

Original Article

Differential expression profile analysis of miRNAs with HER-2 overexpression and intervention in breast cancer cells

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Abstract: Receptor tyrosine-protein kinase erbB-2 (HER-2) as a member of the human epidermal growth factor receptor family, plays a key role in the development and the progression of certain aggressive types of breast cancer, and has become an important biomarker and target of therapy for breast cancer. Recently, liquid biopsy biomarker of tumor cells, such as miRNAs, has been validated and approved as a useful prognostic method for cancer, and however of which in breast cancer is not fully elucidated. To address it, human breast cancer cell stains of MCF-7, BT474, MCF-7/HER-2, BT474/HER-2 (siRNA) were chosen, and the mRNA and protein expression level of HER-2 was analyzed by real-time quantitative polymerase chain reaction (RT-qPCR) and Western Blot respectively. Subsequently, the differential expression profile of miRNA was analyzed by miRNA gene-chip with bioinformatics analysis. As expected, the mRNA and protein expression level of HER-2 was significantly increased with HER-2 overexpression in MCF-7 cells, and significantly decreased with HER-2 intervention in BT474 cells. Significantly, a total of 217 miRNAs with 123 up-regulated miRNAs and 94 down-regulated miRNAs was identified with HER-2 overexpression in MCF-7 cells, and also a total of 374 miRNAs with 121 up-regulated miRNAs and 253 down-regulated miRNAs were identified with HER-2 intervention in BT474 cells. Wherein, 10 miRNAs were up-regulated with HER-2 overexpression in MCF-7 cells, and down-regulated with HER-2 intervention in BT474 cells synchronously, and 17 miRNAs were down-regulated with HER-2 overexpression in MCF-7 cells, and up-regulated with HER-2 intervention in BT474 cells synchronously, and the expression level of hsa-miR-877-3p was consistent with gene-chip results, and not only provided a significant reference on the differential expression of miRNAs in breast cancer cells and its culturing supernatant, and also provided a significant potentially liquid biopsy biomarker for breast cancer in clinic.

Keywords: Breast cancer, miRNA, receptor tyrosine-protein kinase erbB-2, overexpression, intervention, gene-chip, differential expression

Introduction

Breast cancer is a kind of cancer of which originates from breast tissue of male and female, and often occurs in female with the signs of a lump in the breast, a change in breast shape and size, dimpling of the skin, fluid coming from the nipple, and a red scaly patch of skin, and often combines with bone pain, swollen lymph nodes, shortness of breath, and yellow skin following its distant spread [1-4]. Several risk factors are associated with its arising, such as female sex, obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age

at first menstruation, older age, and family history, etc [5-8]. Several receptors on cell surface and in cell cytoplasm and nucleus of breast cancer cells were reported, including estrogen receptor (ER) [9-11], progesterone receptor (PR) [12-14], and tyrosine-protein kinase erbB-2 receptor (HER-2) [15-17].

HER-2 is a member of human epidermal growth factor receptor family, and could encode a tyrosine kinase-like transmembrane glycoprotein with a molecular weight of 185 kDa, and highly expresses in several cancer cells, such as breast cancer, gastric cancer, and salivary duct carcinomas, etc [17-20]. In breast cancer cells,

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HER-2 was mainly located in the cell membrane to act as a tumorigenesis, and could maintain and/or accelerate the growth, the invasion, the angiogenesis of tumor cells, and also inhibit cell apoptosis and damage tissue barrier, and its over-expression implies an adverse biological behavior and prognosis [21-25]. Therefore, how to effectively inhibit the over-expression of HER-2 in breast cancer cells has been become a hot topic, and logically microRNA (miRNA) was chosen as a potentially target for inhibiting of the expression of HER-2 in breast cancer cells due to its down-regulated characteristic for target gene [26-28].

miRNAs are endogenous, non-coding, small RNAs of 21-25 nucleotides, and are widely distributed in tissues and organs of different species [29-31]. miRNAs strongly affect their target genes by forming RNA-induced silencing complexes (RISCs) that partially bind to the 3' untranslated region (3'-UTR) of target genes to induce mRNA degradation or to inhibit mRNA translation [32, 33]. Recently, as a variety of studies have documented, several miRNAs were closely associated with HER-2 in breast cancer cells, such as hsa-miR-205 [33], hsa-miR-497 [34], hsa-miR-125a/hsa-miR-125b [35], hsa-miR-331-3p [36-38], hsa-miR-23b [39], hsa-miR-27b [40], and hsa-miR-21 [41], etc. However, the detailed differential expression profile of miRNA in breast cancer cells with HER-2 overexpression and intervention has been no reported so far. Based on it, the MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cell strains were chosen, and the differential expression of miRNAs were identified by gene-chip and bioinformatics analysis, and several significantly up-regulated and down-regulated miRNAs were screened, and exhibited a significant application value on the treatment of breast cancer in clinic.

Materials and methods

Cell strains and culturing

The MCF-7 and BT474 cell strains were kindly presented by professor Zhu Tao of the University of Science and Technology of China, and resurrected from liquid nitrogen using Roswell Park Memorial Institute-1640 medium (RPMI-1640, Hyclone, USA) with 10% (v/v) fetal bovine serum, and cultured to the logarithmic phase in

a carbon dioxide (CO₂)-incubator with 5% CO₂ at 37°C. Similarly, MCF-7/HER-2 cell strain of which was over-expressed HER-2 gene, and BT474/HER-2 (siRNA) cell strain of which was intervened HER-2 gene, was kindly presented by professor Liu Jing of the University of Science and Technology of China, and resurrected from liquid nitrogen using Dulbecco minimum essential high glucose medium (DMEM, Hyclone, USA) with 10% (v/v) fetal bovine serum and antibiotics of G418 (final concentration: 0.5 mM), and cultured to the logarithmic phase in a CO₂-incubator with 5% CO₂ at 37°C.

Total RNA extraction of MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cells

The above-mentioned MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cells were diluted to 2×10^6 cell/ml, and inoculated to a T-25 cm² flask, and separately cultured in a CO₂-incubator with 5% CO₂ at 37°C till 80% confluence, and cells were collected to extract the total RNA according to the manufacturer's instructions of a Total RNA Extraction Kit (Tiagen, Beijing, China), and the purity and the concentration of total RNA was confirmed by ultraviolet visible spectrophotometer (Bio-Rad, USA). After then, the total RNA was reversely transferred with a Reverse Transcription Kit (Toyobo, Japan) according to the manufacturer's instructions. The reaction mixture, including 10 µL $2 \times$ loading buffer, 1.2 µL oligo (dT), 2 µL RNA, 0.2 µL MMLV, and 6.6 µL DEPC ddH₂O, was prepared and reacted at 65°C for 30 min, followed by 42°C for 30 min, and then by 85°C for 10 min.

Real time quantitative polymerase chain reaction (RT-qPCR) assay

The above-extracted of a total of 100 ng cDNA was used as a template in a RT-qPCR reaction using SYBR[®] Premix Ex TaqTM kit (TAKARA, Japan), according to the manufacturers' instructions. The reaction mixture, including 12.5 µL $2 \times$ Primer Taq Mix, 1 µL forward primer, 1 µL reverse primer, 1 µL cDNA, and 9.5 µL ddH₂O, was prepared and performed RT-qPCR according to the program: one cycle of 94°C for 5 min; 40 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 30 s, using primers as following: HER-2: pU: AGTACCTGGGTCTGGACGTG-3' and pD: 5'-CTGGGAAGCAAGCAGGAAG-3'; β-actin: pU:

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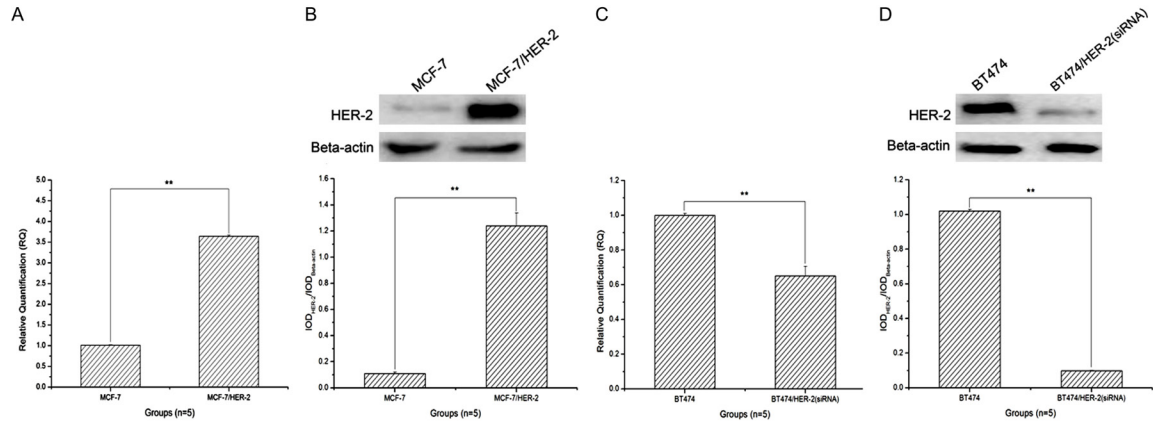


Figure 1. The mRNA and protein expression level assay of the over-expressed and the intervened HER-2 in MCF-7 and BT474 cells by RT-qPCR and Western Blot, and histogram analysis. A. The mRNA expression level assay of the over expressed HER-2 in MCF-7 by RT-qPCR; B. The protein expression level assay of the over expressed HER-2 in MCF-7 by Western blot, and histogram analysis; C. The mRNA expression level assay of the over expressed HER-2 in BT474 by RT-qPCR; D. The protein expression level assay of the over expressed HER-2 in BT474 by Western blot, and histogram analysis. The images indicated that the mRNA and protein expression level of the over-expressed HER-2 was significantly increased (**: $P < 0.01$, compared to MCF-7 cells alone), and the intervened HER-2 was significantly decreased (**: $P < 0.01$, compared to BT474 cells alone).

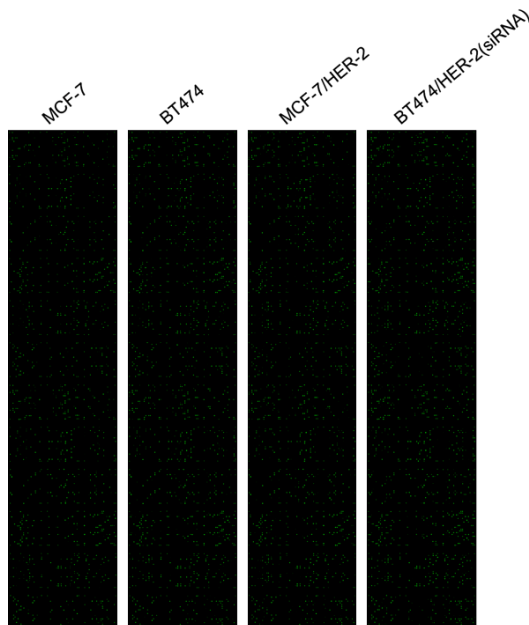


Figure 2. The fluorescent hybridization signal of miRNAs in breast cancer cells. The images indicated the obvious fluorescent hybridization signal of miRNAs was displayed in breast cancer cells.

5'-AGCCATGTACGTAGCCATCC-3' and pD: 5'-CTCTCAGCTGTGGTGGTGAA-3'. The results were analyzed using the SDS 1.41 software (Applied Biosystems, USA) based on $2^{-\Delta\Delta Ct}$, and histogram analysis using the Origin 9.4 software (<http://www.originlab.com/>).

Western blot assay

The above-mentioned MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) were diluted to 2×10^6 cell/ml, and inoculated to a T-25 cm^2 flask, and cultured in a CO_2 -incubator with 5% CO_2 at 37°C till 80% confluence, and total proteins were extracted to measure the concentration by a BCA protein quantification kit (Thermo, USA), and about 35 mg total proteins was fractionated by electrophoresis through 12.5% polyacrylamide gels, and transferred to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare) following the manufacturers' instructions. The membrane was probed with the first antibody, anti-HER-2 monoclonal antibody (ab16901, Abcam, USA) and anti- β -actin monoclonal antibody (ab8226, Abcam, USA) for 1.5 hr at room temperature (RT). Afterward, the membrane was incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000 in TBST; Beijing Golden Bridge Biotechnology Company Ltd, China) at RT for 1 hrs. The chemiluminescence luminol reagent (ZKP-C150044-1, Suzhou Zeke biotech Co., LTD, Jiangsu, China) were used to develop the immune-labeled bands on X-ray film, and the optical density of the bands was quantified using the ImageJ 1.46 software (<http://rsb.info.nih.gov/ij/download.html>), and a histogram was generated using the Origin 9.5 software (<http://www.originlab.com/>).

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Table 1. The up-regulated miRNAs of MCF-7 cells with HER-2 gene overexpression

