OncoTargets and Therapy

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ORIGINAL RESEARCH

Construction of prognostic microRNA signature for human invasive breast cancer by integrated analysis

This article was published in the following Dove Medical Press journal:
OncoTargets and Therapy

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Background: Despite the advances in early detection and treatment methods, breast cancer still has a high mortality rate, even in those patients predicted to have a good prognosis. The purpose of this study is to identify a microRNA signature that could better predict prognosis in breast cancer and add new insights to the current classification criteria.

Materials and methods: We downloaded microRNA sequencing data along with corresponding clinicopathological data from The Cancer Genome Atlas (TCGA). Of 1,098 breast cancer patients identified, 253 patients with fully characterized microRNA profiles were selected for analysis. A three-microRNA signature was generated in the training set. Subsequently, the performance of the signature was confirmed in a validation set. After construction of the signature, we conducted additional experiments, including flow cytometry and the Cell Counting Kit-8 assay, to illustrate the correlation of this microRNA signature with breast cancer cell cycle, apoptosis, and proliferation.

Results: Three microRNAs (*hsa-mir-31*, *hsa-mir-16-2*, and *hsa-mir-484*) were identified to be significantly and independently correlated with patient prognosis, and performed with good stability. Our results suggest that higher expression of *hsa-mir-484* indicated worse prognosis, while higher expression of *hsa-mir-31* and *hsa-mir-16-2* indicated better prognosis. Moreover, additional experiments confirmed that this microRNA signature was related to breast cancer cell cycle and proliferation.

Conclusion: Our results indicate a three-microRNA signature that can accurately predict the prognosis of breast cancer, especially in basal-like and hormone receptor-positive breast cancer subtypes. We recommend more aggressive therapy and more frequent follow-up for high-risk groups.

Keywords: microRNA, breast cancer, TCGA, prognosis

Introduction

Breast cancer is one of the most common malignancies among women, and despite the discovery of early detection methods and effective treatment therapies, it is still the second leading cause of cancer-related death in females.[1] Breast cancer is a group of molecularly distinct neoplasms classified into four main subgroups based on their expression of estrogen receptor (ER),[2] progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2). These subgroups require different treatment therapies and experience different clinical outcomes. However, even within the subgroups, there are different subsets of genetic and epigenetic abnormalities leading to different patient prognoses;[3] thus, more research is needed to understand the mechanisms related to the prognosis within different breast cancer subgroups.

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http://dx.doi.org/10.2147/OTT.S189265

MicroRNAs are a class of endogenously expressed small, single-stranded, non-coding RNAs. Over the past decade, the aberrant expression of microRNAs has been increasingly reported in human cancers and has often been associated with diagnosis,[4] prognosis, and response to clinical therapies.[5] They are involved in the post-transcriptional regulation of gene expression via base pairing with target mRNAs (usually in the 3′ untranslated region), causing degradation and translation repression of mRNAs.[6] MicroRNAs are now widely regarded as the most powerful regulators of gene expression in complex cellular processes including cancer cell proliferation, metastasis, migration, and apoptosis.[7] Of particular importance is the association with cancer cell proliferation and metastasis, as these are two hallmarks of malignancy and the leading causes of cancer-related death.[5] In addition, many studies have shed light on tumor-targeting therapies using microRNAs as novel diagnostic and therapeutic tools.[8,9]

The Cancer Genome Atlas (TCGA) project provides researchers with a set of comprehensive tools that can be used to analyze clinical and genetic signatures of a variety of cancers including breast carcinoma. In this study, we retrieved breast carcinoma data from TCGA to construct a three-microRNA signature that can be used to predict the prognosis of breast cancer, and we verified the signature using both statistical and experimental methods.

Materials and methods

TCGA breast invasive carcinoma data set

The clinical information and expression levels from 1,158 microRNAs of 1,098 patients with breast as the primary cancer site were downloaded from TCGA (https://cancergenome.nih.gov/) on May 4, 2017. Patients were screened by the following criteria for inclusion: 1) the patients were female; 2) the patients had no preoperative treatment; 3) the patients' sample types were primary tumor; 4) the patients had fully characterized microRNA profiles; and 5) the percentage of necrosis in samples was <40% on both the top and bottom slides. Patients who were alive but missing the date of last contact were excluded. A total of 253 breast invasive carcinoma patients were identified for further study according to the selection criteria. The total set was randomly separated into a training set (153 patients) and a validation set (100 patients).

Construction and validation of the integrated microRNA signature

The microRNA signature was constructed in the training set. A total of 1,158 microRNA expression levels were presented as reads per million (RPM) microRNA mapped data. Any microRNA expression level reads where microRNAs equaled 0 RPM in >40% observations were excluded. After transformation into binary variables according to the median expression level, univariate Cox models were generated for preliminary screening of microRNAs that were significantly correlated with overall survival (OS). A cut-off *P*-value of <0.05 was used to filter out significant parameters. Clinical characteristics that were previously reported to be associated with prognosis, including age at diagnosis, N stage, T stage, metastasis, ER, PR, and Her2, were also similarly evaluated in the univariate Cox models. We then generated general multivariate stepwise Cox regression models to determine which of the significant microRNA identified by univariate proportional hazards regression was an independent predictor of prognosis. OS time was calculated from the date of the initial pathological diagnosis to the date of death.

The permutation test was used to evaluate the performance and randomness of the final multivariate model. Using the combination of patient OS time and vital status as a label, each patient was assigned a label and risk score under the microRNA scoring system. A random system was constructed by assigning labels while the risk score was kept consistent within each individual. The random system was tested for significance in predicting survival. If the model performed well, the random system was not a predictor of prognosis, and the area under the curve (AUC) of the receiver operating characteristics (ROC) curve would approach 0.5. We generated 1,000 random systems. A cut-off *P*-value of <0.05 was used to indicate a significant association between AUCs of the random system and the label system. We would conclude that the label system had no effect on outcome unless the calculated *P*-value was smaller than 0.05. A validation set containing 100 patients was used to test the prognostic value of the microRNA signature. These analyses were performed using R software (version 3.3.2, https://www.r-project.org/).

