, 24; miR-20-44980-3p, 13015*, -121, 86, 23; miR-15-38560-5p, 13053*, -123, 85, 24; miR-12-33610-3p, 13174*, -132, 89, 24; miR-5-15733-3p, 13174*, -127, 86, 24; miR-22-45834-5p, 13176*, -123, 88, 23; miR-X-48265-3p, 13415*, -129, 87, 24
STAR: miR-5-13733-5p, 1001*, -115, 89, 23
TIMP3: miR-14-15069-5p, 659, -119, 87, 22; miR-6-17519-3p, 1102, -121, 90, 22; miR-11-27076-3p, 3153**, -119, 86, 24; miR-5-15026-5p, 3176**, -119, 86, 23
TNF: miR-20-42898-3p, 230*, -121, 92, 23
Notes: miRNA; in brackets, the number of binding sites; the beginning of the binding site; without * – 5'UTR; * – CDS; ** – 3'UTR; binding energy, kJ / mole; the value ΔG / ΔGm,%; length, nt

In mRNA of *ADAM10* metallopeptidase gene, the binding sites of three miRNAs are located in the 5'UTR. The starts of binding sites of two miRNAs are coinciding. miR-5-15733-3p binds with the highest free binding energy equal to -132 kJ/mole. These data indicate the dependence of the expression of *ADAM10* gene on miRNAs. Note that when mutations occur in the binding site of two miRNAs, the gene leaves their control and can become an oncogene. It was found that the expression of *ADAM10* gene is higher in the tumor than in normal tissue and suppression of gene expression significantly reduces the *in vitro* migration of cells [33]. Therefore, the gene can serve as a marker for the HER2 subtype and the therapeutic target [34].

The mRNA of *BRCA2* gene has one binding site in the 5'UTR and one site in the 3'UTR. The free energy of miRNA binding with mRNA varies from -113 kJ/mole to -115 kJ/mole, which indicates their weak interaction. Interestingly, the CDS mRNA of *BRCA2* gene has more than 10,000 nucleotides and does not contain any miRNA binding site, which also reflects a weak dependence of the expression of *BRCA2* gene on miRNA. Since the *BRCA2* gene is considered as a tumor suppressor [35; 36], its weak dependence on the direct effect of miRNA reflects the preservation of its function and only mutations in the gene can affect on its function as a tumor suppressor.

The *BRIP1* gene is a target for five miRNAs, of which three binding sites are located in the 3'UTR and two sites in the 5'UTR (Table 5). miR-18-39953-5 and miR-16-20406-3p bind with mRNA of *BRIP1* gene with a free energy value varies from -129 kJ/mole to -125 kJ/mole, respectively. The free energy value of the interaction of miRNA in the 3'UTR is significantly lower: from -115 kJ/mole to -119 kJ/mole. *BRIP1* is a candidate gene for the development of BC [37].

The mRNA of *CDK2* gene has only one miRNA binding site in the 5'UTR. The *CDK6* gene is a target for five miRNAs. Three miRNA binding sites are located in two regions in the 5'UTRs: 258 nt (one miRNA) and 466 nt and 483 nt (two miRNAs). MiRNAs which bind in the 3'UTR are also bind in two regions: from 1900 nt to 1,906 nt (two sites for miR-15-36862-3p) and at 7773 nt. The *CDK6* gene encodes a cyclin dependent kinase. CDK4/6 can be used as a target for inhibition of oncogenesis.

The *EPOR* gene can affect on the growth of the tumor. The *EPOR* gene serves as a target for four miRNAs that bind in the 5'UTR mRNA in one site from 79 nt to 83 nt. Four miRNAs interact with mRNA in the CDS. The free energy of miRNA binding with mRNA varies from -117 kJ/mole to -129 kJ/mole. EPOR expression has been detected in several cancer forms [38].

The *EPO* gene is involved in the proliferation of BC cells. The mRNA of *EPO* gene has two binding sites in one 5'UTR region. The mRNA of *ERBB3* gene has three binding sites in the 5'UTR. The mRNA of

FKBPL gene has binding sites for three miRNAs in one 5'UTR region and one site in the CDS (Table 5).