ID	Name	Fore Ground		Fore Ground/Back Ground		Normalized		Fold change
		E	C	E	C	E	C	
169320	hsa-miR-4468	134.50	2729.50	42.50	2130.00	0.0594	3.5708	60.1580
17358	ebv-miR-BART16	137.00	2176.00	36.50	1621.50	0.0510	2.7184	53.3245
147595	hsa-miR-3178	174.00	2814.00	56.50	2215.00	0.0789	3.7133	47.0574
148377	hsa-miR-3653	200.00	3796.50	97.50	3276.00	0.1362	5.4920	40.3313
42522	ebv-miR-BART19-3p	1529.50	47393.00	1423.50	46810.00	1.9881	78.4744	39.4715
42524	hsa-miR-21-3p	151.00	2192.00	56.50	1665.00	0.0789	2.7913	35.3727
42865	hsa-miR-181a-5p	229.00	3886.50	125.50	3313.50	0.1753	5.5549	31.6917
168973	hsa-miR-1268b	105.00	872.00	13.50	343.50	0.0189	0.5759	30.5419
168682	hsa-miR-4502	110.00	711.50	7.50	189.00	0.0105	0.3168	30.2484
168971	hsa-miR-4449	165.00	2060.00	68.00	1516.50	0.0950	2.5423	26.7692
30687	hsa-miR-93-5p	327.50	5317.00	238.50	4795.50	0.3331	8.0394	24.1350
148000	hsa-miR-3195	156.50	1461.00	47.50	908.50	0.0663	1.5231	22.9580
10946	hsa-miR-141-3p	578.50	9061.50	485.00	8553.50	0.6774	14.3395	21.1692
10964	hsa-miR-155-5p	133.50	1139.00	41.00	602.00	0.0573	1.0092	17.6244
17427	hsa-miR-200c-3p	1204.50	16108.00	1110.00	15577.00	1.5503	26.1140	16.8447
42827	hsa-miR-652-3p	119.00	706.00	13.50	188.00	0.0189	0.3152	16.7158
31026	hsa-miR-101-3p	149.00	1279.00	50.50	702.50	0.0705	1.1777	16.6977
17561	ebv-miR-BART6-3p	302.00	3397.00	211.00	2903.00	0.2947	4.8667	16.5146
169363	hsa-miR-320c	187.50	1583.50	79.00	1030.50	0.1103	1.7276	15.6575
147817	hsa-miR-3196	129.50	812.00	19.50	247.00	0.0272	0.4141	15.2042
27533	hsa-miR-320a	165.50	1437.00	72.50	897.50	0.1013	1.5046	14.8593
13171	hsa-miR-429	126.50	909.00	35.00	409.50	0.0489	0.6865	14.0439
17854	hsa-miR-106b-3p	200.50	1735.50	103.00	1172.50	0.1439	1.9656	13.6640
10986	hsa-miR-193a-3p	127.00	898.50	31.00	351.00	0.0433	0.5884	13.5909
169420	hsa-miR-193b-5p	98.50	660.00	8.50	93.50	0.0119	0.1567	13.2037
19596	hsa-miR-30d-5p	126.00	866.50	30.00	303.50	0.0419	0.5088	12.1434
168586	hsa-miR-34a-5p	158.50	1165.50	66.50	660.00	0.0929	1.1065	11.9131
168819	hsa-miR-200a-3p	212.50	1568.50	105.50	1018.50	0.1473	1.7075	11.5881
42682	hsa-miR-25-3p	322.00	2674.00	227.50	2110.50	0.3177	3.5381	11.1354
148657	hsa-miR-381-5p	279.00	2166.00	185.50	1658.50	0.2591	2.7804	10.7318
148420	hsa-miR-3607-3p	500.00	4159.50	396.00	3539.00	0.5531	5.9329	10.7272
147805	hsa-miR-3183	324.50	2563.00	225.00	2010.50	0.3142	3.3705	10.7257
147165	hsa-let-7b-5p	262.50	2022.00	156.50	1391.50	0.2186	2.3328	10.6726
169123	hsa-miR-3184-3p	142.50	1054.00	58.00	513.00	0.0810	0.8600	10.6168
168776	hsa-miR-4795-3p	1378.00	11383.50	1282.50	10772.00	1.7912	18.0587	10.0819
11134	hsa-miR-502-5p	146.50	909.50	45.00	328.00	0.0628	0.5499	8.7491
10972	hsa-miR-181b-5p	254.50	1724.00	166.50	1206.50	0.2325	2.0226	8.6979
46324	hsa-miR-320b	263.50	1692.00	158.00	1117.00	0.2207	1.8726	8.4859
42570	hsa-miR-194-3p	112.00	688.50	22.00	143.50	0.0307	0.2406	7.8295
19582	hsa-miR-106b-5p	425.50	2629.50	324.00	2073.00	0.4525	3.4753	7.6799
168637	hsa-miR-3940-5p	827.00	4930.50	724.50	4383.50	1.0119	7.3487	7.2625
145984	ebv-miR-BART2-3p	106.00	615.00	12.50	73.00	0.0175	0.1224	7.0100
169409	hsa-miR-4286	300.50	1682.00	200.00	1161.00	0.2793	1.9464	6.9679
42744	hsa-miR-23a-3p	563.50	3256.50	462.50	2678.00	0.6459	4.4895	6.9503
168868	hsa-miR-5681b	1183.50	6710.50	1071.50	6138.00	1.4965	10.2900	6.8760
11020	hsa-miR-22-3p	283.00	1655.50	193.50	1104.50	0.2703	1.8516	6.8515
42702	hsa-miR-30c-1-3p	606.00	3365.50	508.00	2803.50	0.7095	4.6999	6.6243
42739	hsa-miR-339-5p	227.00	1191.00	125.00	681.50	0.1746	1.1425	6.5442
168806	hsa-miR-4659a-3p	110.00	648.50	10.00	54.50	0.0140	0.0914	6.5418

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169399	hsa-miR-4750-5p	475.00	2453.00	358.00	1881.50	0.5000	3.1542	6.3085
17608	hsa-miR-425-5p	113.00	593.00	10.00	50.00	0.0140	0.0838	6.0017
168605	hsa-miR-4653-3p	1456.50	7201.00	1355.50	6634.50	1.8932	11.1224	5.8750
27565	hsa-miR-423-5p	1075.50	5336.00	963.50	4709.50	1.3457	7.8952	5.8671
17418	kshv-miR-K12-4-3p	166.00	864.00	66.50	324.50	0.0929	0.5440	5.8573
10987	hsa-miR-193b-3p	382.00	1911.50	295.50	1415.00	0.4127	2.3722	5.7478
145820	hsa-let-7c-5p	207.50	1077.00	111.00	531.00	0.1550	0.8902	5.7421
42514	hsa-miR-937-3p	195.00	1007.00	98.50	470.00	0.1376	0.7879	5.7275
46336	hsa-miR-1284	1241.50	5909.50	1147.00	5361.50	1.6020	8.9883	5.6108
42951	ebv-miR-BHRF1-2-3p	212.00	1084.50	124.00	565.50	0.1732	0.9480	5.4741
10998	hsa-miR-19b-3p	186.50	960.00	91.00	415.00	0.1271	0.6957	5.4741
27720	hsa-miR-15a-5p	230.50	1178.00	139.50	636.00	0.1948	1.0662	5.4725
169238	hsa-miR-4654	275.50	1301.00	164.50	737.00	0.2297	1.2355	5.3778
169376	hsa-miR-5701	1727.00	7795.50	1634.50	7264.50	2.2828	12.1785	5.3349
11004	hsa-miR-203a	229.00	1130.50	121.50	536.00	0.1697	0.8986	5.2953
17546	hsa-miR-585-3p	493.50	2270.50	395.00	1741.00	0.5517	2.9187	5.2906
10928	hsa-miR-125a-5p	1130.50	4968.50	1032.50	4407.50	1.4420	7.3889	5.1239
146111	hsa-miR-767-5p	1778.00	7594.00	1674.50	6993.50	2.3387	11.7242	5.0132
10985	hsa-miR-191-5p	200.00	953.50	111.50	460.00	0.1557	0.7712	4.9521
169260	hsa-miR-4436b-3p	152.50	706.00	53.50	204.00	0.0747	0.3420	4.5770
9938	hsa-let-7i-5p	218.50	994.00	116.50	433.00	0.1627	0.7259	4.4613
168686	hsa-miR-4768-5p	279.00	1217.00	183.50	656.50	0.2563	1.1006	4.2944
168709	hsa-miR-4429	430.50	1744.50	338.50	1197.00	0.4728	2.0067	4.2446
10967	hsa-miR-16-5p	641.00	2429.50	535.50	1861.00	0.7479	3.1199	4.1715
147162	hsa-let-7a-5p	401.50	1596.00	309.00	1070.50	0.4316	1.7946	4.1584
147506	hsa-miR-21-5p	4100.00	14348.00	4007.50	13832.00	5.5971	23.1886	4.1430
168836	hsa-miR-4496	195.50	859.50	97.00	331.00	0.1355	0.5549	4.0960
168802	hsa-miR-4516	440.50	1681.00	325.00	1100.50	0.4539	1.8449	4.0645
169204	hsa-miR-4709-3p	796.50	2759.50	687.00	2195.00	0.9595	3.6798	3.8351
169079	hsa-miR-4667-5p	846.50	2887.00	734.00	2327.00	1.0251	3.9011	3.8054
42887	hsa-miR-331-3p	277.50	1078.00	170.50	521.50	0.2381	0.8743	3.6714
147186	hsa-miR-200b-3p	877.50	3008.00	790.00	2379.50	1.1034	3.9891	3.6154
168619	hsa-miR-1260b	1580.00	5016.00	1473.00	4432.50	2.0573	7.4308	3.6120
13143	hsa-miR-301a-3p	153.50	718.50	59.50	178.50	0.0831	0.2992	3.6010
46215	hsa-miR-1301-3p	231.00	922.50	122.00	365.50	0.1704	0.6127	3.5961
169188	hsa-miR-4443	2672.00	7922.00	2560.00	7336.50	3.5754	12.2992	3.4399
46869	hsa-miR-1258	262.00	1030.50	152.50	435.00	0.2130	0.7293	3.4239
10919	hsa-miR-103a-3p	648.50	2158.50	538.50	1522.00	0.7521	2.5516	3.3926
168844	hsa-miR-4532	1441.50	4326.50	1338.50	3748.50	1.8694	6.2842	3.3616
169395	hsa-miR-4484	795.00	2492.00	700.50	1944.50	0.9784	3.2598	3.3320
11078	hsa-miR-365a-3p/hsa-miR-365b-3p	291.50	1047.50	205.00	546.00	0.2863	0.9153	3.1970
17493	hsa-miR-622	338.00	1141.50	246.00	637.50	0.3436	1.0687	3.1106
148327	hsa-miR-3651	218.00	862.50	122.00	315.50	0.1704	0.5289	3.1041
169110	hsa-miR-4497	881.00	2523.50	793.50	2022.00	1.1082	3.3898	3.0587
145968	hsa-let-7d-5p	356.00	1225.00	261.00	664.50	0.3645	1.1140	3.0560
145745	hsa-miR-335-3p	3293.00	8624.00	3184.50	8031.50	4.4476	13.4644	3.0273
146158	hsa-miR-3202	1050.50	2936.50	954.50	2376.00	1.3331	3.9832	2.9879
168631	hsa-miR-4723-5p	250.00	944.50	151.50	372.00	0.2116	0.6236	2.9474
169274	hsa-miR-5196-3p	225.00	884.50	139.00	338.50	0.1941	0.5675	2.9231
169015	hsa-miR-4454	4817.50	11954.00	4729.00	11478.00	6.6047	19.2422	2.9134
13132	hsa-miR-519e-5p	575.50	1730.00	465.00	1116.50	0.6494	1.8718	2.8821
169412	hsa-miR-1260a	295.50	1030.50	177.00	421.50	0.2472	0.7066	2.8584

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147631	hsa-miR-4258	842.00	2323.50	754.50	1785.50	1.0538	2.9933	2.8406
148085	hsa-miR-3687	826.00	2283.00	716.00	1684.50	1.0000	2.8240	2.8240
169082	hsa-miR-1275	591.00	1732.50	495.00	1164.00	0.6913	1.9514	2.8226
42516	kshv-miR-K12-12-5p	221.50	932.50	133.50	307.00	0.1865	0.5147	2.7603
147199	hsa-miR-27b-3p	174.50	786.00	66.00	151.50	0.0922	0.2540	2.7553
169330	hsa-miR-23b-3p	951.50	2415.00	866.00	1927.50	1.2095	3.2313	2.6716
169185	hsa-miR-5187-3p	937.00	2381.00	849.00	1887.50	1.1858	3.1643	2.6686
168928	hsa-miR-4431	1170.00	2950.00	1064.00	2355.00	1.4860	3.9480	2.6568
11164	hsa-miR-519e-3p	231.00	869.00	129.50	283.00	0.1809	0.4744	2.6231
147794	kshv-miR-K12-8-3p	173.00	732.00	77.00	164.00	0.1075	0.2749	2.5566
168696	hsa-miR-4739	681.50	1715.00	585.00	1236.50	0.8170	2.0729	2.5371
45764	hsa-miR-302e	210.00	751.00	121.00	254.00	0.1690	0.4258	2.5197
148668	hsa-miR-378a-3p	551.50	1428.00	435.00	913.00	0.6075	1.5306	2.5193
147600	hsa-miR-4292	668.50	1721.50	572.00	1187.50	0.7989	1.9908	2.4920
148504	hsa-miR-874-5p	287.50	939.50	192.50	398.50	0.2689	0.6681	2.4848
146112	hsa-miR-30b-5p	163.50	642.50	67.00	136.00	0.0936	0.2280	2.4365
169358	hsa-miR-4417	591.50	1582.50	485.50	971.00	0.6781	1.6278	2.4007
169199	hsa-miR-4518	260.00	870.00	167.00	329.50	0.2332	0.5524	2.3683
168933	hsa-miR-5581-3p	1665.00	3627.00	1556.00	3039.50	2.1732	5.0956	2.3447
169070	hsa-miR-4695-3p	2118.00	4523.50	2017.50	3934.00	2.8177	6.5951	2.3406
168882	hsa-miR-664b-3p	1709.50	3584.50	1617.50	3103.50	2.2591	5.2028	2.3031
42661	hsa-miR-492	127.50	570.50	24.50	44.50	0.0342	0.0746	2.1802
169028	hsa-miR-4708-3p	3371.50	6417.50	3259.00	5875.00	4.5517	9.8491	2.1638
11182	hsa-miR-98-5p	327.50	959.00	214.00	375.50	0.2989	0.6295	2.1062
17506	hsa-miR-24-3p	670.50	1607.50	562.00	985.50	0.7849	1.6521	2.1049
169159	hsa-miR-4521	176.00	720.50	66.50	116.00	0.0929	0.1945	2.0938
147891	hsa-miR-3175	625.00	1519.00	518.00	901.50	0.7235	1.5113	2.0890
168672	hsa-miR-1587	584.50	1353.50	492.00	836.00	0.6872	1.4015	2.0396
42703	hsa-miR-490-3p	381.50	1060.50	278.50	472.50	0.3890	0.7921	2.0365
169087	hsa-miR-149-3p	2519.00	4596.50	2425.00	4058.00	3.3869	6.8030	2.0086

miRNA extraction of MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cells

The above-mentioned MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cells were diluted to 2×10^6 cell/ml, and inoculated to a T-25 cm² flask, and separately cultured in a CO₂-incubator with 5% CO₂ at 37°C till 80% confluence, and cells and its culturing supernatant were collected to extract miRNA according to the manufacturer's instructions of miRNA extraction kit (Tiagen, Beijing, China). After grinding, 1 mL of TRIzol was added, and the samples were mixed for 30 s and then incubated at room temperature for 5 min. Chloroform (200 µL, Invitrogen, USA) was added to the samples, which were mixed for 15 s, incubated at room temperature for 5 min, and centrifuged at 4°C, at 12,000 rpm for 10 min. The supernatant was collected, mixed with 1.5 volumes of absolute ethanol, transferred to a miRspin column, and centrifuged at room temperature, at

12,000 rpm for 30 s. The effluent was removed, and 500 µL MRD buffer was added to the column, which was incubated at room temperature for 2 min followed by centrifugation at room temperature, at 12,000 rpm for 30 s. The effluent was removed and 700 µL RW buffer was added to the column, which was incubated at room temperature for 2 min followed by centrifugation at room temperature at 12,000 rpm for 30 s, and repeated once with 700 µL RW buffer. The effluent was removed and 500 µL RW buffer was added to the column, which was then incubated at room temperature for 2 min, followed by centrifugation at room temperature, at 12,000 rpm for 30 s. The column was transferred to a new collection tube and centrifuged at 12,000 rpm for 1 min to remove any residual liquid. After air-drying at room temperature, 30 µL of RNase-free double-distilled H₂O (ddH₂O) was added to the miRspin column. The column was centrifuged at room temperature,