Bioinformatics analysis

Targetscan7.1 (http://www.targetscan.org/vert_71/), DIANA-microT,[10] miRWalk,[11] miRanda (http://www.microrna.org/microrna/home.do), PicTar (http://www.pictar.org/), and miRDB[12] were used to identify the target genes of three microRNAs. To increase accuracy, only target genes predicted by a minimum of three programs were retained for further analysis. Lists of target genes were submitted to DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/)

to annotate the biological functions of the candidate microRNAs. Subsequently, Gene Ontology (GO) function, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis,[13] and PANTHER™ Version 11 analyses were conducted. Pathways with fold enrichment >1.5 and $P<0.05$ were considered to be of interest.[14]

Cell lines and culturing method

After evaluating qRT-PCR (data not shown) for the expression of the three microRNAs together with our statistical analysis results, we ultimately chose the cell line MDA-MB-231 to continue further study. MDA-MB-231 was obtained from the American Type Culture Collection (Manassas, VA, USA), cultured according to the instructions, and used within 6 months after recovery from liquid nitrogen.

Transfection, cell proliferation assay, and flow cytometry

Cells were plated in six-well plates, transfected with microRNA mimic, microRNA inhibitor, and their corresponding negative controls using Lipofectamine™ 3000 Transfection Reagent (Thermo Fisher Scientific, Waltham, MA, USA) following established protocols (transfection efficiency was at least 60% as confirmed by qRT-PCR; data not shown). All microRNA oligonucleotides were synthesized by RiboBio (Guangzhou, China) and quantification was performed with a stem-loop real-time PCR microRNA kit (RiboBio, Guangzhou, China). Transfected MDA-MB-231 was seeded at a density of 5×10^3 cells per well into 96-well plates and incubated at 37°C for 72 hours. Cell viability was assessed using the Cell-Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan); absorbance values were determined at 450 nm using a microplate spectrophotometer. Flow cytometry was performed using propidium iodide (PI) staining solution (Chinese Academy of Sciences, Shanghai, China) and Annexin V: fluorescein isothiocyanate (FITC) Apoptosis Detection Kit I (BD Bioscience) following the instructions provided.

Statistical analyses

Apart from the above methods, other statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Survival analysis was conducted using the Kaplan–Meier method with the log-rank test. Means ± SDs of continuous variables were calculated from at least three independent experiments. Student's *t*-test was used to compare groups and Pearson's chi-squared test to assess the correlation between variables. All statistical tests were two-sided and a *P*-value <0.05 was considered statistically significant.

Table 1 Univariate Cox analysis of 1,158 microRNAs

MicroRNA	*P*-value	Coefficient	Type
hsa-mir-31	0.008361862	–0.625612446	Protective
hsa-mir-16-2	0.007335068	–0.629745321	Protective
hsa-mir-484	0.007238498	0.636249043	Increased risk
hsa-mir-877	0.00619359	0.652427525	Increased risk
hsa-let-7b	0.00126726	–0.781058038	Protective
hsa-mir-937	0.001580468	0.777204799	Increased risk

Results

Construction of microRNA prognostic signature

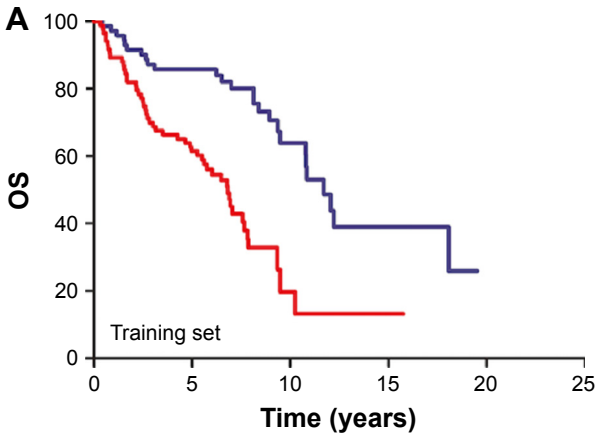
Six microRNAs were identified as prognostic markers after univariate Cox model screening (Table 1). Three microRNAs (*hsa-mir-31*, *hsa-mir-16-2*, and *hsa-mir-484*) were identified to be independently correlated with patient prognosis in multivariate Cox regression (Table 2); higher expression of *hsa-mir-484* indicated worse prognosis, while higher expression of *hsa-mir-31* and *hsa-mir-16-2* indicated improved prognosis. The β-coefficients (microRNA weight on OS) and status of every selected microRNA were used to calculate the risk score, as follows: risk score = (0.494 * Status of *hsa-mir-484*) – (0.786 * Status of *hsa-mir-16-2*) – (0.620 * Status of *hsa-mir-31*). The patients were assigned to the high-risk group if their risk score was greater than the median; otherwise, they were assigned to the low-risk group.

Performance of microRNA signature

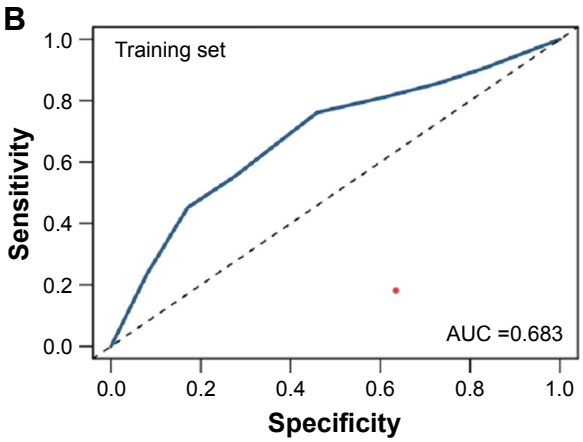
The Kaplan–Meier and ROC analyses were applied to test the performance of the three-microRNA signature in the training set. The patients in the high-risk group had significantly worse OS than those in the low-risk group ($P<0.0001$) (Figure 1A). The AUC of the signature was 0.683 (Figure 1B). These results confirmed that the three-microRNA signature was powerful enough to divide breast cancer patients into high-risk and low-risk groups.