The mRNA of *H2AFX* gene binds 12 miRNAs that are located in the 3'UTR. In the region from 632 to 688 nt, the binding sites of seven miRNAs are located arranged with nucleotide sequences. The *H2AFX* gene was considered as a prognostic marker of breast cancer.

The *MAPK3* gene participates in proliferation, differentiation, cell cycle. At the beginning of MAPK3 protein, there is the oligopeptide AAAAAQGGGGGE, which is encoded by the miR-11-29461-3p, miR-13-32613-3p and miR-7-21142-5p binding sites. The sites of these miRNAs binding with mRNA of *MAPK3* gene are located in one cluster in the CDS and two miRNAs are bound in the 3'UTR.

Transcription factor MAZ is associated with participation in the development of BC. The protein contains oligopeptides: AAAAAAAAAAAA, PPPPPP, GAGGGGG. 11 miRNA bind in the 5'UTR with nucleotide sequence overlap in two sites from 16 nt to 47 nt and from 107 to 118 nt. The binding sites of six miRNAs form a cluster from 495 nt in the CDS. Another cluster of binding sites for nine miRNAs starts at 457 nt. miR-18-41189-3p, miR-3-8100-5p, miR-2-3313-3p, miR-19-21199-3p, miR-1-2121-3p, miR-19-33623-3p bind with mRNA of *MAZ* gene with free energy from -132 kJ/mole to -140 kJ/mole.

The mRNA of *NHS* gene binds with 18 miRNAs only in the CDS, the binding sites are located in three regions: from 426 nt to 431 nt (2 miRNA), from 532 nt to 544 nt (12 miRNA) and from 608 to 674 nt (4 miRNA). The free energy of interaction is large in all binding sites varies from -121 kJ/mole to -146 kJ/mole. miR-19-21199-3p binds in three sites with a free energy value equal to -134 kJ/mole ÷ -138 kJ/mole. Naturally, the question arises again how this gene can be expressed with such a number of miRNAs that potentially can interact with mRNA of *NHS* gene. This gene has complex pattern of temporally and spatially regulated expression, which, together with the pleiotropic features of NHS, suggests that this gene has key functions in the regulation of eye, tooth, brain, and craniofacial development [39].

The synthesis of NISCH protein is lower in BC than in normal tissue, while the overexpression induces apoptosis, inhibits cell migration and invasion, decreases tumor growth and metastases. A gene can be a potential target for breast cancer therapy. In the NISCH protein, there are two domains consisting of polyglutamic acid, which are encoded by miRNA binding sites. Nine sites of miRNA binding with mRNA of *NISCH* gene are located in the same 5'UTR cluster with beginning from 31 nt to 47 nt. The free energy of the interaction of miRNA with mRNA varies from -121 kJ/mole to -127 kJ/mole, which indicates a strong binding of these molecules. Two binding sites for two miRNAs are available in the CDS. miR-1-2121-3p binds with mRNA of *NISCH* gene with free interaction energy equal to -134 kJ/mole. In general, all miRNAs can significantly reduce the expression of the *NISCH* gene even in the absence of several miRNAs. Expression of NISCH significantly negatively correlated with estrogen receptor status [40].

The mRNAs of *RAD21* and *RASSF1* genes have miRNA binding sites only in the 5'UTR. The mRNA of *RYR1* gene has binding site for 13 miRNAs, of which 11 are bound in the CDS and two in the 5'UTR [41]. In all sites the free energy of binding fluctuates from -121 kJ/mole to -136 kJ/mole. There are several domains of polyglutamic acid in the protein RYR1. It is described the relationship of *RYR1* gene with the calcification at BC is and the gene is proposed as a target for BC therapy.

The *TIMP3* gene is a target for four miRNAs, two of which bind in the 5'UTR and two in the 3'UTR with a free energy value from -119 kJ/mole to -121 kJ/mole [42]. The *TIMP3* gene regulates apoptosis. The *TNF* gene tumor necrosis factor regulates apoptosis.

Based on the results of establishing the characteristics of the interaction of miRNAs with mRNAs of studied candidate genes of different subtypes of the breast cancer, several features of these interactions can be identified.