Differential expression profile analysis of miRNAs in breast cancer cells

Table 2. The down-regulated miRNAs of MCF-7 cells with HER-2 gene overexpression

ID	Name	Fore Ground		Fore Ground/ Back Ground		Normalized		Fold change
		E	C	E	C	E	C	
29460	hsa-miR-553	1733.50	481.50	1645.00	0.50	2.2975	0.0008	0.0004
169319	hsa-miR-3136-3p	3076.00	586.00	2991.50	13.50	4.1781	0.0226	0.0054
13137	hsa-miR-518e-5p/hsa-miR-519a-5p/hsa-miR-519b-5p/ hsa-miR-519c-5p/hsa-miR-522-5p/hsa-miR-523-5p	1995.00	536.50	1903.00	18.50	2.6578	0.0310	0.0117
169308	hsa-miR-4503	1597.50	581.50	1500.50	15.50	2.0957	0.0260	0.0124
168986	hsa-miR-4677-3p	1958.50	571.50	1859.50	21.50	2.5971	0.0360	0.0139
17327	hsa-miR-630	6036.00	626.50	5942.50	69.50	8.2996	0.1165	0.0140
169227	hsa-miR-3064-5p	180.50	553.50	83.50	1.00	0.1166	0.0017	0.0144
148234	hsa-miR-3667-5p	3897.50	652.00	3811.00	48.00	5.3226	0.0805	0.0151
168852	hsa-miR-4764-5p	888.00	579.00	788.00	10.50	1.1006	0.0176	0.0160
168597	hsa-miR-5699-3p	3398.00	534.00	3311.50	44.50	4.6250	0.0746	0.0161
13178	hsa-miR-18a-3p	210.00	575.00	101.00	1.50	0.1411	0.0025	0.0178
27568	hsa-miR-744-5p	458.50	593.00	360.50	5.50	0.5035	0.0092	0.0183
145675	hsa-miR-501-5p	1807.00	665.50	1718.50	30.00	2.4001	0.0503	0.0210
17892	hsa-miR-372-5p	1121.00	578.00	1026.00	19.00	1.4330	0.0319	0.0222
146179	hsa-miR-2113	808.00	525.00	720.50	15.00	1.0063	0.0251	0.0250
145826	hsa-miR-18b-3p	253.50	596.00	144.00	3.00	0.2011	0.0050	0.0250
14301	hsa-miR-361-5p	2361.50	622.50	2256.50	51.00	3.1515	0.0855	0.0271
42475	hsa-miR-221-5p	1886.00	587.00	1792.00	59.50	2.5028	0.0997	0.0399
169262	hsa-miR-5009-5p	618.00	586.00	504.00	17.50	0.7039	0.0293	0.0417
46788	hsa-miR-1299	701.00	593.50	582.50	22.50	0.8135	0.0377	0.0464
169379	hsa-miR-4694-3p	1774.50	602.50	1684.00	67.00	2.3520	0.1123	0.0478
42730	hsa-miR-423-3p	1445.00	616.50	1336.00	58.00	1.8659	0.0972	0.0521
169010	hsa-miR-2681-3p	2217.50	619.00	2127.00	98.00	2.9707	0.1643	0.0553
147667	hsa-miR-3182	632.50	513.00	542.50	27.50	0.7577	0.0461	0.0608
147926	hsa-miR-4329	3495.00	666.50	3406.50	185.50	4.7577	0.3110	0.0654
169076	hsa-miR-345-3p	393.50	617.00	288.00	16.00	0.4022	0.0268	0.0667
169168	hsa-miR-5580-3p	334.50	623.50	227.50	13.00	0.3177	0.0218	0.0686
27672	hsa-miR-615-3p	1938.50	655.50	1842.00	109.00	2.5726	0.1827	0.0710
11151	hsa-miR-516b-5p	1639.50	626.50	1551.50	96.50	2.1669	0.1618	0.0747
169167	hsa-miR-4451	1826.50	697.00	1717.50	107.50	2.3987	0.1802	0.0751
169211	hsa-miR-5704	577.00	633.00	465.00	32.50	0.6494	0.0545	0.0839
17646	ebv-miR-BHRF1-3	3315.00	754.00	3225.00	234.00	4.5042	0.3923	0.0871
148652	hsa-miR-620	1211.50	566.00	1123.00	86.50	1.5684	0.1450	0.0925
42906	ebv-miR-BHRF1-1	1069.50	656.50	961.00	80.50	1.3422	0.1350	0.1005
146010	hsa-miR-2116-5p	2755.00	784.00	2667.00	236.50	3.7249	0.3965	0.1064
42954	ebv-miR-BART7-5p	1386.50	661.50	1273.50	116.00	1.7786	0.1945	0.1093
168768	hsa-miR-4423-5p	3149.50	837.50	3057.00	293.50	4.2696	0.4920	0.1152
168727	hsa-miR-4426	2084.00	778.00	1988.50	191.50	2.7772	0.3210	0.1156
148495	hsa-miR-3915	2398.50	868.50	2285.50	228.50	3.1920	0.3831	0.1200
169307	hsa-miR-4685-3p	532.50	604.50	438.50	44.50	0.6124	0.0746	0.1218
146090	hsv1-miR-H7-3p	656.50	650.00	555.00	56.50	0.7751	0.0947	0.1222
17942	hsa-miR-125a-3p	887.00	620.50	795.00	82.50	1.1103	0.1383	0.1246
11007	hsa-miR-206	1797.50	768.00	1706.50	186.00	2.3834	0.3118	0.1308
168964	hsa-miR-4450	4461.50	1083.50	4361.50	511.00	6.0915	0.8567	0.1406
169407	hsa-miR-4301	1333.50	727.00	1224.50	144.50	1.7102	0.2422	0.1416
17810	hsa-miR-29b-1-5p	1922.50	781.00	1825.00	218.50	2.5489	0.3663	0.1437
148247	hsa-miR-2355-3p	2285.50	818.00	2181.00	263.00	3.0461	0.4409	0.1447
17578	kshv-miR-K12-5-3p	2163.00	788.50	2075.00	252.00	2.8980	0.4225	0.1458
46789	hsa-miR-513b-5p	1139.00	720.50	1029.50	128.50	1.4378	0.2154	0.1498
17863	hsa-miR-934	635.00	629.50	538.50	67.50	0.7521	0.1132	0.1505
168980	hsa-miR-4324	1375.50	718.00	1279.50	161.50	1.7870	0.2707	0.1515

Differential expression profile analysis of miRNAs in breast cancer cells

168769	hsa-miR-5002-5p	3064.00	887.00	2977.50	378.50	4.1585	0.6345	0.1526
148057	hsa-miR-186-3p	1683.00	768.00	1585.00	211.00	2.2137	0.3537	0.1598
169170	hsa-miR-4472	2228.00	879.50	2122.50	285.50	2.9644	0.4786	0.1615
169411	hsa-miR-205-3p	2682.00	891.50	2589.00	358.00	3.6159	0.6002	0.1660
168941	hsa-miR-4501	1098.00	678.50	1007.00	145.00	1.4064	0.2431	0.1728
28047	hsa-miR-890	711.50	621.00	617.50	89.00	0.8624	0.1492	0.1730
21702	hsa-miR-219a-1-3p	342.50	658.50	235.50	34.00	0.3289	0.0570	0.1733
168989	hsa-miR-4447	381.00	606.00	281.00	41.00	0.3925	0.0687	0.1751
169311	hsa-miR-4714-5p	665.50	667.50	556.00	82.00	0.7765	0.1375	0.1770
42654	hsa-miR-483-5p	400.00	647.50	284.00	44.50	0.3966	0.0746	0.1881
148316	hsa-miR-134-3p	637.00	627.50	547.00	86.50	0.7640	0.1450	0.1898
42749	hsa-miR-659-3p	607.50	600.00	507.50	87.50	0.7088	0.1467	0.2070
147889	hsv1-miR-H14-3p	120.00	543.50	37.50	6.50	0.0524	0.0109	0.2081
168994	hsa-miR-3591-5p	1814.00	863.00	1702.50	303.00	2.3778	0.5080	0.2136
46380	hsa-miR-1255a	2609.00	1002.00	2508.50	447.50	3.5035	0.7502	0.2141
29872	hsa-miR-340-5p	395.50	667.50	291.50	54.00	0.4071	0.0905	0.2224
17328	ebv-miR-BART8-3p	1684.50	815.00	1594.00	295.50	2.2263	0.4954	0.2225
168911	hsa-miR-4682	887.50	651.50	803.00	152.50	1.1215	0.2557	0.2280
168722	hsa-miR-4742-3p	783.50	681.50	686.50	137.00	0.9588	0.2297	0.2395
168955	hsa-miR-4756-3p	534.00	650.00	442.50	91.50	0.6180	0.1534	0.2482
168606	hsa-miR-4633-5p	215.00	594.50	118.00	26.50	0.1648	0.0444	0.2696
169035	hsa-miR-4658	775.00	703.50	674.00	152.00	0.9413	0.2548	0.2707
168660	hsa-miR-5000-5p	3521.50	1287.50	3432.00	818.00	4.7933	1.3713	0.2861
46810	hsa-miR-1827	1623.50	906.50	1530.50	366.50	2.1376	0.6144	0.2874
148335	hsa-miR-3606-5p	2131.50	1136.50	2017.00	503.50	2.8170	0.8441	0.2996
147632	hsa-miR-4297	705.50	660.00	606.00	154.00	0.8464	0.2582	0.3050
168998	hsa-miR-4508	480.00	696.50	379.00	97.00	0.5293	0.1626	0.3072
148465	hsa-miR-3611	1541.00	965.50	1431.50	368.00	1.9993	0.6169	0.3086
42932	hsa-miR-920	327.00	696.00	217.50	56.00	0.3038	0.0939	0.3091
42795	kshv-miR-K12-3-5p	1026.00	859.00	918.00	239.00	1.2821	0.4007	0.3125
19585	hsa-miR-148b-3p	186.50	585.00	89.00	23.50	0.1243	0.0394	0.3169
17898	hsa-miR-99b-3p	1295.50	858.50	1205.50	322.50	1.6837	0.5407	0.3211
17488	kshv-miR-K12-6-3p	3111.50	1371.00	3018.50	833.50	4.2158	1.3973	0.3314
147900	hsv2-miR-H6-3p	893.50	847.00	786.00	218.00	1.0978	0.3655	0.3329
147588	hsa-miR-4288	604.50	683.00	512.50	142.50	0.7158	0.2389	0.3338
148481	hsa-miR-3646	816.50	742.50	722.50	209.50	1.0091	0.3512	0.3481
168557	hsa-miR-4777-5p	2071.00	1152.00	1968.50	587.00	2.7493	0.9841	0.3579
42446	hsa-miR-576-5p	783.00	793.50	684.50	205.50	0.9560	0.3445	0.3604
168661	hsa-miR-4531	2117.00	1217.00	2013.00	604.50	2.8115	1.0134	0.3605
168915	hsa-miR-4780	4821.00	1945.00	4731.50	1426.00	6.6082	2.3906	0.3618
169232	hsa-miR-3156-3p	1056.00	835.00	965.50	296.00	1.3485	0.4962	0.3680
17338	hsa-miR-660-5p	276.00	578.00	182.00	57.50	0.2542	0.0964	0.3792
148622	hsa-miR-877-3p	600.50	711.00	477.50	155.00	0.6669	0.2598	0.3896
10923	hsa-miR-107	379.00	621.00	289.50	99.50	0.4043	0.1668	0.4126
168805	hsa-miR-4478	1827.00	1225.00	1713.00	596.50	2.3925	1.0000	0.4180
169282	hsa-miR-4290	1146.50	921.00	1052.00	368.00	1.4693	0.6169	0.4199
147947	hsa-miR-4308	2158.00	1258.50	2068.00	729.50	2.8883	1.2230	0.4234
148156	hsa-miR-3686	3826.50	1850.50	3734.00	1326.50	5.2151	2.2238	0.4264
147836	hsv2-miR-H7-5p	3040.00	1625.50	2936.50	1063.00	4.1013	1.7821	0.4345
168887	hsa-miR-5089-5p	352.50	600.00	247.00	91.50	0.3450	0.1534	0.4447
42808	hsa-miR-874-3p	555.50	759.50	454.50	174.00	0.6348	0.2917	0.4595
42442	hsa-miR-498	1055.00	987.50	939.00	361.00	1.3115	0.6052	0.4615
148032	hsa-miR-3685	1482.00	1079.00	1390.00	549.50	1.9413	0.9212	0.4745
169130	hsa-miR-4764-3p	540.00	805.50	436.00	177.00	0.6089	0.2967	0.4873

at 12,000 rpm for 2 min. The effluent was collected, and the purity and the concentration of

extracted miRNA were determined by ultraviolet visible spectrophotometer (Bio-Rad, USA).

Differential expression profile analysis of miRNAs in breast cancer cells

Table 3. The up-regulated miRNAs of BT474 cells with HER-2 gene intervention

ID	Name	Fore Ground		Fore Ground/ Back Ground		Normalized		Fold change E vs C
		E	C	E	C	E	C	
42696	hsa-miR-943	165.00	1220.50	108.50	1132.50	0.6272	6.2917	10.0319
10919	hsa-miR-103a-3p	95.00	481.50	41.00	391.50	0.2370	2.1750	9.1774
145977	hsa-miR-1247-5p	59.00	114.50	4.00	30.50	0.0231	0.1694	7.3285
145838	hsa-miR-125b-1-3p	230.50	1297.00	174.00	1209.00	1.0058	6.7167	6.6781
147165	hsa-let-7b-5p	126.50	452.00	70.50	364.50	0.4075	2.0250	4.9691
148635	hsa-miR-933	100.00	306.00	44.00	218.50	0.2543	1.2139	4.7728
148599	hsa-miR-3680-5p	72.50	168.50	17.00	81.00	0.0983	0.4500	4.5794
148206	hsa-miR-3664-5p	527.00	2319.50	472.00	2233.00	2.7283	12.4056	4.5470
168640	hsa-miR-4475	270.00	1097.00	213.50	1009.00	1.2341	5.6056	4.5422
168861	hsa-miR-4754	74.00	162.50	16.50	70.50	0.0954	0.3917	4.1066
169221	hsa-miR-4748	69.50	148.50	15.00	60.50	0.0867	0.3361	3.8765
169076	hsa-miR-345-3p	155.50	501.00	103.50	417.00	0.5983	2.3167	3.8723
169031	hsa-miR-4726-5p	81.00	198.00	27.00	108.50	0.1561	0.6028	3.8622
169170	hsa-miR-4472	121.50	341.50	65.00	254.00	0.3757	1.4111	3.7557
14313	hsa-miR-499a-5p	64.00	119.50	9.00	35.00	0.0520	0.1944	3.7377
147203	hsa-miR-302a-3p	77.50	174.00	20.00	77.50	0.1156	0.4306	3.7243
168648	hsa-miR-4687-5p	69.00	137.50	11.50	44.50	0.0665	0.2472	3.7191
169188	hsa-miR-4443	171.00	523.50	114.50	435.50	0.6618	2.4194	3.6556
42965	hsa-miR-424-5p	75.00	157.50	19.50	73.00	0.1127	0.4056	3.5980
147840	hsv2-miR-H9-3p	69.00	132.00	13.00	48.50	0.0751	0.2694	3.5857
147767	hsa-miR-4279	127.50	358.50	74.00	271.50	0.4277	1.5083	3.5262
147836	hsv2-miR-H7-5p	87.50	205.00	32.00	116.50	0.1850	0.6472	3.4990
168878	hsa-miR-5100	741.50	2559.50	686.50	2473.50	3.9682	13.7417	3.4629
168570	hsa-miR-2682-3p	178.00	525.50	121.50	437.00	0.7023	2.4278	3.4568
169034	hsa-miR-642b-5p	69.50	131.50	12.00	43.00	0.0694	0.2389	3.4440
46438	hsa-let-7g-5p	265.50	803.50	208.00	717.50	1.2023	3.9861	3.3154
10977	hsa-miR-183-5p	237.00	715.50	182.50	628.00	1.0549	3.4889	3.3073
10990	hsa-miR-196a-5p	2684.00	9076.50	2630.00	8987.50	15.2023	49.9306	3.2844
31026	hsa-miR-101-3p	79.50	167.00	23.50	78.00	0.1358	0.4333	3.1901
146161	hsa-miR-2115-3p	67.00	123.00	11.00	35.50	0.0636	0.1972	3.1018
148625	hsa-miR-941	360.50	1050.00	304.50	964.00	1.7601	5.3556	3.0427
169113	hsa-miR-4755-5p	75.00	140.50	16.50	52.00	0.0954	0.2889	3.0290
17858	hsa-miRPlus-A1073	648.00	1940.50	592.50	1855.00	3.4249	10.3056	3.0090
147864	hsv2-miR-H24	2510.50	7766.00	2453.50	7673.50	14.1821	42.6306	3.0059
168919	hsa-miR-4456	840.00	2544.00	786.50	2459.50	4.5462	13.6639	3.0055
168745	hsa-miR-4667-3p	71.50	131.00	13.00	40.50	0.0751	0.2250	2.9942
168661	hsa-miR-4531	118.00	283.50	62.50	193.50	0.3613	1.0750	2.9756
168989	hsa-miR-4447	414.00	1201.50	359.50	1112.00	2.0780	6.1778	2.9729
168998	hsa-miR-4508	112.00	259.00	53.50	164.00	0.3092	0.9111	2.9462
145859	hsa-miR-33a-5p	200.50	524.50	143.50	438.50	0.8295	2.4361	2.9369
145950	hsa-miR-33b-5p	114.00	268.50	59.50	181.50	0.3439	1.0083	2.9318
148038	hsa-miR-3679-3p	1741.00	5211.00	1685.00	5124.50	9.7399	28.4694	2.9230
168943	hsa-miR-4769-3p	104.50	236.50	49.50	150.50	0.2861	0.8361	2.9222
169312	hsa-miR-548an	69.50	130.50	13.50	41.00	0.0780	0.2278	2.9189
169050	hsa-miR-4787-5p	250.00	673.00	194.50	584.50	1.1243	3.2472	2.8883