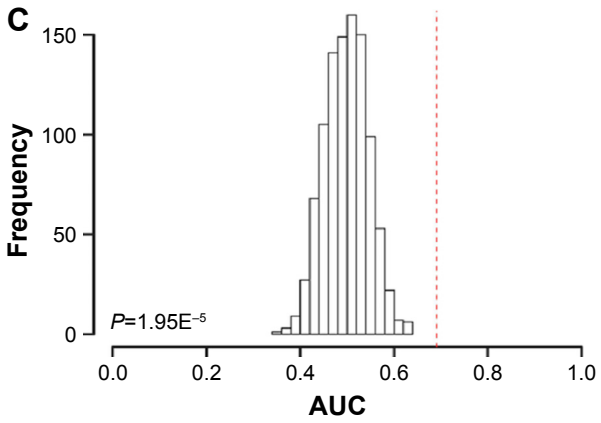
Table 2 Multivariate Cox analysis of 1,158 microRNAs

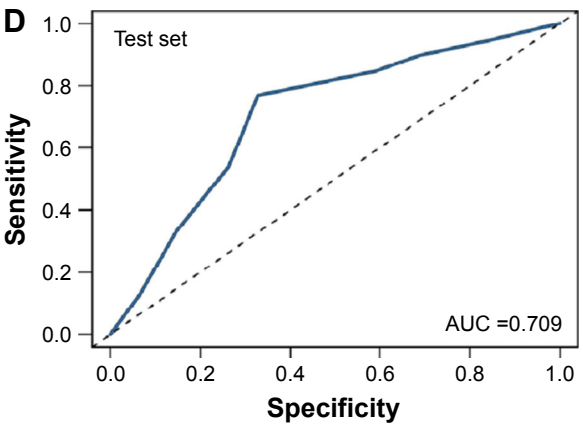
MicroRNA	*P*-value	Coefficient	Type
hsa-mir-31	0.011486	–0.62045	Protective
hsa-mir-16-2	0.001398	–0.78621	Protective
hsa-mir-484	0.042246	0.493782	Increased risk

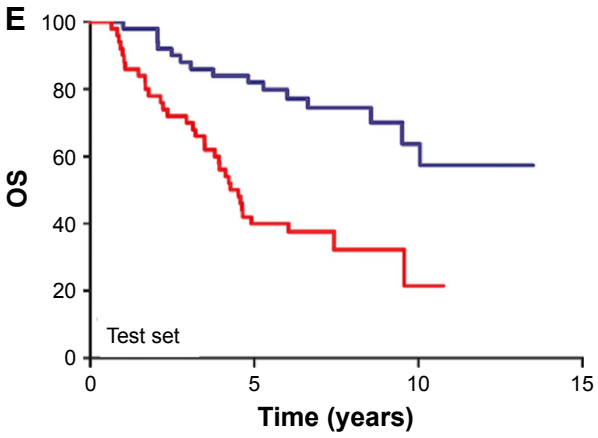


	N	Median (year)	Log-rank *P*
Low risk	70	11.707	*P*<0.0001
High risk	83	6.803	









	N	Median (year)	Log-rank *P*
Low risk	50	–	*P*<0.0001
High risk	50	4.400	

Figure 1 (**A**) Kaplan–Meier analysis of OS in the training set: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test ($P<0.0001$); (**B**) ROC curve of the training set. (**C**) The permutation test found that the AUC of the random system showed great significance with high-risk and low-risk groups ($P=1.95E-05$); (**D**) ROC curves of the validation set, AUC =0.709. (**E**) Kaplan–Meier analysis of OS in the test set: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test ($P<0.0001$). All of these results suggest that our three-microRNA signature can be used as a better diagnostic marker to distinguish breast cancer patients into high-risk and low-risk groups.

Abbreviations: AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristics.

Next, we conducted a permutation test and leave-one-out cross-validation (LOO-CV) to test whether the three-microRNA signature was applicable to other breast cancer patients in the test set.[15] The permutation test found that the AUC of the random system showed great significance with high-risk and low-risk groups (P=1.95E-05) (Figure 1C). In addition, the LOO-CV AUC was 0.709 (Figure 1D) and the Kaplan–Meier curve indicated that the high-risk patients had significantly worse OS ($P<0.0001$) (Figure 1E), which together validated the performance of the three-microRNA signature.

Subgroup analysis

After the construction and validation of the three-microRNA signature, we constructed Kaplan–Meier and ROC curves of OS in the total set (Figure 2). We then divided these patients into different subgroups according to their clinicopathological features to assess the performance of the three-microRNA signature in different groups.

First, the patients were separated into three groups based on their age at diagnosis (≤45 years, 46–65 years, and >65 years). In the ≤45-year-old group, the AUC of the signature was 0.715 with a Kaplan–Meier curve P-value <0.0001 (Figure 3A and D). However, in the 46–65-year-old and >65-year-old groups, the AUCs were 0.57 and 0.561, respectively, and the Kaplan–Meier curve P-values were 0.0798 and 0.422, respectively (Figure 3B, C, E, and F).