The studied genes have a different number of miRNAs that bind with mRNAs. The largest number of miRNAs bind with mRNA of *FOXA1* (56 miRNA), *MAZ* (41 miRNA), *TGFB1* (25), *SOX4* (23 miRNA) genes. Sites for miRNA binding with mRNA are predominantly clustered. That is, in a small region of mRNA, several different miRNAs can bind with overlapping nucleotide sequences of binding sites. These sites more often are located in the 5'UTR at the triple negative subtype, in the CDS at the luminal A, B subtype and in the 3'UTR at the her2 subtype. This indicates a non-random distribution of miRNA binding sites throughout the mRNA sequence. For example, in mRNA of *GTF21RD1* and *HMGA2* genes, miRNA binding sites are located only in the 5'UTR, in mRNA of *NHS* gene only in the CDS and in mRNA of *H2AFX* gene only in the 3'UTR (Tables 4, 5).

A role of a large number of miRNA binding sites in mRNA of one gene is still not clarified. It is likely that such a gene is necessary for life, but it should be poorly expressed, at least in most organs. An increase in its expression leads to various pathologies. Above mentioned *FOXA1, MAZ* and *SOX4* genes, having in their mRNAs a large number of binding sites for miRNAs, are transcription factors and poorly expressed in the norm. The *TGFB1* gene is functionally associated with the SMAD transcription factor family.

The miRNA binding sites are more often located in the 5'UTR, then in the CDS and less in the 3'UTR. This preference can be explained by the biological role of miRNA – stopping the translation process. It is energetically more advantageous to stop protein synthesis at the beginning of the process, than to interrupt it later, resulting in abortive proteins, the synthesis of which was spent energy.

Subtypes of breast cancer differ in the candidate genes, whose mRNAs in the triple negative subtype bind miRNAs preferably in the 5'UTR. At the luminal A,B subtype, miRNAs is preferably bind in the CDS and, in the her2 subtype, miRNAs is preferably bind to 3'UTR (Tables 4, 5). Based on this, one of the functions of 5'UTR and 3'UTR is the need to include miRNA binding sites. For example, genes with an extended nucleotide sequence contain more miRNA binding sites.

Selection of associations of miRNA with their target genes for the diagnosis of subtypes of breast cancer is a complex task, since there are from one to several dozens of miRNA which influence on each gene. Of these, the most specific should be selected to reduce the likelihood of including miRNAs, which may have other target genes. To do this, it is necessary to determine the expected impact of each candidate miRNA on all genes of the human genome.

Conclusion

In the present study, we performed a bioinformatics analysis of interaction of miRNA with mRNA of breast cancer candidate genes. We selected 33 genes specific for the triple negative subtype, 16 genes specific for the luminal A, B subtype and 28 genes specific for the her2 subtype. It was identified the features of interactions of these genes with miRNAs. The miRNA binding sites are more often located in the 5'UTR, then in the CDS and less in the 3'UTR. Genes *CBL, DRAM1, FGFR2, LAMC1, MMP2, NTRK2, PFN1, PTGS2, PRRT2, RAB5A* responsible for the triple negative subtype of breast cancer can be characterized as candidate target genes for miRNAs which binding sites are located in the 5'UTR of mRNA. For candidate genes *JHDM1D, RUNX1* miRNAs binding sites are located in the CDS and for *RUNX1* – in the 3'UTR of mRNA. Genes *FOXA1, GTF2IRD1, HMGA2, ITGA6, MAPT, SMAD3, TGFB1* responsible for the luminal A,B subtype of breast cancer can be characterized as candidate target genes for miRNAs which binding sites are located in the 5'UTR of mRNA. For candidate genes *FOXA1, ITGB1, SOX4* miRNAs binding sites are located in the CDS and for *SMAD3, TGFB1* – in the 3'UTR of mRNA. For the her2 subtype of breast cancer, candidate genes for binding miRNAs in the 5'UTR are *A4GALT, EPOR, MAZ, NISCH, RAD21*. Candidate genes for miRNA binding in the CDS are *EPOR, MAPK3, MAZ, NHS, RYR1* and in the 3'UTR – *H2AFX*.

In summary, our study provides associations of the above miRNAs and their target genes that can be used to develop a method for diagnosis subtypes of breast cancer.

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