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31867	hsa-miR-145-3p	1033.00	3023.50	978.00	2936.50	5.6532	16.3139	2.8858
145742	hsa-miR-935	13649.50	40865.50	13593.00	40777.00	78.5723	226.5389	2.8832
147600	hsa-miR-4292	1637.00	4813.50	1582.50	4730.00	9.1474	26.2778	2.8727
148371	hsa-miR-3620-3p	221.00	575.00	164.50	489.50	0.9509	2.7194	2.8600
168631	hsa-miR-4723-5p	190.00	485.50	134.50	398.50	0.7775	2.2139	2.8476
169087	hsa-miR-149-3p	2122.00	6180.00	2065.00	6089.00	11.9364	33.8278	2.8340
146103	hsa-miR-1913	846.00	2417.50	790.50	2323.50	4.5694	12.9083	2.8250
145826	hsa-miR-18b-3p	411.00	1117.50	352.50	1027.50	2.0376	5.7083	2.8015
145976	hsa-miR-663b	687.50	1929.50	632.00	1840.00	3.6532	10.2222	2.7982
46259	hsa-miR-885-5p	177.50	439.50	121.00	351.50	0.6994	1.9528	2.7920
146010	hsa-miR-2116-5p	1899.00	5394.50	1844.00	5305.00	10.6590	29.4722	2.7650
145676	hsa-miR-30e-3p	66.00	118.50	11.50	32.00	0.0665	0.1778	2.6744
147947	hsa-miR-4308	1067.00	2881.50	1012.00	2795.50	5.8497	15.5306	2.6549
168768	hsa-miR-4423-5p	196.50	480.00	142.00	391.50	0.8208	2.1750	2.6498
168973	hsa-miR-1268b	69.50	121.00	11.50	31.50	0.0665	0.1750	2.6326
169307	hsa-miR-4685-3p	2737.00	7430.00	2682.50	7344.00	15.5058	40.8000	2.6313
169179	hsa-miR-4650-5p	107.00	229.00	50.50	138.00	0.2919	0.7667	2.6264
169411	hsa-miR-205-3p	144.00	331.50	89.00	242.00	0.5145	1.3444	2.6134
169393	hsa-miR-4747-5p	74.00	133.50	16.00	43.50	0.0925	0.2417	2.6130
10986	hsa-miR-193a-3p	296.00	744.50	242.00	657.50	1.3988	3.6528	2.6113
17460	hsa-miR-657	74.00	133.50	14.50	39.00	0.0838	0.2167	2.5851
46258	hsa-miR-1184	87.00	166.50	31.00	83.00	0.1792	0.4611	2.5733
146042	hsv1-miR-H8-3p	301.00	739.50	242.50	644.00	1.4017	3.5778	2.5524
146115	hsa-miR-940	189.00	441.50	133.00	351.50	0.7688	1.9528	2.5401
148247	hsa-miR-2355-3p	347.50	856.00	289.50	765.00	1.6734	4.2500	2.5397
17327	hsa-miR-630	116.00	247.50	60.50	159.00	0.3497	0.8833	2.5259
17488	kshv-miR-K12-6-3p	244.00	583.50	188.50	491.50	1.0896	2.7306	2.5060
168882	hsa-miR-664b-3p	122.00	259.50	66.50	172.00	0.3844	0.9556	2.4859
169102	hsa-miR-4639-3p	248.50	587.50	194.00	498.50	1.1214	2.7694	2.4697
169285	hsa-miR-4467	91.00	188.00	37.50	96.00	0.2168	0.5333	2.4604
169049	hsa-miR-3162-3p	2268.00	5730.00	2211.00	5638.50	12.7803	31.3250	2.4510
146072	hsa-miR-1469	2402.00	6040.50	2348.00	5952.00	13.5723	33.0667	2.4363
147942	hsa-miR-4268	89.50	172.50	30.00	75.50	0.1734	0.4194	2.4188
148622	hsa-miR-877-3p	752.00	1826.50	698.00	1744.00	4.0347	9.6889	2.4014
168637	hsa-miR-3940-5p	751.50	1814.50	698.00	1728.00	4.0347	9.6000	2.3794
42761	hsa-miR-675-5p	82.00	155.00	25.00	61.00	0.1445	0.3389	2.3451
46810	hsa-miR-1827	167.50	357.00	111.00	270.50	0.6416	1.5028	2.3422
148504	hsa-miR-874-5p	121.00	246.00	65.00	158.00	0.3757	0.8778	2.3362
169375	hsa-miR-660-3p	198.50	435.50	144.00	349.50	0.8324	1.9417	2.3327
169380	hsa-miR-3124-3p	426.50	985.00	369.00	894.00	2.1329	4.9667	2.3285
169183	hsa-miR-4644	155.50	330.00	99.00	239.50	0.5723	1.3306	2.3251
169360	hsa-miR-4445-5p	70.50	122.00	14.50	35.00	0.0838	0.1944	2.3199
9938	hsa-let-7i-5p	291.50	657.00	237.50	570.00	1.3728	3.1667	2.3067
27720	hsa-miR-15a-5p	135.50	276.50	76.50	183.00	0.4422	1.0167	2.2991
46800	hsa-miR-1224-3p	231.50	507.00	175.00	418.50	1.0116	2.3250	2.2984
29575	hsa-miR-32-3p	80.00	151.50	26.50	63.00	0.1532	0.3500	2.2849
146068	hsa-miR-1915-3p	1579.00	3687.50	1523.00	3601.00	8.8035	20.0056	2.2725
145846	hsa-let-7e-5p	123.50	248.50	68.00	160.50	0.3931	0.8917	2.2685
147631	hsa-miR-4258	99.50	186.50	42.50	100.00	0.2457	0.5556	2.2614

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147904	hsa-miR-3148	77.50	134.00	20.50	48.00	0.1185	0.2667	2.2504
148140	hsa-miR-181d-3p	1089.00	2499.00	1035.00	2410.50	5.9827	13.3917	2.2384
148156	hsa-miR-3686	1098.00	2503.50	1041.00	2411.00	6.0173	13.3944	2.2260
148118	hsa-miR-676-5p	73.50	131.00	19.00	44.00	0.1098	0.2444	2.2257
17328	ebv-miR-BART8-3p	197.50	415.00	142.50	329.00	0.8237	1.8278	2.2190
169214	hsa-miR-4638-5p	287.50	616.50	230.00	527.50	1.3295	2.9306	2.2043
169145	hsa-miR-5000-3p	1286.00	2905.00	1231.00	2817.50	7.1156	15.6528	2.1998
46806	hsa-miR-1227-3p	1502.50	3390.00	1446.50	3306.50	8.3613	18.3694	2.1970
147731	hsa-miR-3189-3p	183.50	377.00	128.50	289.50	0.7428	1.6083	2.1653
169024	hsa-miR-3960	85.50	154.50	27.50	61.50	0.1590	0.3417	2.1494
168904	hsa-miR-4473	86.50	159.00	31.50	70.00	0.1821	0.3889	2.1358
168672	hsa-miR-1587	394.00	821.00	333.50	734.00	1.9277	4.0778	2.1153
17492	sv40-miR-S1-5p	97.50	182.00	41.50	91.00	0.2399	0.5056	2.1075
145845	hsa-miR-20a-5p	1016.00	2191.00	960.00	2105.00	5.5491	11.6944	2.1074
168978	hsa-miR-371b-5p	100.50	183.50	45.50	99.50	0.2630	0.5528	2.1018
169305	hsa-miR-4455	75.50	131.50	21.50	46.50	0.1243	0.2583	2.0787
146121	hsa-miR-1470	197.50	389.50	139.50	301.50	0.8064	1.6750	2.0772
169313	hsa-miR-4800-3p	226.50	461.50	172.50	372.50	0.9971	2.0694	2.0754
169323	hsa-miR-4723-3p	158.50	312.00	104.00	224.50	0.6012	1.2472	2.0747
10985	hsa-miR-191-5p	90.50	163.50	35.50	76.50	0.2052	0.4250	2.0711
10987	hsa-miR-193b-3p	1565.50	3335.00	1509.00	3244.50	8.7225	18.0250	2.0665
147667	hsa-miR-3182	319.50	654.50	264.50	567.50	1.5289	3.1528	2.0621
147806	hsa-miR-3149	81.50	140.00	26.00	55.00	0.1503	0.3056	2.0331
168689	hsa-miR-361-3p	218.50	428.00	164.00	345.00	0.9480	1.9167	2.0218
168639	hsa-miR-4533	3033.00	6323.50	2975.50	6230.00	17.1994	34.6111	2.0123
168657	hsa-miR-5001-3p	84.00	143.50	28.00	58.50	0.1618	0.3250	2.0080
169263	hsa-miR-4634	75.50	131.00	18.00	37.50	0.1040	0.2083	2.0023

miRNA gene-chip assay

The above-validated miRNA was fluorescently labeled by Hy3 or Hy5 using miRCURY Array Power Labeling Kit (Exiqon, Denmark), and hybridized with microarray within 1-2 hrs. The chips above-hybridized was washed using Wash Buffer Kit (Exiqon, Denmark). The fluorescent signal was detected with a Gene Pix 4000B laser scanner, and differential expression of miRNA was assigned according to Student's t-test and the fold change (FC) using the significance analysis of Gene Pixpro V6.0 software.

Quantitative reverse transcription-polymerase chain reaction assay of miRNA

miRNA was used as the template in a reverse transcription reaction using a kit (TOYOBO, Japan), according to the manufacturer's instructions. The miRNA reverse transcription reaction mixture included 10 μ L 2 \times loading buffer, 1.2

μ L miRNA RT primer/U6 small nuclear RNA primer, 2 μ L miRNA template, 0.2 μ L MMLV reverse transcriptase, and 6.6 μ L DEPC-treated H₂O. The reaction was incubated at 26°C for 30 min, followed by 42°C for 30 min, and then by 85°C for 10 min. For quantitative PCR (qPCR), 100 ng cDNA was used as the template in a reaction mixture that included 10 μ L 2 \times Master Mix, 0.08 μ L forward primer, 0.08 μ L reverse primer, 2 μ L cDNA template, 0.4 μ L Taq DNA polymerase, and 7.44 μ L ddH₂O. The qPCR amplification conditions were as follows: one cycle of 95°C for 3 min; 40 cycles of 95°C for 12 s, 62°C for 30 s, and 72°C for 30 s. The results were analyzed using SDS 1.4 software (Applied Biosystems).

Gene ontology (GO) clustering

The GO database (<http://geneontology.org/>) includes three functional categories: biological process, cellular component, and molecular

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Table 4. The down-regulated miRNAs of BT474 cells with HER-2 intervention