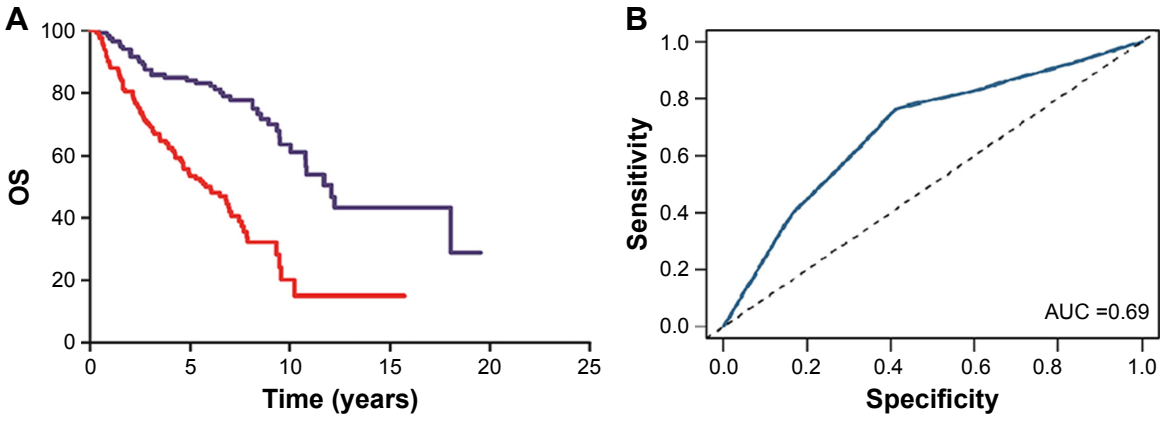
Next, we grouped the patients based on their molecular subtype. For basal-like carcinoma patients, the AUC and P-value were 0.755 and 0.003, respectively (Figure 4A and B). For luminal carcinoma patients, the AUC and P-value were 0.688 and <0.0001, respectively (Figure 4C and D). However, in the Her2-enriched subgroup, the AUC and P-value were 0.545 and 0.5532, respectively (Figure 4E and F).

Finally, we analyzed the relationship between tumor stage and the microRNA signature. In the American Joint Committee on Cancer (AJCC) stage I and II group, the AUC and P-value were 0.724 and <0.0001, respectively (Figure 5A and B); in the stage III and IV group, the AUC and P-value were 0.673 and <0.013, respectively (Figure 5C and D). There was no significant difference between these two groups.

Clinical and pathological features and microRNA signature

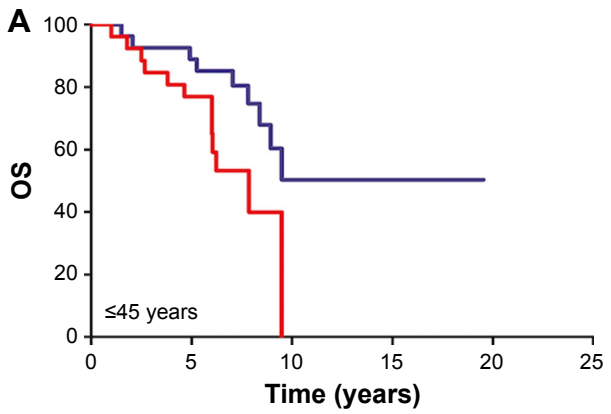
The clinical characteristics that were utilized to fit the univariate Cox model are shown in Table 3. In our study, age at diagnosis, ER status, PR status, Her2 status, and T stage were not associated with prognosis. N stage and metastasis had significant prognostic value, with P-values of 0.000 and 0.000, respectively. After adjustment for N stage and metastasis, *hsa-mir-31*, *hsa-mir-16-2*, and *hsa-mir-484* were all still independent prognostic factors (Table 4).

The correlation between patient clinicopathological characteristics and the microRNA signature is presented in Table 5. The microRNA signature was not associated with age at diagnosis, ER status, PR status, Her2 status, T stage, N stage or metastasis.

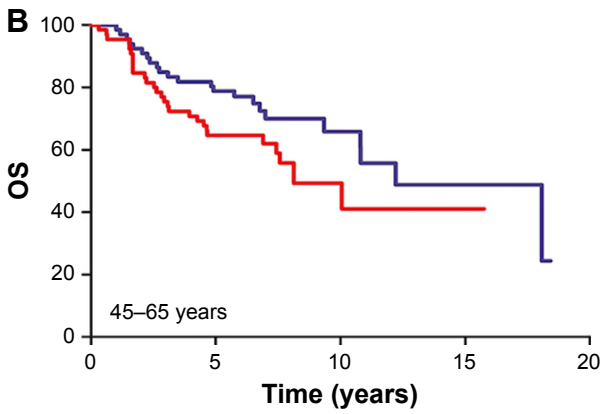


	N	Median (year)	Log-rank P
Low risk	120	12.049	P<0.0001
High risk	50	6.005	

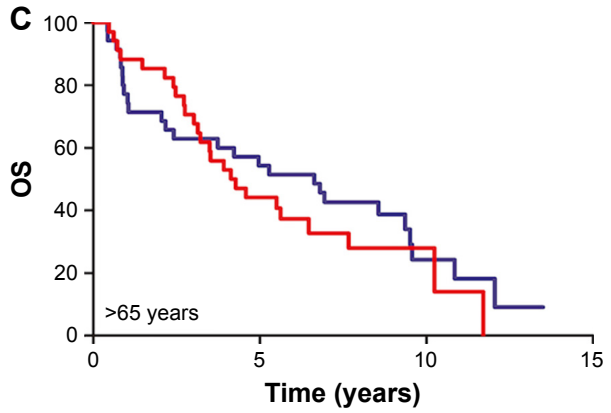
Figure 2 (**A**) Kaplan–Meier analysis of OS in the total set; (**B**) the ROC curve of the total set AUC was 0.69.
Abbreviations: AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristics.