ID	Name	Fore Ground		Fore Ground/ Back Ground		Normalized		Fold change
		E	C	E	C	E	C	E vs C
17585	ebv-miR-BART20-3p	101.00	91.00	45.50	0.50	0.2630	0.0028	0.0106
42681	hsa-miR-1307-3p	259.50	90.00	203.00	2.50	1.1734	0.0139	0.0118
42610	hcmv-miR-UL36-3p	88.50	89.00	35.00	0.50	0.2023	0.0028	0.0137
147535	hsa-miR-4309	180.00	91.50	124.00	2.00	0.7168	0.0111	0.0155
147705	hsa-miR-4320	254.50	96.00	197.50	4.50	1.1416	0.0250	0.0219
147891	hsa-miR-3175	89.00	86.50	35.50	1.00	0.2052	0.0056	0.0271
146024	hsa-miR-383-5p	101.50	90.50	46.50	1.50	0.2688	0.0083	0.0310
168574	hsa-miR-4428	375.00	104.00	316.50	10.50	1.8295	0.0583	0.0319
168667	hsa-miR-4999-3p	147.50	99.50	90.00	3.00	0.5202	0.0167	0.0320
168569	hsa-miR-5088-5p	95.50	91.50	42.00	1.50	0.2428	0.0083	0.0343
169335	hsa-miR-4699-5p	261.00	102.00	202.50	7.50	1.1705	0.0417	0.0356
169086	hsa-miR-4786-5p	108.50	93.00	52.50	2.00	0.3035	0.0111	0.0366
28191	hsa-miR-30e-5p	154.00	98.50	97.00	4.00	0.5607	0.0222	0.0396
11113	hsa-miR-448	234.00	96.00	178.50	8.00	1.0318	0.0444	0.0431
42811	hsa-miR-542-5p	122.00	88.50	66.50	3.00	0.3844	0.0167	0.0434
46791	hsa-miR-1204	143.50	96.00	86.00	4.00	0.4971	0.0222	0.0447
11240	hsa-miR-409-3p	116.50	91.50	61.50	3.00	0.3555	0.0167	0.0469
145638	hsa-miR-29a-5p	165.00	102.00	105.00	6.00	0.6069	0.0333	0.0549
147837	hsa-miR-3119	468.00	109.00	414.00	24.50	2.3931	0.1361	0.0569
147715	hsa-miR-4251	90.50	87.50	33.50	2.00	0.1936	0.0111	0.0574
148042	hsa-miR-3662	106.50	95.00	49.50	3.00	0.2861	0.0167	0.0582
147595	hsa-miR-3178	163.00	96.50	106.50	6.50	0.6156	0.0361	0.0587
168814	hsa-miR-4463	226.50	103.50	169.00	10.50	0.9769	0.0583	0.0597
169222	hsa-miR-4760-5p	187.00	103.00	129.50	8.50	0.7486	0.0472	0.0631
169353	hsa-miR-5582-3p	84.00	90.50	30.00	2.00	0.1734	0.0111	0.0641
169412	hsa-miR-1260a	360.00	109.50	304.50	20.50	1.7601	0.1139	0.0647
146091	hsa-miR-1914-5p	183.00	101.00	125.50	9.00	0.7254	0.0500	0.0689
145905	hsa-miR-518a-5p/hsa-miR-527	104.00	96.00	48.00	3.50	0.2775	0.0194	0.0701
147835	hsa-miR-3143	94.50	93.50	39.00	3.00	0.2254	0.0167	0.0739
147901	kshv-miR-K12-7-5p	86.00	91.00	31.50	2.50	0.1821	0.0139	0.0763
147941	hsa-miR-3160-3p	133.50	98.50	75.50	6.00	0.4364	0.0333	0.0764
168558	hsa-miR-5585-3p	99.50	91.00	44.00	3.50	0.2543	0.0194	0.0765
168918	hsa-miR-4491	143.00	99.50	85.00	8.00	0.4913	0.0444	0.0905
169099	hsa-miR-4790-3p	85.00	93.50	31.50	3.00	0.1821	0.0167	0.0915
169289	hsa-miR-4692	99.00	94.50	46.00	4.50	0.2659	0.0250	0.0940
17625	hsa-miR-627-5p	128.00	100.50	71.00	7.00	0.4104	0.0389	0.0948
11040	hsa-miR-29b-3p	90.00	90.00	35.50	3.50	0.2052	0.0194	0.0948
13130	hsa-miR-517-5p	113.50	101.00	55.50	5.50	0.3208	0.0306	0.0952
147608	hsa-miR-4307	126.00	98.50	67.50	7.00	0.3902	0.0389	0.0997
147872	hsv2-miR-H13	97.00	94.00	42.00	4.50	0.2428	0.0250	0.1030
147830	hsv1-miR-H18	126.00	94.50	72.50	8.00	0.4191	0.0444	0.1061
147897	hsa-miR-3136-5p	115.00	91.00	57.00	6.50	0.3295	0.0361	0.1096
147318	hsa-miR-3660	231.50	109.00	177.50	20.50	1.0260	0.1139	0.1110
148284	hsa-miR-208b-3p	213.00	106.00	159.50	18.50	0.9220	0.1028	0.1115
168684	hsa-miR-5010-5p	179.50	101.00	124.00	14.50	0.7168	0.0806	0.1124
168932	hsa-miR-5094	112.00	92.00	55.50	6.50	0.3208	0.0361	0.1126
168812	hsa-miR-875-3p	128.00	96.50	72.50	8.50	0.4191	0.0472	0.1127
169321	hsa-miR-4691-3p	489.50	144.50	431.00	51.50	2.4913	0.2861	0.1148

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42717	hsa-miR-92b-5p	196.50	111.00	137.50	16.50	0.7948	0.0917	0.1153
10937	hsa-miR-132-3p	118.00	102.50	60.00	7.50	0.3468	0.0417	0.1201
42700	hsa-miR-631	95.00	89.00	39.50	5.00	0.2283	0.0278	0.1217
17508	hcmv-miR-UL112-3p	217.50	110.50	160.00	20.50	0.9249	0.1139	0.1231
46860	hsa-miR-1205	174.50	108.50	116.50	15.50	0.6734	0.0861	0.1279
11058	hsa-miR-325	140.00	100.50	83.00	12.00	0.4798	0.0667	0.1390
46416	hsa-miR-1293	105.50	97.50	47.50	7.00	0.2746	0.0389	0.1416
46789	hsa-miR-513b-5p	206.00	114.50	149.00	22.00	0.8613	0.1222	0.1419
17612	hsa-miR-555	89.00	90.50	33.50	5.00	0.1936	0.0278	0.1434
146005	hsa-miR-3129-5p	110.00	92.00	53.50	8.00	0.3092	0.0444	0.1437
145677	hsa-miR-139-5p	90.50	92.50	36.50	5.50	0.2110	0.0306	0.1448
145643	hsa-miR-382-5p	92.00	94.00	36.50	5.50	0.2110	0.0306	0.1448
147817	hsa-miR-3196	90.00	92.50	33.00	5.00	0.1908	0.0278	0.1456
147799	hsa-miR-3156-5p	201.50	111.00	145.00	22.00	0.8382	0.1222	0.1458
148593	hsa-miR-3605-3p	176.00	105.50	121.50	18.50	0.7023	0.1028	0.1463
148434	hsa-miR-3692-3p	106.00	95.00	51.50	8.00	0.2977	0.0444	0.1493
148380	hsa-miR-3913-5p	131.50	107.50	73.50	11.50	0.4249	0.0639	0.1504
148480	hsa-miR-494-5p	274.50	130.00	215.50	34.00	1.2457	0.1889	0.1516
148089	hsa-miR-208a-3p	126.50	101.50	69.50	11.00	0.4017	0.0611	0.1521
42629	hsa-miR-376c-3p	117.50	97.00	62.50	10.00	0.3613	0.0556	0.1538
46863	hsa-miR-3157-3p	116.00	100.50	62.00	10.00	0.3584	0.0556	0.1550
168644	hsa-miR-4775	203.00	116.00	144.50	23.50	0.8353	0.1306	0.1563
168770	hsa-miR-4699-3p	3877.50	724.00	3818.50	627.50	22.0723	3.4861	0.1579
169107	hsa-miR-4782-5p	95.00	99.00	36.50	6.00	0.2110	0.0333	0.1580
169194	hsa-miR-4513	126.50	103.50	66.50	11.00	0.3844	0.0611	0.1590
169126	hsa-miR-4486	89.50	99.50	32.00	5.50	0.1850	0.0306	0.1652
29529	hsa-miR-369-3p	102.50	97.50	46.50	8.00	0.2688	0.0444	0.1654
17415	ebv-miR-BART10-3p	133.00	104.00	74.50	13.00	0.4306	0.0722	0.1677
46300	hiv1-miR-TAR-3p	162.50	112.50	104.50	18.50	0.6040	0.1028	0.1701
147537	hsa-miR-4264	180.50	116.00	122.50	22.50	0.7081	0.1250	0.1765
147673	hsa-miR-4262	95.50	92.00	40.50	7.50	0.2341	0.0417	0.1780
148239	hsa-miR-3916	90.50	90.50	35.00	6.50	0.2023	0.0361	0.1785
42755	hcmv-miR-US33-3p	102.50	100.50	42.50	8.00	0.2457	0.0444	0.1809
46808	hsa-miR-4485	97.00	101.50	39.00	7.50	0.2254	0.0417	0.1848
168872	hsa-miR-24-1-5p	101.50	99.50	44.00	8.50	0.2543	0.0472	0.1857
33596	hsa-miR-126-5p	279.50	134.00	219.50	42.50	1.2688	0.2361	0.1861
146096	hsa-miR-764	116.00	98.50	61.50	12.00	0.3555	0.0667	0.1875
17932	hsa-miR-381-5p	101.50	96.50	44.50	9.00	0.2572	0.0500	0.1944
147979	hsa-miR-3150a-3p	88.50	95.00	34.50	7.00	0.1994	0.0389	0.1950
148454	hsa-miR-3682-3p	300.50	136.50	245.00	50.00	1.4162	0.2778	0.1961
148039	hsa-miR-3925-5p	93.00	92.50	39.00	8.00	0.2254	0.0444	0.1972
168875	hsa-miR-5091	224.00	125.00	165.50	34.00	0.9566	0.1889	0.1974
169055	hsa-miR-3529-5p	333.50	142.00	277.00	57.00	1.6012	0.3167	0.1978
169141	hsa-miR-4423-3p	97.50	96.00	43.00	9.00	0.2486	0.0500	0.2012
46869	hsa-miR-1258	123.50	103.00	64.00	13.50	0.3699	0.0750	0.2027
19588	hsa-miR-17-3p	162.00	110.00	106.00	22.50	0.6127	0.1250	0.2040
11065	hsa-miR-335-5p	122.00	109.00	65.50	14.00	0.3786	0.0778	0.2054
146046	jcv-miR-J1-5p	315.00	145.50	258.50	58.00	1.4942	0.3222	0.2156
146149	hsv2-miR-H2	97.00	99.50	40.00	9.00	0.2312	0.0500	0.2163
145974	hsa-miR-200b-5p	319.50	145.00	263.50	59.50	1.5231	0.3306	0.2170
148384	hsa-miR-3648	162.00	119.00	105.50	24.00	0.6098	0.1333	0.2186
148402	hsa-miR-3920	139.00	115.50	81.00	18.50	0.4682	0.1028	0.2195

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148064	hsa-miR-3926	89.00	101.00	30.50	7.00	0.1763	0.0389	0.2206
148187	hsa-miR-410-5p	113.50	106.00	58.50	13.50	0.3382	0.0750	0.2218
168632	hsa-miR-5188	94.00	99.00	38.00	9.00	0.2197	0.0500	0.2276
168559	hsa-miR-15a-3p	99.50	101.00	42.00	10.00	0.2428	0.0556	0.2288
169056	hsa-miR-4669	103.00	100.00	46.00	11.00	0.2659	0.0611	0.2298
169253	hsa-miR-4690-5p	224.50	125.50	168.50	40.50	0.9740	0.2250	0.2310
169266	hsa-miR-378d	117.50	104.50	62.00	15.00	0.3584	0.0833	0.2325
169304	hsa-miR-4793-5p	113.50	101.00	59.00	14.50	0.3410	0.0806	0.2362
169020	hsa-miR-4506	193.50	127.00	135.00	33.50	0.7803	0.1861	0.2385
145789	hsa-miR-550a-3-5p/hsa-miR-550a-5p	87.00	100.00	32.00	8.00	0.1850	0.0444	0.2403
145746	hsa-let-7i-3p	1095.00	345.00	1040.50	262.00	6.0145	1.4556	0.2420
147613	hsa-miR-3145-3p	105.50	106.50	47.50	12.00	0.2746	0.0667	0.2428
147262	hsa-miR-548h-3p/hsa-miR-548z	105.00	101.50	50.50	13.00	0.2919	0.0722	0.2474
148228	hsa-miR-3656	180.00	120.50	123.00	32.00	0.7110	0.1778	0.2500
168692	hsa-miR-3688-5p	119.00	109.00	61.50	16.00	0.3555	0.0889	0.2500
168589	hsa-miR-4671-3p	101.50	100.00	46.00	12.00	0.2659	0.0667	0.2507
168923	hsa-miR-4688	98.50	104.00	40.00	10.50	0.2312	0.0583	0.2523
168578	hsa-miR-5190	146.00	115.00	87.00	23.00	0.5029	0.1278	0.2541
168627	hsa-miR-4678	562.00	227.00	506.50	135.00	2.9277	0.7500	0.2562
169257	hsa-miR-4740-5p	97.00	105.50	37.50	10.00	0.2168	0.0556	0.2563
169010	hsa-miR-2681-3p	206.50	133.00	147.50	39.50	0.8526	0.2194	0.2574
169417	hsa-miR-551b-5p	162.50	117.50	106.00	28.50	0.6127	0.1583	0.2584
42483	hsa-miR-522-3p	195.00	125.00	138.00	37.50	0.7977	0.2083	0.2612
17529	hcmv-miR-US25-2-3p	91.50	95.50	34.50	9.50	0.1994	0.0528	0.2647
46623	hsa-miR-1273a	184.00	127.50	125.00	34.50	0.7225	0.1917	0.2653
17530	hsa-miR-641	171.50	124.50	114.00	31.50	0.6590	0.1750	0.2656
148393	hsa-miR-676-3p	133.00	106.00	77.50	21.50	0.4480	0.1194	0.2666
148379	hsa-miR-3654	320.50	163.50	267.50	75.00	1.5462	0.4167	0.2695
168921	hsa-miR-4718	220.00	139.50	163.50	46.00	0.9451	0.2556	0.2704
168666	hsa-miR-4659a-5p	210.50	137.00	152.50	43.00	0.8815	0.2389	0.2710
168703	hsa-miR-4716-3p	154.50	120.50	97.00	27.50	0.5607	0.1528	0.2725
169181	hsa-miR-5191	564.50	231.00	508.50	145.00	2.9393	0.8056	0.2741
169245	hsa-miR-2467-3p	184.00	125.50	129.00	37.00	0.7457	0.2056	0.2757
169115	hsa-miR-450a-2-3p	1309.00	449.50	1253.50	360.00	7.2457	2.0000	0.2760
169225	hsa-miR-640	146.50	113.00	91.50	26.50	0.5289	0.1472	0.2784
42501	hcmv-miR-US33-5p	176.00	121.00	119.50	35.00	0.6908	0.1944	0.2815
42538	hsa-miR-196a-3p	137.50	114.00	81.50	24.00	0.4711	0.1333	0.2830
42672	hsa-miR-323b-5p	87.50	96.00	30.50	9.00	0.1763	0.0500	0.2836
42950	hsa-miR-24-2-5p	85.00	101.00	30.00	9.00	0.1734	0.0500	0.2883
46729	hsa-miR-302d-5p	270.00	156.00	214.50	66.50	1.2399	0.3694	0.2980
146052	hsa-miR-1471	140.00	114.00	85.00	26.50	0.4913	0.1472	0.2996
147735	hsa-miR-4289	110.00	103.50	54.50	17.00	0.3150	0.0944	0.2998
56873	hsa-miR-3124-5p	172.50	126.00	113.50	35.50	0.6561	0.1972	0.3006
147530	hsa-miR-378b	108.00	104.00	49.50	15.50	0.2861	0.0861	0.3010
148639	hsa-miR-4298	130.00	111.50	75.00	23.50	0.4335	0.1306	0.3011
168583	hsa-miR-539-3p	283.00	161.50	225.50	71.00	1.3035	0.3944	0.3026
168887	hsa-miR-5089-5p	124.50	113.50	68.00	21.50	0.3931	0.1194	0.3039
168747	hsa-miR-4520b-3p	241.00	147.00	185.00	58.50	1.0694	0.3250	0.3039
168942	hsa-miR-4636	2418.50	841.00	2360.00	748.50	13.6416	4.1583	0.3048
168847	hsa-miR-4740-3p	91.00	100.00	33.00	10.50	0.1908	0.0583	0.3058
168682	hsa-miR-4502	235.50	148.50	180.50	58.50	1.0434	0.3250	0.3115
169226	hsa-miR-4419a	106.50	109.00	50.50	16.50	0.2919	0.0917	0.3140

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169200	hsa-miR-4677-5p	132.50	108.50	78.00	25.50	0.4509	0.1417	0.3142
46402	hsa-miR-1255b-5p	295.00	168.00	237.50	78.00	1.3728	0.4333	0.3156
17520	hcmv-miR-US5-2-3p	99.50	104.00	44.00	14.50	0.2543	0.0806	0.3167
42556	hsa-miR-889-3p	133.00	117.00	76.50	25.50	0.4422	0.1417	0.3204
11091	hsa-miR-377-3p	96.50	101.00	41.50	14.00	0.2399	0.0778	0.3242
46675	hsa-miR-1181	288.00	172.00	232.00	78.50	1.3410	0.4361	0.3252
145798	hsa-miR-142-5p	111.50	107.00	56.00	19.00	0.3237	0.1056	0.3261
147911	hsa-miR-4280	89.00	103.50	32.00	11.00	0.1850	0.0611	0.3304
147886	hsa-miR-3122	147.00	118.00	91.50	31.50	0.5289	0.1750	0.3309
148106	hsa-miR-3678-3p	201.50	136.50	147.00	51.00	0.8497	0.2833	0.3334
148052	hsa-miR-374c-3p	150.00	122.00	95.00	33.00	0.5491	0.1833	0.3339
168896	hsa-miR-548ao-3p	114.00	111.50	59.00	20.50	0.3410	0.1139	0.3339
168851	hsa-miR-4650-3p	479.50	240.50	422.50	147.00	2.4422	0.8167	0.3344
168724	hsa-miR-4529-3p	160.00	131.50	102.00	35.50	0.5896	0.1972	0.3345
168854	hsa-miR-4498	155.50	125.00	96.50	34.50	0.5578	0.1917	0.3436
169040	hsa-miR-4753-5p	132.00	112.50	76.50	27.50	0.4422	0.1528	0.3455
169189	hsa-miR-4795-5p	585.00	280.50	527.50	190.00	3.0491	1.0556	0.3462
46295	hsa-miR-1286	115.50	111.00	61.00	22.00	0.3526	0.1222	0.3466
42630	hsa-miR-140-3p	469.00	244.00	411.00	149.00	2.3757	0.8278	0.3484
4610	hsa-miR-126-3p	130.00	120.00	73.00	26.50	0.4220	0.1472	0.3489
42476	hsa-miR-374b-3p	121.50	119.50	65.50	24.00	0.3786	0.1333	0.3522
146110	hsa-miR-3164	152.00	122.00	90.00	33.00	0.5202	0.1833	0.3524
145833	hsa-miR-596	151.00	123.00	96.00	35.50	0.5549	0.1972	0.3554
147923	hsa-miR-3142	103.50	106.00	48.50	18.00	0.2803	0.1000	0.3567
147818	hsa-miR-4270	125.00	120.50	67.00	25.00	0.3873	0.1389	0.3586
148688	hsa-miR-765	237.00	159.00	179.50	67.00	1.0376	0.3722	0.3587
42764	hsa-miR-412-3p	244.50	165.50	188.50	70.50	1.0896	0.3917	0.3595
168903	hsa-miR-4439	156.50	123.00	102.00	38.50	0.5896	0.2139	0.3628
168967	hsa-miR-4476	139.50	125.00	83.00	31.50	0.4798	0.1750	0.3648
169310	hsa-miR-3913-3p	125.50	115.50	69.50	26.50	0.4017	0.1472	0.3665
169362	hsa-miR-5591-3p	274.00	178.50	217.50	83.00	1.2572	0.4611	0.3668
169369	hsa-miR-4490	189.50	145.00	131.50	51.00	0.7601	0.2833	0.3728
42692	hsa-miR-127-5p	126.50	113.50	69.50	27.00	0.4017	0.1500	0.3734
29379	hsa-miR-452-5p	167.50	134.00	109.50	43.00	0.6329	0.2389	0.3774
17613	hsa-miR-645	138.50	118.00	83.50	33.00	0.4827	0.1833	0.3798
46440	hsa-miR-1287-5p	104.00	106.50	50.50	20.00	0.2919	0.1111	0.3806
28889	hsa-miR-888-5p	502.00	268.50	444.50	180.00	2.5694	1.0000	0.3892
146044	hsa-miR-2115-5p	106.50	110.50	50.00	20.50	0.2890	0.1139	0.3941
17820	hsa-miRPlus-C1066	147.50	128.00	89.00	36.50	0.5145	0.2028	0.3942
145757	hsa-miR-33a-3p	110.50	114.00	52.00	21.50	0.3006	0.1194	0.3974
147187	hsa-miR-215-5p	122.00	116.50	67.50	28.00	0.3902	0.1556	0.3987
148666	hsa-miR-938	149.50	129.00	91.50	38.00	0.5289	0.2111	0.3991
146123	hsv1-miR-H2-3p	265.50	181.50	206.50	86.00	1.1936	0.4778	0.4003
168871	hsa-miR-151a-5p/hsa-miR-151b	4526.00	1951.50	4469.00	1861.50	25.8324	10.3417	0.4003
168959	hsa-miR-4778-5p	517.50	285.50	459.50	192.00	2.6561	1.0667	0.4016
168929	hsa-miR-4648	90.00	102.50	33.50	14.00	0.1936	0.0778	0.4017
169068	hsa-miR-513c-3p	117.50	118.00	59.50	25.00	0.3439	0.1389	0.4038
169026	hsa-miR-4679	100.00	105.00	44.00	18.50	0.2543	0.1028	0.4041
42786	hsa-miR-188-3p	106.50	110.50	48.50	20.50	0.2803	0.1139	0.4062
17499	hcmv-miR-US5-1	94.00	104.50	40.00	17.00	0.2312	0.0944	0.4085
42458	hcmv-miR-US25-1-3p	176.50	147.50	118.50	50.50	0.6850	0.2806	0.4096
146169	mcv-miR-M1-3p	115.00	113.00	59.50	25.50	0.3439	0.1417	0.4119

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146179	hsa-miR-2113	257.50	181.50	199.50	85.50	1.1532	0.4750	0.4119
146127	hsa-miR-449b-3p	258.50	175.50	204.00	88.00	1.1792	0.4889	0.4146
147843	hsa-miR-3144-5p	149.50	125.00	93.50	41.00	0.5405	0.2278	0.4214
28534	hsa-miRPlus-D1058	104.00	110.00	49.00	21.50	0.2832	0.1194	0.4217
168792	hsa-miR-4434	104.00	109.50	49.00	21.50	0.2832	0.1194	0.4217
169089	hsa-miR-4470	1020.50	516.50	963.00	423.00	5.5665	2.3500	0.4222
169161	hsa-miR-4489	1979.00	931.50	1922.50	844.50	11.1127	4.6917	0.4222
17532	ebv-miR-BART5-5p	87.00	101.50	33.00	14.50	0.1908	0.0806	0.4223
46272	hsa-miR-1200	202.00	156.50	144.50	63.50	0.8353	0.3528	0.4224
14270	hsa-miR-493-3p	97.00	106.50	42.00	18.50	0.2428	0.1028	0.4233
168746	hsa-miR-4756-5p	204.50	155.50	149.50	66.00	0.8642	0.3667	0.4243
168906	hsa-miR-4703-3p	112.50	111.00	57.00	25.50	0.3295	0.1417	0.4300
168990	hsa-miR-5688	284.00	193.00	223.50	100.50	1.2919	0.5583	0.4322
17641	hsa-miR-573	90.50	97.50	35.50	16.00	0.2052	0.0889	0.4332
46398	hsa-miR-513c-5p	257.00	178.50	202.00	91.50	1.1676	0.5083	0.4354
17570	hsa-miR-589-3p	145.50	125.50	91.50	41.50	0.5289	0.2306	0.4359
42466	ebv-miR-BART18-3p	99.50	113.00	42.50	19.50	0.2457	0.1083	0.4410
42783	hsa-miR-197-3p	93.50	106.00	38.00	17.50	0.2197	0.0972	0.4426
42747	hsa-miR-93-3p	123.00	119.50	67.00	31.00	0.3873	0.1722	0.4447
46707	hsa-miR-1253	156.00	131.50	100.00	46.50	0.5780	0.2583	0.4469
146029	hsa-miR-365b-5p	151.50	132.50	96.50	45.00	0.5578	0.2500	0.4482
146159	hsv1-miR-H4-3p	96.00	109.50	39.50	18.50	0.2283	0.1028	0.4501
147988	hsa-miR-3135a	143.50	132.50	89.50	42.00	0.5173	0.2333	0.4510
147988	hsa-miR-3135a	143.50	132.50	89.50	42.00	0.5173	0.2333	0.4510
147996	hsa-miR-3127-5p	466.00	283.50	408.50	192.00	2.3613	1.0667	0.4517
147935	hsa-miR-3125	351.00	227.50	295.00	139.50	1.7052	0.7750	0.4545
148377	hsa-miR-3653	143.00	130.00	88.00	42.00	0.5087	0.2333	0.4587
148059	hsa-miR-493-5p	374.00	245.00	317.00	151.50	1.8324	0.8417	0.4593
168899	hsa-miR-1285-5p	1001.50	540.50	943.50	451.50	5.4538	2.5083	0.4599
168922	hsa-miR-5584-3p	819.00	454.00	761.00	366.00	4.3988	2.0333	0.4622
168836	hsa-miR-4496	141.50	131.50	87.00	42.50	0.5029	0.2361	0.4695
168841	hsa-miR-5588-3p	150.50	138.00	96.00	47.00	0.5549	0.2611	0.4705
168866	hsa-miR-4771	106.00	115.00	53.00	26.00	0.3064	0.1444	0.4715
168960	hsa-miR-4436a	123.50	123.50	68.00	33.50	0.3931	0.1861	0.4735
168879	hsa-miR-4460	263.50	191.50	210.00	103.50	1.2139	0.5750	0.4737
169296	hsa-miR-5004-5p	136.50	130.50	81.50	40.50	0.4711	0.2250	0.4776
169260	hsa-miR-4436b-3p	260.00	195.50	204.00	101.50	1.1792	0.5639	0.4782
169326	hsa-miR-451b	189.50	154.50	133.50	66.50	0.7717	0.3694	0.4788
169212	hsa-miR-514a-5p	108.50	114.00	52.00	26.00	0.3006	0.1444	0.4806
29460	hsa-miR-553	118.00	120.00	59.00	29.50	0.3410	0.1639	0.4806
46439	hsa-miR-1243	686.00	408.00	629.50	315.00	3.6387	1.7500	0.4809
21501	hsa-miR-891b	91.00	107.00	37.50	19.00	0.2168	0.1056	0.4870
145914	hsa-miR-135b-5p	87.50	101.50	33.50	17.00	0.1936	0.0944	0.4877
145857	hsa-miR-154-5p	601.00	368.00	546.00	279.50	3.1561	1.5528	0.4920
147943	hsa-miR-3074-3p	146.00	135.50	88.50	45.50	0.5116	0.2528	0.4941
148445	hsa-miR-3622b-5p	556.00	341.50	501.50	258.00	2.8988	1.4333	0.4944
168685	hsa-miR-4432	89.00	103.00	34.00	17.50	0.1965	0.0972	0.4947
168660	hsa-miR-5000-5p	133.00	126.00	77.50	40.00	0.4480	0.2222	0.4961
169302	hsa-miR-4695-5p	84.50	102.00	30.00	15.50	0.1734	0.0861	0.4966

function. Genes could be further organized by directed acyclic graph according to their scope.

In GO clustering, genes are considered significantly enriched based on the ratio of the

Differential expression profile analysis of miRNAs in breast cancer cells

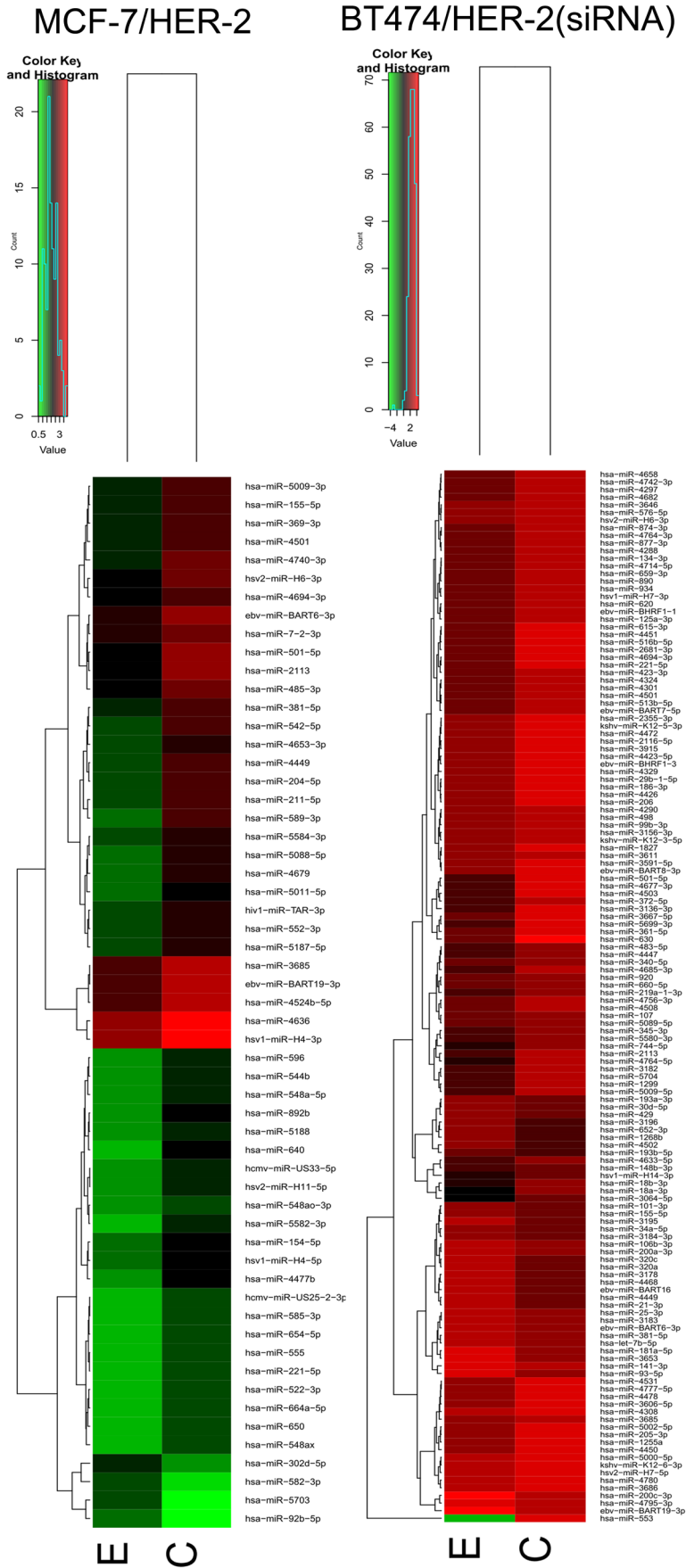


Figure 3. The heatmap of differential expression miRNA in MCF-7, BT474, MCF-7/HER-2, and BT474/HER-2 (siRNA) cells.

observed GO term for all genes/GO term for a single gene set. First, each gene that was assigned a particular GO term was broadly noted in the upper father node, then the *p*-value of each enriched GO term was determined using a hypergeometric distribution, and the *p*-value was adjusted using the false discovery rate (FDR), with P = 0.05 selected as the threshold value. Subsequently, redundant GO terms were removed, and the hierarchy chart's terminal nodes were selected as the final significantly enriched GO terms.

Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The KEGG database (<http://www.genome.jp/kegg/pathway.html>) is used to systematically analyze gene function and genomic information from biological pathways, and to further group biological pathways according to metabolism, enzyme, biochemical reaction, gene regulation, and protein-protein interaction. Here, KEGG signaling pathway analysis was applied, followed by hypergeometric distribution analysis and the FDR method to give an adjusted *p*-value (P = 0.05 as a threshold value). To identify the KEGG signaling pathway for differentially expressed miRNAs or mRNAs, we carried out KEGG pathway enrichment of their up-regulated or down-regulated genes.

Differential expression profile analysis of miRNAs in breast cancer cells

Table 5. The differential expression miRNAs with HER-2 overexpression or intervention

ID	Name	Fore Ground (E/C)	
		MCF-7/HER-2	BT474/HER-2 (siRNA)
147595	hsa-miR-3178	47.0570↑	0.0587↓
148377	hsa-miR-3653	40.3310↑	0.4587↓
168682	hsa-miR-4502	30.2480↑	0.3115↓
147817	hsa-miR-3196	15.2040↑	0.1456↓
148657	hsa-miR-381-5p	10.7320↑	0.1944↓
169260	hsa-miR-4436b-3p	4.5770↑	0.4735↓
168836	hsa-miR-4496	4.0960↑	0.4695↓
46869	hsa-miR-1258	3.4239↑	0.2027↓
169412	hsa-miR-1260a	2.8584↑	0.0647↓
147891	hsa-miR-3175	2.0890↑	0.0271↓
147836	hsv2-miR-H7-5p	0.4345↓	3.4990↑
148156	hsa-miR-3686	0.4264↓	2.2260↑
147947	hsa-miR-4308	0.4234↓	2.6549↑
148622	hsa-miR-877-3p	0.3896↓	2.4014↑
168661	hsa-miR-4531	0.3605↓	2.9756↑
168998	hsa-miR-4508	0.3072↓	2.9462↑
46810	hsa-miR-1827	0.2874↓	2.3422↑
168989	hsa-miR-4447	0.1751↓	2.9729↑
169411	hsa-miR-205-3p	0.1660↓	2.6134↑
169170	hsa-miR-4472	0.1615↓	3.7557↑
148247	hsa-miR-2355-3p	0.1447↓	2.5397↑
169307	hsa-miR-4685-3p	0.1218↓	2.6313↑
168768	hsa-miR-4423-5p	0.1152↓	2.6498↑
146010	hsa-miR-2116-5p	0.1064↓	2.7650↑
169076	hsa-miR-345-3p	0.0667↓	3.8723↑
147667	hsa-miR-3182	0.0608↓	2.0621↑
145826	hsa-miR-18b-3p	0.0250↓	2.8015↑

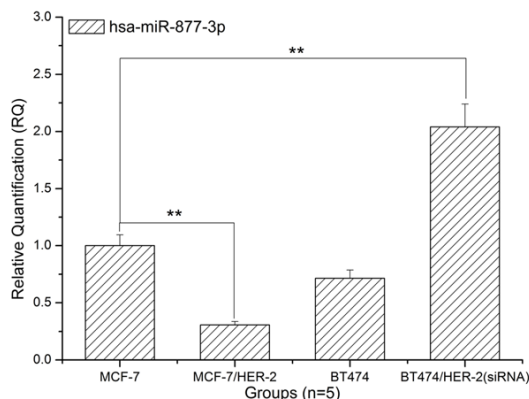


Figure 4. The differential expression miRNAs assay by RT-qPCR. The image indicated that with HER-2 overexpression, the expression level of hsa-miR-877-3p was significantly increased, and synchronously decreased (**: $P < 0.01$, when compared to control).

Statistical analysis

All data are expressed as the mean \pm standard deviation (SD). Statistical analysis was carried out by one-way analysis of variance using the SPSS software (version 21.0, <http://spss.en.softonic.com/>). Student's t-test was carried out on groups of two samples, and $P < 0.05$ and $P < 0.01$ were considered to indicate a significant difference and a highly significant difference, respectively.

Results

The mRNA and protein expression level of HER-2 was significantly increased with HER-2 overexpression, and significantly decreased with HER-2 intervention

As exhibiting of RT-qPCR result of **Figure 1A**, when compared to MCF-7 cells alone, the mRNA expression level of HER-2 was significantly increased in MCF-7/HER-2 cells (**: $P < 0.01$), and increased by about 3.6 times. Similarly, the protein expression level of HER-2 was also significantly increased in MCF-7/HER-2 cells when compared to that of MCF-7 cells alone (**Figure 1B**, **: $P < 0.01$). Synchronously, as exhibiting of RT-qPCR result of

Figure 1C, when compared to BT474 cells alone, the mRNA expression level of HER-2 was significantly decreased in BT474/HER-2 (siRNA) cells (**: $P < 0.01$), and decreased by about 30%. Similarly, the protein expression level of HER-2 was also significantly decreased in BT474/HER-2 (siRNA) cells when compared to that of BT474 cells alone (**Figure 1D**, **: $P < 0.01$).

miRNA gene-chip assay

To elucidate the differential expression profile of miRNAs in breast cancer cells, the miRNA gene-chip was chosen, and the gene-chip image of breast cancer cells was shown in the **Figure 2**.

Differential expression profile analysis of miRNAs in breast cancer cells

Table 6. The primers used for amplification of miRNAs in this study

miRNA	Sequence of primers (5'→3')
hsa-miR-877-3p-frs	ACACTCCAGCTGGGTCCUUCTTCTCCCT
hsa-miR-877-3p-rvs	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTTGAGCTGGGAGG

Differential expression profiles analysis of miRNAs with HER-2 overexpression and intervention by miRNA gene-chip

In breast cancer cells of MCF7 with HER-2 overexpression, a total of 217 miRNAs were identified. Wherein, a total of 123 up-regulated miRNAs (**Table 1**), and a total of 94 down-regulated miRNAs (**Table 2**) were validated. Synchronously, with HER-2 intervention in BT474 cells, a total of 374 miRNAs were identified. Wherein, a total of 121 up-regulated miRNAs (**Table 3**), and a total of 253 down-regulated miRNAs (**Table 4**) were validated, and the heatmap was shown in the **Figure 3**. As further analysis in **Table 5**, a total of 10 miRNAs were up-regulated with HER-2 overexpression in MCF-7 cells, and down-regulated with HER-2 intervention in BT474 cells synchronously, and 17 miRNAs were down-regulated with HER-2 overexpression in MCF-7 cells, and up-regulated with HER-2 intervention in BT474 cells synchronously.

hsa-miR-877-3p was significantly decreased with HER-2 overexpression, and significantly increased with HER-2 intervention

As exhibiting of RT-qPCR result of **Figure 4**, based on the previous study and the biological functions of screened miRNAs, hsa-miR-877-3p was chosen for RT-qPCR identification using primers as showing in **Table 6**, and manifested as a significant decrease with HER-2 gene overexpression, and a significant increase with HER-2 gene intervention, and accordance with gene-chip results.

Furthermore, as exhibiting of **Figure 5A**, hsa-miR-877-3p regulated target genes were predicted by software of TargetScan, miRDB, and miRanda, and a total of 11 cross genes were obtained with GO and KEGG pathway clustering analysis. As exhibiting of **Figure 5B**, hsa-miR-877-3p is associated with the molecular function of translation regulator activity, binding, receptor activity, structural molecule activity, signal transducer activity, catalytic activity,

antioxidant activity, and transporter activity, and the biological process of cellular component organization, cellular process, localization, biological

regulation, response to stimulus, developmental process, multicellular organismal process, locomotion, biological adhesion, metabolic process, growth, and immune system process, and the cellular component of synapse, cell junction, membrane, macromolecular complex, extracellular matrix, cell part, organelle, and extracellular region, and its regulated KEGG pathway was exhibiting in **Figure 6**.

Discussion

The present study demonstrated that with the over-expression and intervention of HER-2, several differential expression miRNAs were screened in breast cancer cells, and manifested as a total of 217 miRNAs were identified in breast cancer cells, including 123 up-regulated miRNAs and 94 down-regulated miRNAs, and a total of 374 miRNAs were identified in breast cancer cells, including 121 up-regulated miRNAs and 253 down-regulated miRNAs. Wherein, a total of 10 miRNAs were up-regulated with HER-2 overexpression in MCF-7 cells, and down-regulated with HER-2 intervention in BT474 cells synchronously, and 17 miRNAs were down-regulated with HER-2 overexpression in MCF-7 cells, and up-regulated with HER-2 intervention in BT474 cells synchronously, and provided a significantly reference on liquid biopsy of breast cancer cells.

Breast cancer has been become a leading type cancer of women with a high incidence and a high mortality [15, 42, 43], and about 40,450 women in the U.S. died in 2015 from breast cancer, and will be increasing day by day in the future [16, 17]. In breast cancer patients, with the aggravation of the disease, the HER-2 often over-expressed, and implied a significant biomarker for breast cancer diagnosis in clinic, and however the differential expression profile of miRNAs in breast cancer cells has been no reported so far. Therefore, the MCF-7 cells and its stable strain with HER-2 overexpression were chosen, and the HER-2 mRNA and protein expression level was analyzed. After transfec-

Differential expression profile analysis of miRNAs in breast cancer cells

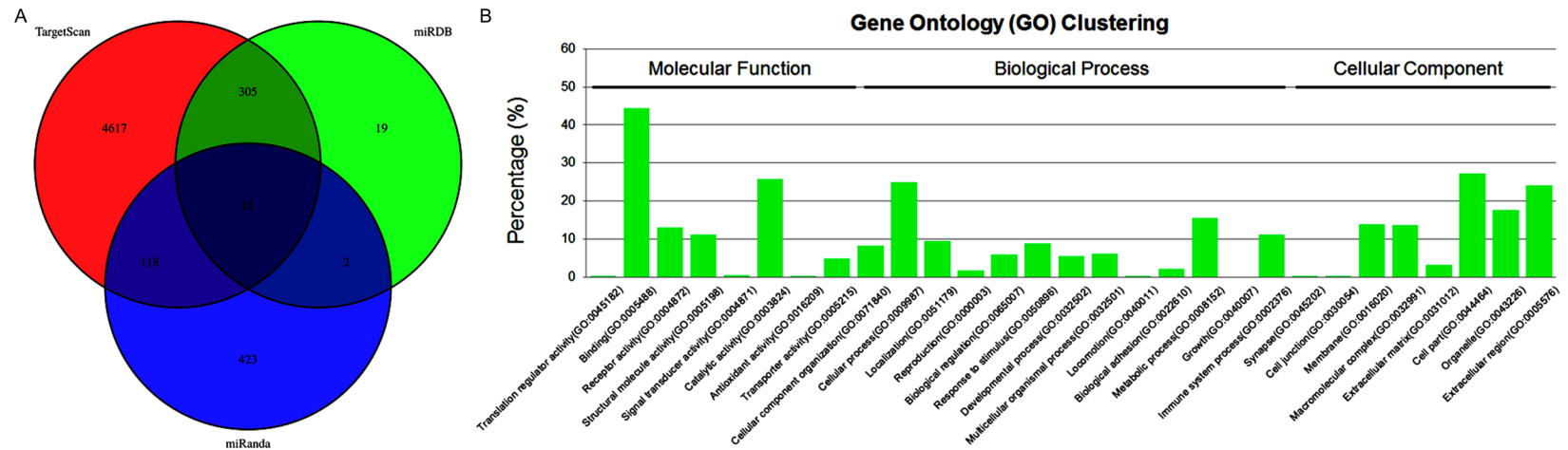


Figure 5. The cross-over analysis hsa-miR-777-3p regulated target genes by Venn image, and GO clustering. A. The cross-over analysis hsa-miR-777-3p regulated target genes by Venn image; B. The GO clustering of hsa-miR-777-3p regulated target genes. The images indicated that in three databases of TargetScan, miRDB, and miRanda, a total of five cross-over genes were identified, and involved in several significant molecular function, biological process, and cellular component.

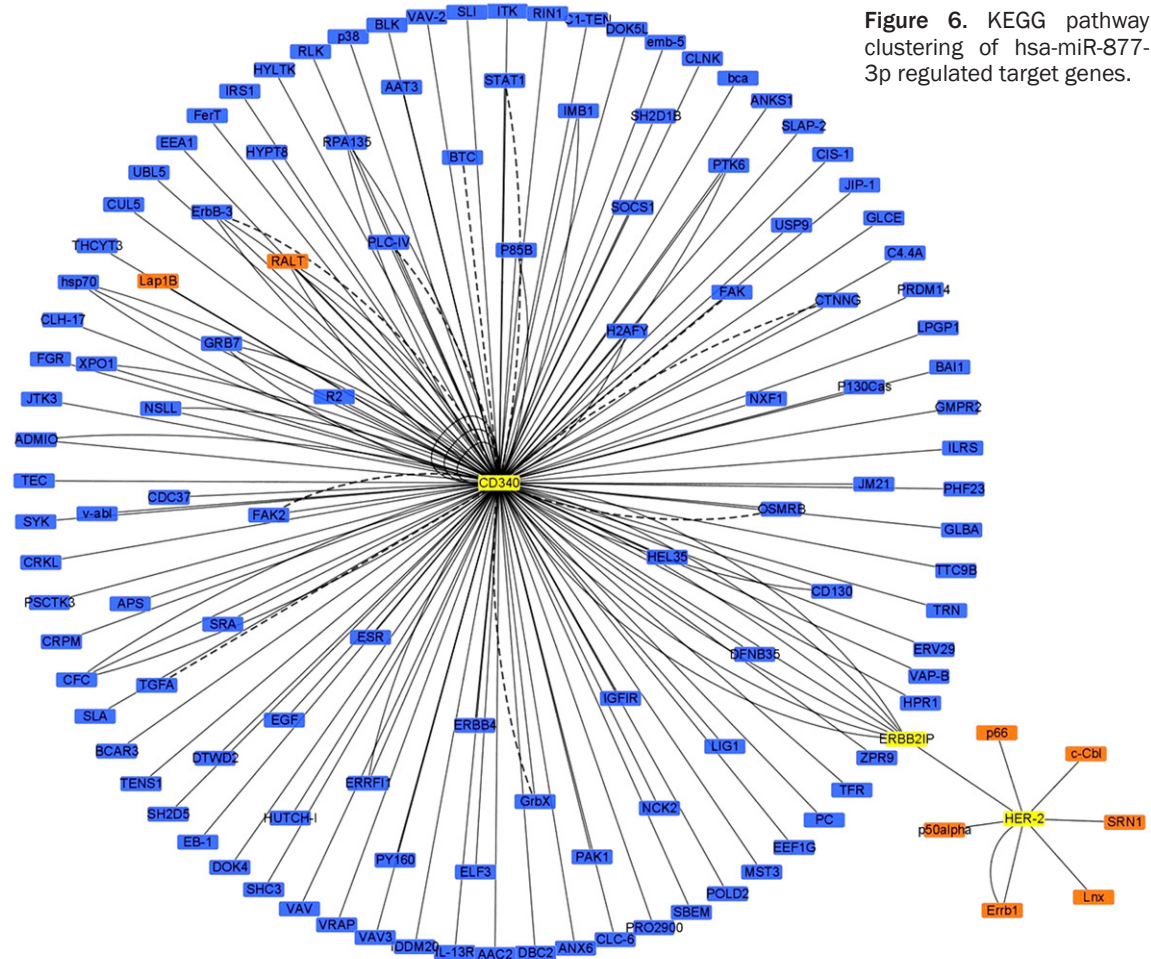


Figure 6. KEGG pathway clustering of hsa-miR-877-3p regulated target genes.