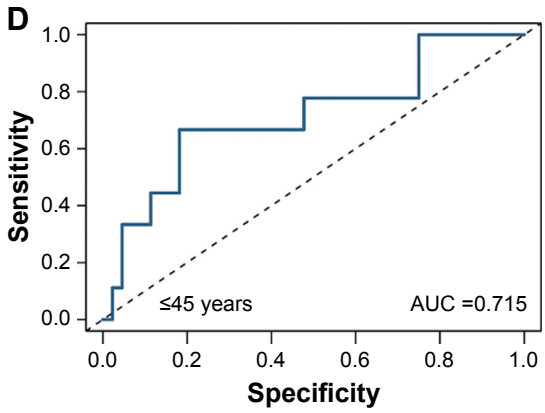
	N	Median (year)	Log-rank *P*
Low risk	27	–	*P*<0.0001
High risk	26	7.852	

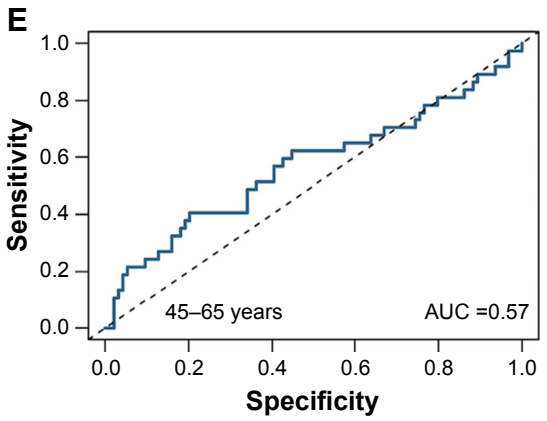


	N	Median (year)	Log-rank *P*
Low risk	66	12.21	*P*=0.0798
High risk	65	8.123	



	N	Median (year)	Log-rank *P*
Low risk	35	6.636	*P*=0.422
High risk	34	4.207	





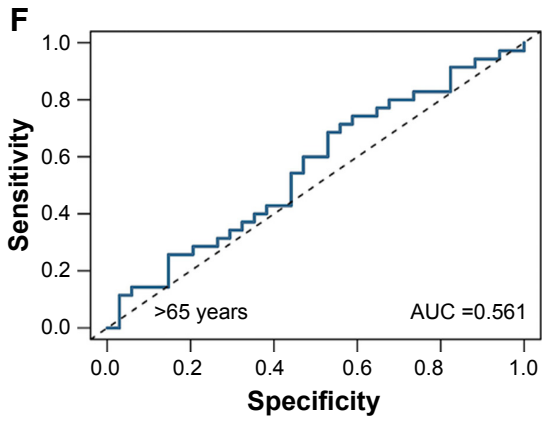
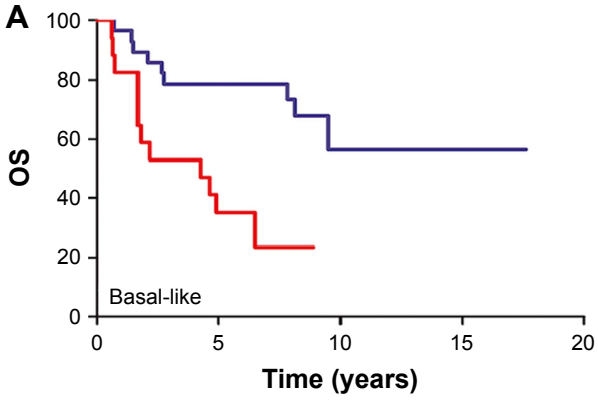
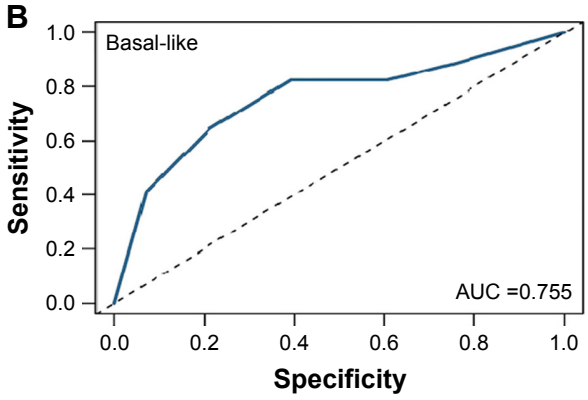


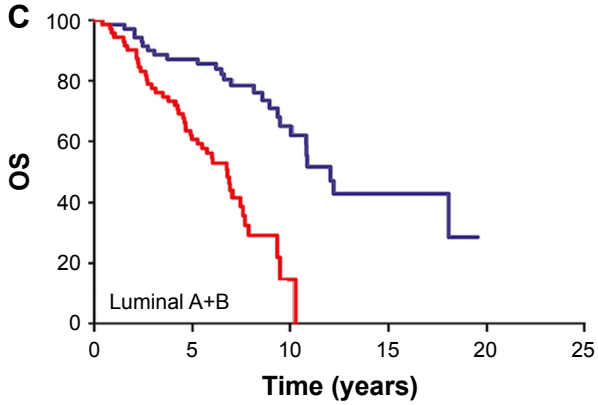
Figure 3 (**A**) Kaplan–Meier analysis of OS in the ≤45-year age group: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test (*P*<0.0001); (**B**) the ROC curve AUC was 0.715. (**C**) Kaplan–Meier analysis of OS in the 46–65-year age group: OS rates between the high-risk group and low-risk group showed no significant differences (*P*=0.0798); (**D**) the ROC curve AUC was 0.57. (**E**) Kaplan–Meier analysis of OS in the >65-year age group: OS rates between the high-risk group and low-risk group showed no significant differences (*P*=0.561); (**F**) the ROC curve AUC was 0.422. This signature performs better in younger patients (≤45 years) than older patients (>65 years).

Abbreviations: AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristics.