tion, the HER-2 could significantly express in MCF-7 cells, and subsequently subcellular location indicated it was mainly located in the cell membrane as previous study described (Data was not provided). In addition, to knock-down the endogenous HER-2 gene, the BT474 cell strain with HER-2 gene expression was chosen. After transfection, the HER-2 could significantly knockdown in BT474 cells.

miRNAs are a kind of no-coding RNA with length of 19-25 nt, and could be down-regulated the target gene expression in transcription and translation level, and as several previous studies documented, some miRNAs may be closely associated with HER-2 in breast cancer patients [28, 44, 45]. For instance, hsa-miR-205 and has-miR-497 could significantly inhibit the expression of HER-2 to intervene the invasion and the tumorigenicity of breast cancer [34, 46], and the over-expressed hsa-miR-

125a/hsa-miR-125b could inhibited the activity of HER-2 over-expressed breast cancer cells [35], etc. In this study, several differential expression miRNAs were identified, and indicated that with the over-expression of HER-2, a total of 217 miRNAs containing 123 up-regulated miRNAs and 94 down-regulated miRNAs were significantly changed, and also with the intervention of HER-2, a total of 374 miRNAs containing 121 up-regulated miRNAs and 253 down-regulated miRNAs were significantly changed. Wherein, a total of 10 miRNAs were up-regulated with HER-2 overexpression in MCF-7 cells, and down-regulated with HER-2 intervention in BT474 cells synchronously, and 17 miRNAs were down-regulated with HER-2 overexpression in MCF-7 cells, and up-regulated with HER-2 intervention in BT474 cells synchronously. To further identifying the regulation network, based on our previous study and several literature report, 1 down-regulated miRNAs, hsa-

Differential expression profile analysis of miRNAs in breast cancer cells

miR-877-3p, was chosen, and detected by RT-qPCR. In accordance with gene-chip results demonstrating, the expression of hsa-miR-877-3p was significantly decreased with HER-2 gene over-expression, and significantly increased with HER-2 intervention, and involved in several biological process as GO and KEGG pathway analysis.

This study using miRNA gene-chip screened several significantly miRNA in breast cancer cells after HER-2 overexpression and intervention, and implied several potentially regulated process of miRNA to HER-2, and exhibited a significant application value in clinic.

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Disclosure of conflict of interest

None.

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References

- [1] Mauro GP, de Andrade Carvalho H, Stuart SR, Mano MS and Marta GN. Effects of locoregional radiotherapy in patients with metastatic breast cancer. *Breast* 2016; 28: 73-78.
- [2] Bedognetti D, Maccalli C, Bader SB, Marincola FM and Seliger B. Checkpoint inhibitors and their application in breast cancer. *Breast Care (Basel)* 2016; 11: 108-115.
- [3] Han Y, Sun S, Zhao M, Zhang Z, Gong S, Gao P, Liu J, Zhou J, Ma D, Gao Q and Wu P. CYC1 predicts poor prognosis in patients with breast cancer. *Dis Markers* 2016; 2016: 3528064.
- [4] Garcia-Fernandez A, Barco I, Fraile M, Lain JM, Carmona A, Gonzalez S, Pessarrodona A, Gimenez N and Garcia-Font M. Factors predictive of mortality in a cohort of women surgically treated for breast cancer from 1997 to 2014. *Int J Gynaecol Obstet* 2016; 134: 212-216.
- [5] Denkert C and Schutz F. Immunological mechanisms in breast cancer - from bench to bedside. *Breast Care (Basel)* 2016; 11: 93-94.
- [6] Feiten S, Dunnebacke J, Friesenhahn V, Heymanns J, Koppler H, Meister R, Thomalla J, van Roye C, Wey D and Weide R. Follow-up reality for breast cancer patients - standardised survey of patients and physicians and analysis of treatment data. *Geburtshilfe Frauenheilkd* 2016; 76: 557-563.
- [7] Thill M, Pisa G and Isbary G. Targets for neoadjuvant therapy - The Preferences of patients with early breast cancer. *Geburtshilfe Frauenheilkd* 2016; 76: 551-556.
- [8] Marchese A, Barbieri R, Pesce M, Franchelli S and De Maria A. Breast implant infection due to *Paenibacillus residui* in a cancer patient. *Clin Microbiol Infect* 2016; 22: 743-4.
- [9] De Marchi T, Foekens JA, Umar A and Martens JW. Endocrine therapy resistance in estrogen receptor (ER)-positive breast cancer. *Drug Discov Today* 2016; 21: 1181-1188.
- [10] Kwa M, Plottel CS, Blaser MJ and Adams S. The intestinal microbiome and estrogen receptor-positive female breast cancer. *J Natl Cancer Inst* 2016; 108.
- [11] Holst F. Estrogen receptor alpha gene amplification in breast cancer: 25 years of debate. *World J Clin Oncol* 2016; 7: 160-173.
- [12] Piasecka D, Skladanowski AC, Kordek R, Romanska HM and Sadej R. [Aspects of progesterone receptor (PR) activity regulation - impact on breast cancer progression]. *Postepy Biochem* 2015; 61: 198-206.
- [13] Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Birrell SN, Bruna A, Saadi A, Menon S, Hadfield J, Pugh M, Raj GV, Brown GD, D'Santos C, Robinson JL, Silva G, Launchbury R, Perou CM, Stingl J, Caldas C, Tilley WD and Carroll JS. Corrigendum: Progesterone receptor modulates ERalpha action in breast cancer. *Nature* 2015; 526: 144.
- [14] Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AA, Birrell SN, Bruna A, Saadi A, Menon S, Hadfield J, Pugh M, Raj GV, Brown GD, D'Santos C, Robinson JL, Silva G, Launchbury R, Perou CM, Stingl J, Caldas C, Tilley WD and Carroll JS. Progesterone receptor modulates ERalpha action in breast cancer. *Nature* 2015; 523: 313-317.
- [15] Asif HM, Sultana S, Ahmed S, Akhtar N and Tariq M. HER-2 positive breast cancer - a mini-review. *Asian Pac J Cancer Prev* 2016; 17: 1609-1615.
- [16] Jonjic N, Mustac E, Tomic S, Razumovic JJ, Sarcevic B, Blazicevic V, Labinac LP, Svagelj D, Kopjar A, Sikic NL, Vrbic B and Boric I. Interlaboratory concordance in Her-2 positive breast cancer. *Acta Clin Croat* 2015; 54: 479-485.
- [17] Ishikawa T, Ichikawa Y, Shimizu D, Sasaki T, Tanabe M, Chishima T, Takabe K and Endo I. The

Differential expression profile analysis of miRNAs in breast cancer cells

- role of HER-2 in breast cancer. *J Surg Sci* 2014; 2: 4-9.
- [18] Li MH, Hou CL, Wang C and Sun AJ. HER-2, ER, PR status concordance in primary breast cancer and corresponding metastatic lesion in lymph node in Chinese women. *Pathol Res Pract* 2016; 212: 252-257.
- [19] Nishimura R, Okamoto N, Satou M, Kojima K and Tanaka S. HER 2 immunohistochemistry for breast cancer cell blocks can be used in the same way as that used for histological specimens. *Diagn Cytopathol* 2016; 44: 274-279.
- [20] Jia H, Jia W, Yang Y, Li S, Feng H, Liu J, Rao N, Jin L, Wu J, Gu R, Zhu L, Chen K, Deng H, Zeng Y, Liu Q, Song E and Su F. HER-2 positive breast cancer is associated with an increased risk of positive cavity margins after initial lumpectomy. *World J Surg Oncol* 2014; 12: 289.
- [21] Kim JY, Jung WH and Koo JS. Expression of autophagy-related proteins according to androgen receptor and HER-2 status in estrogen receptor-negative breast cancer. *PLoS One* 2014; 9: e105666.
- [22] Quintela-Fandino M, Urruticochea A, Guerra J, Gil M, Gonzalez-Martin A, Marquez R, Hernandez-Agudo E, Rodriguez-Martin C, Gil-Martin M, Bratos R, Escudero MJ, Vlassak S, Hilberg F and Colomer R. Phase I clinical trial of nintedanib plus paclitaxel in early HER-2-negative breast cancer (CNIO-BR-01-2010/GEICAM-2010-10 study). *Br J Cancer* 2014; 111: 1060-1064.
- [23] Cao XZ, Xiang HL, Quan MF and He LH. Inhibition of cell growth by BrMC through inactivation of Akt in HER-2/neu-overexpressing breast cancer cells. *Oncol Lett* 2014; 7: 1632-1638.
- [24] Singh R, Gupta S, Pawar SB, Pawar RS, Gandham SV and Prabhudesai S. Evaluation of ER, PR and HER-2 receptor expression in breast cancer patients presenting to a semi urban cancer centre in Western India. *J Cancer Res Ther* 2014; 10: 26-28.
- [25] Garcia-Munoz C, Cortijo-Cascajares S, Canamares-Orbis I, Goyache-Goni MP and Ferrari-Piquero JM. [Lapatinib plus trastuzumab for HER-2 positiva metastatic breast cancer: experience of use]. *Farm Hosp* 2014; 38: 130-134.
- [26] Liang AL, Zhang TT, Zhou N, Wu CY, Lin MH and Liu YJ. MiRNA-10b sponge: An anti-breast cancer study in vitro. *Oncol Rep* 2016; 35: 1950-1958.
- [27] Ruiz Esparza-Garrido R, Torres-Marquez ME, Viedma-Rodriguez R, Velazquez-Wong AC, Salamanca-Gomez F, Rosas-Vargas H and Velazquez-Flores MA. Breast cancer cell line MDA-MB-231 miRNA profile expression after BIK interference: BIK involvement in autophagy. *Tumour Biol* 2016; 37: 6749-6759.
- [28] Zaleska K. miRNA-Therapeutic tool in breast cancer? Where are we now? *Rep Pract Oncol Radiother* 2015; 20: 79-86.
- [29] Lv J, Xia K, Xu P, Sun E, Ma J, Gao S, Zhou Q, Zhang M, Wang F, Chen F, Zhou P, Fu Z and Xie H. miRNA expression patterns in chemoresistant breast cancer tissues. *Biomed Pharmacother* 2014; 68: 935-942.
- [30] Mulrane L, McGee SF, Gallagher WM and O'Connor DP. miRNA dysregulation in breast cancer. *Cancer Res* 2013; 73: 6554-6562.
- [31] Riaz M, van Jaarsveld MT, Hollestelle A, Prager-van der Smissen WJ, Heine AA, Boersma AW, Liu J, Helmijs J, Ozturk B, Smid M, Wiemer EA, Foekens JA and Martens JW. miRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs. *Breast Cancer Res* 2013; 15: R33.
- [32] Vrba L, Munoz-Rodriguez JL, Stampfer MR and Futscher BW. miRNA gene promoters are frequent targets of aberrant DNA methylation in human breast cancer. *PLoS One* 2013; 8: e54398.
- [33] Martin EC, Bratton MR, Zhu Y, Rhodes LV, Tilghman SL, Collins-Burow BM and Burow ME. Insulin-like growth factor-1 signaling regulates miRNA expression in MCF-7 breast cancer cell line. *PLoS One* 2012; 7: e49067.
- [34] Luo Q, Li X, Gao Y, Long Y, Chen L, Huang Y and Fang L. MiRNA-497 regulates cell growth and invasion by targeting cyclin E1 in breast cancer. *Cancer Cell Int* 2013; 13: 95.
- [35] Karbasy SH, Taheriazam A, Mirghasemi A, Sedaghati F, Shakeri M, Yahaghi E and Bahador R. Upregulation of miR-300 and downregulation of miR-125b act as potential predictor biomarkers in progression, metastasis, and poor prognosis of osteosarcoma. *Tumour Biol* 2015; [Epub ahead of print].
- [36] Zhao D, Sui Y and Zheng X. MiR-331-3p inhibits proliferation and promotes apoptosis by targeting HER2 through the PI3K/Akt and ERK1/2 pathways in colorectal cancer. *Oncol Rep* 2016; 35: 1075-1082.
- [37] Liu XH, Sun M, Nie FQ, Ge YB, Zhang EB, Yin DD, Kong R, Xia R, Lu KH, Li JH, De W, Wang KM and Wang ZX. Lnc RNA HOTAIR functions as a competing endogenous RNA to regulate HER2 expression by sponging miR-331-3p in gastric cancer. *Mol Cancer* 2014; 13: 92.
- [38] Epis MR, Giles KM, Barker A, Kendrick TS and Leedman PJ. miR-331-3p regulates ERBB-2 expression and androgen receptor signaling in prostate cancer. *J Biol Chem* 2009; 284: 24696-24704.
- [39] Avci CB, Susluer SY, Caglar HO, Balci T, Aygunes D, Dodurga Y and Gunduz C. Genistein-

Differential expression profile analysis of miRNAs in breast cancer cells

- induced mir-23b expression inhibits the growth of breast cancer cells. *Contemp Oncol (Pozn)* 2015; 19: 32-35.
- [40] Jin L, Wessely O, Marcusson EG, Ivan C, Calin GA and Alahari SK. Prooncogenic factors miR-23b and miR-27b are regulated by Her2/Neu, EGF, and TNF-alpha in breast cancer. *Cancer Res* 2013; 73: 2884-2896.
- [41] Fragni M, Bonini SA, Bettinsoli P, Bodei S, Generali D, Bottini A, Spano PF, Memo M and Sigala S. The miR-21/PTEN/Akt signaling pathway is involved in the anti-tumoral effects of zoledronic acid in human breast cancer cell lines. *Naunyn Schmiedebergs Arch Pharmacol* 2016; 389: 529-538.
- [42] Breast cancer risk variants affect estrogen receptor. *Cancer Discov* 2016; 6: OF3.
- [43] Alves C, Mendes D, Andrade S and Batel MF. The benefit of Her-2 targeted therapies on overall survival of patients with metastatic breast cancer-a systematic review. *Value Health* 2014; 17: A620.
- [44] Yang F, Lyu S, Dong S, Liu Y, Zhang X and Wang O. Expression profile analysis of long noncoding RNA in HER-2-enriched subtype breast cancer by next-generation sequencing and bioinformatics. *Oncotargets Ther* 2016; 9: 761-772.
- [45] Yang F, Wang Y, Xue J, Ma Q, Zhang J, Chen YF, Shang ZZ, Li QQ, Zhang SL and Zhao L. Effect of Corilagin on the miR-21/smad7/ERK signaling pathway in a schistosomiasis-induced hepatic fibrosis mouse model. *Parasitol Int* 2016; 65: 308-315.
- [46] Wang Z, Liao H, Deng Z, Yang P, Du N, Zhang Y and Ren H. miRNA-205 affects infiltration and metastasis of breast cancer. *Biochem Biophys Res Commun* 2013; 441: 139-143.