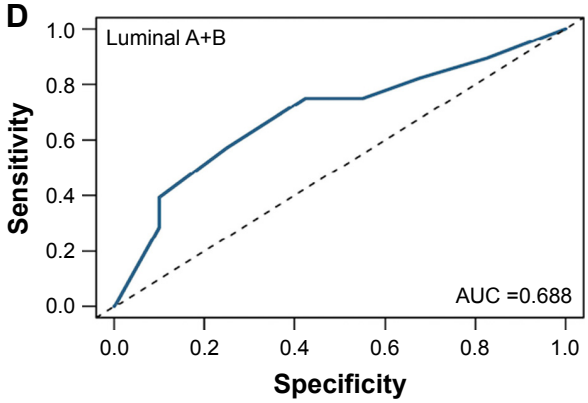


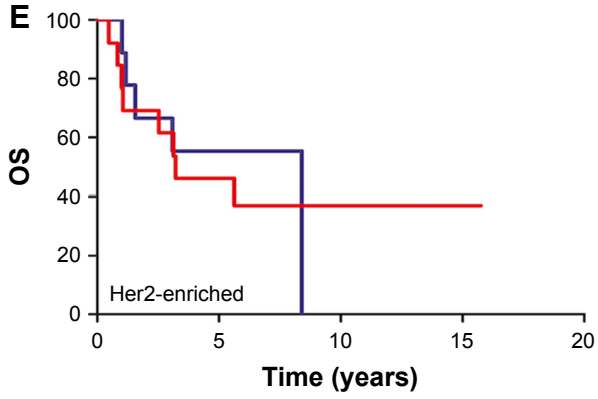
	N	Median (year)	Log-rank *P*
Low risk	28	–	*P*=0.003
High risk	17	4.263	





	N	Median (year)	Log-rank *P*
Low risk	70	12.049	*P*<0.0001
High risk	71	6.803	





	N	Median (year)	Log-rank *P*
Low risk	9	8.392	*P*=0.5532
High risk	13	3.216	

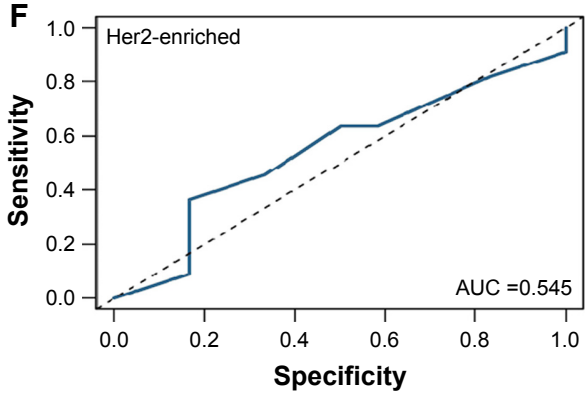
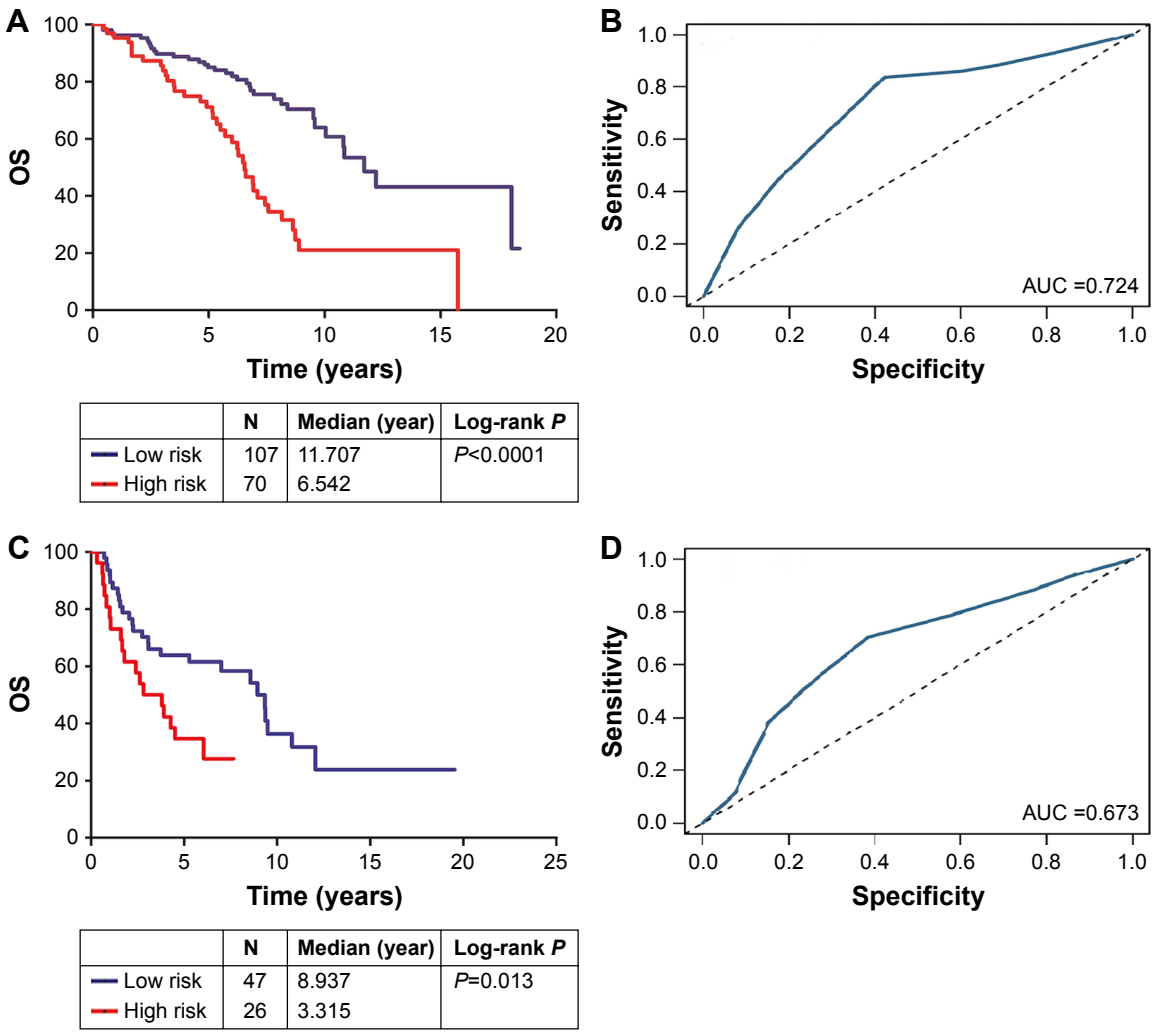


Figure 4 (**A**) Kaplan–Meier analysis of OS in the basal-like carcinoma group: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test (*P*=0.003); (**B**) the ROC curve AUC was 0.755. (**C**) Kaplan–Meier analysis of OS in the luminal carcinoma group: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test (*P*<0.0001); (**D**) the ROC curve AUC was 0.688. (**E**, **F**) Kaplan–Meier analysis of OS in the Her2-enriched subgroup showed no significant differences between the high-risk group and low-risk group; the AUC and *P*-value were 0.545 and 0.5532, respectively. This signature showed better performance in basal-like and luminal patients than in Her2-enriched patients.

Abbreviations: AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristics.



	N	Median (year)	Log-rank *P*
Low risk	107	11.707	*P*<0.0001
High risk	70	6.542	

	N	Median (year)	Log-rank *P*
Low risk	47	8.937	*P*=0.013
High risk	26	3.315	

Figure 5 (**A**) Kaplan–Meier analysis of OS in stage I and II groups: OS rates between the high-risk group and low-risk group showed statistically significant differences (*P*<0.0001); (**B**) the ROC curve AUC was 0.724. (**C**) Kaplan–Meier analysis of OS in stage III and IV groups: OS rates between the high-risk group and low-risk group showed statistically significant differences (*P*=0.0013); (**D**) the ROC curve AUC was 0.673. The performance of the signature was not associated with the AJCC stage of the patients.
Abbreviations: AJCC, American Joint Committee on Cancer; AUC, area under the curve; OS, overall survival; ROC, receiver operating characteristics.

GO annotation and KEGG pathway analysis of *hsa-mir-31*, *hsa-mir-16-2*, and *hsa-mir-484*

Target genes of *hsa-mir-16-2*, *hsa-mir-31*, and *hsa-mir-484*, as predicted by five programs, are listed in Table 6. There were 254, 149, and 336 target genes predicted by at least three programs for *hsa-mir-16-2*, *hsa-mir-31*, and *hsa-mir-484*, respectively. GO annotation analysis included biological processes, cellular components, and molecular function, as shown in Table 7 (fold enrichment >1.5, *P*<0.05). These

Table 3 Univariate Cox analysis of clinicopathological parameters

Variables	n	HR	95% CI	*P*-value
Age	253	1.448	0.905–2.317	0.112
ER	243	0.719	0.481–1.076	0.108
PR	243	0.715	0.491–1.041	0.080
Her2	232	1.165	0.693–1.958	0.565
T stage	250	1.106	0.892–1.371	0.361
N stage	251	1.471	1.205–1.795	0.000
Metastasis	238	3.260	1.787–5.948	0.000

Abbreviations: ER, estrogen receptor; Her2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Table 4 Multivariate Cox analysis of clinicopathological parameters and microRNAs

Variables	n	HR	95% CI	*P*-value
N stage	237	1.355	1.080–1.702	0.009
Metastasis	237	1.845	0.870–3.914	0.110
hsa-mir-16-2	237	0.556	0.379–0.817	0.003
hsa-mir-484	237	1.560	1.043–2.332	0.030
hsa-mir-31	237	0.486	0.333–0.711	0.000
hsa-mir-877	237	1.476	0.968–2.251	0.071
hsa-mir-937	237	1.223	0.815–1.837	0.331
hsa-let-7b	237	0.670	0.437–1.027	0.066

Table 5 Correlation between microRNA expression level and clinical pathological parameters in breast cancer patients

Parameters	Total (n)	MicroRNA score		*P*-value
		Low (n=120)	High (n=133)	
Age, years				0.791
≤45	53	26 (21.7)	27 (20.3)	
>45	200	94 (78.3)	106 (79.7)	
Missing (%)	0			
ER (%)				0.723
Negative	66	30 (45.5)	36 (54.5)	
Positive	177	85 (48.0)	92 (52.0)	
Missing	10			
PR (%)				0.778
Negative	91	42 (46.2)	49 (53.8)	
Positive	152	73 (48.0)	79 (52.0)	
Missing	10			
Her2 (FISH) (%)				0.343
Negative	197	96 (48.7)	101 (51.3)	
Positive	35	14 (40.0)	21 (60.0)	
Missing	21			
T stage (%)				0.177
T1	65	29 (44.6)	36 (55.4)	
T2	133	59 (44.4)	74 (55.6)	
T3	38	22 (57.9)	16 (42.1)	
T4	14	8 (57.1)	6 (42.9)	
Missing	3			
Nodal stage (%)				0.564
N0	114	53 (46.5)	61 (53.5)	
N1	91	49 (53.8)	42 (46.2)	
N2	32	13 (40.6)	19 (59.4)	
N3	14	5 (35.7)	9 (64.3)	
Missing	2			
Metastasis (%)				0.947
M0	225	106 (47.1)	119 (52.9)	
M1	13	6 (46.2)	7 (53.8)	
Missing	15			

Abbreviations: ER, estrogen receptor; FISH, fluorescence in situ hybridization; Her2, human epidermal growth factor receptor 2; PR, progesterone receptor.

results indicate that these candidate targets are significantly related to biosynthesis, metabolic processes, DNA binding, and system development. Furthermore, they could be protein complex or transcription factor complex components. KEGG and PANTHER analyses indicate that the candidate targets were significantly enriched in several oncogenic signaling pathways, including Hippo (P=0.0025), Wnt (P=0.000852), epidermal growth factor (EGF) receptor (P=0.00712), fibroblast growth factor (FGF) (P=0.000458), angiogenesis (P=0.003092), adherens junction (P=0.003865), and cytokine–cytokine receptor interaction (P=0.001133), as shown in Table 8. The three microRNAs are related to breast cancer cell cycle, viability, and apoptosis in vitro.

MDA-MB-231 cells were transfected according to the β-coefficient. One group was transfected with *hsa-mir-484* inhibitor, *hsa-mir-16-2* mimic, and *hsa-mir-31* mimic (low-risk group), a second group was transfected with *hsa-mir-484* mimic, *hsa-mir-16-2* inhibitor, and *hsa-mir-31* inhibitor (high-risk group), and a final group was transfected with control sequences (negative control group). Cell cycle flow cytometry showed that the cell counts of S and G2/M phase were increased in both high-risk and low-risk groups compared to the negative control group (Figure 6A–D). The CCK-8 assay showed that cell viability of the high-risk group was significantly increased compared to the control group, while the viability of the low-risk group was decreased (Figure 6E). We then used an apoptosis assay to confirm whether cell apoptosis was increased in the experimental groups. Our results revealed that the apoptosis rate was 11.07% in the high-risk group (Figure 6F) and 30.49% in low-risk group (Figure 6G), while it was 12.01% in the control group (Figure 6H).

Discussion

Accumulating evidence has shown that microRNA deregulation plays a pivotal role in multiple cellular and biological processes, including cell proliferation and cell apoptosis,[16–19] and targets a variety of pathways as oncogenes or tumor suppressors. Recently, microRNA-based anticancer therapies have been explored, either alone or in combination with other therapies.[20,21] However, only a few articles have constructed a microRNA scoring system to predict the outcome of breast carcinoma.[22,23] Here, we built a three-microRNA signature (*hsa-mir-31*, *hsa-mir-484*, and *hsa-mir-16-2*) that proved powerful enough to be an independent prognostic factor after rounds of statistical analysis.

According to our analysis, all three microRNAs target many cancer-related pathways, including the MAPK signaling pathway,[24] Hippo signaling pathway,[25] EGF receptor signaling pathway,[26] and Wnt signaling pathway;[27] some of these have been confirmed by previous studies.[28] To be specific, *hsa-mir-484* was found to be associated with poor prognosis in patients receiving gemcitabine treatment for breast cancer or sunitinib treatment for metastatic renal cell carcinoma and in ovarian cancer patients demonstrating chemosensitivity.[28–30] In addition, we found that circulating *hsa-mir-484* is significantly differentially expressed, with decreased expression in the tumor tissue and increased expression in plasma compared to healthy volunteers.[28,31–33]

Table 6 Target genes of three microRNAs

MicroRNA	Target gene	Annotation
hsa-mir-31	*NR2C2*	nuclear receptor subfamily 2 group C member 2
hsa-mir-31	*MLXIP*	MLX interacting protein
hsa-mir-31	*STAU2*	staufen double-stranded RNA binding protein 2
hsa-mir-31	*ATF7IP*	activating transcription factor 7 interacting protein
hsa-mir-31	*PRKAA2*	protein kinase AMP-activated catalytic subunit alpha 2
hsa-mir-31	*ZNF16*	zinc finger protein 16
hsa-mir-31	*RHBDL3*	rhomboid like 3
hsa-mir-31	*GPRC5A*	G protein-coupled receptor class C group 5 member A
hsa-mir-31	*ARID1A*	AT-rich interaction domain 1A
hsa-mir-31	*KHDRBS3*	KH RNA binding domain containing, signal transduction associated 3
hsa-mir-31	*UCN2*	urocortin 2
hsa-mir-31	*CTNND2*	catenin delta 2
hsa-mir-31	*KLF13*	Kruppel like factor 13
hsa-mir-31	*IQSEC2*	IQ motif and Sec7 domain 2
hsa-mir-31	*RAB6B*	RAB6B, member RAS oncogene family
hsa-mir-31	*TFRC*	transferrin receptor
hsa-mir-31	*SLC24A3*	solute carrier family 24 member 3
hsa-mir-31	*KCNN3*	potassium calcium-activated channel subfamily N member 3
hsa-mir-31	*APBB2*	amyloid beta precursor protein binding family B member 2
hsa-mir-31	*TACC2*	transforming acidic coiled-coil containing protein 2
hsa-mir-31	*NDRG3*	NDRG family member 3
hsa-mir-31	*DICER1*	dicer 1, ribonuclease III
hsa-mir-31	*SPRED1*	sprouty related EVH1 domain containing 1
hsa-mir-31	*NFAT5*	nuclear factor of activated T-cells 5
hsa-mir-31	*BAHD1*	bromo adjacent homology domain containing 1
hsa-mir-31	*RTL9*	retrotransposon Gag like 9
hsa-mir-31	*KLF7*	Kruppel like factor 7
hsa-mir-31	*PRSS8*	protease, serine 8
hsa-mir-31	*PIK3C2A*	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha
hsa-mir-31	*FNDC5*	fibronectin type III domain containing 5
hsa-mir-31	*ZNHIT6*	zinc finger HIT-type containing 6
hsa-mir-31	*BTBD11*	BTB domain containing 11
hsa-mir-31	*PHF8*	PHD finger protein 8
hsa-mir-31	*ZNF662*	zinc finger protein 662
hsa-mir-31	*TMPRSS11F*	transmembrane protease, serine 11F
hsa-mir-31	*CCNC*	cyclin C
hsa-mir-31	*FZD4*	frizzled class receptor 4
hsa-mir-31	*SATB2*	SATB homeobox 2
hsa-mir-31	*SLC43A2*	solute carrier family 43 member 2
hsa-mir-31	*RSF1*	remodeling and spacing factor 1
hsa-mir-31	*RAP2B*	RAP2B, member of RAS oncogene family
hsa-mir-31	*FMNL3*	formin like 3
hsa-mir-31	*TM9SF4*	transmembrane 9 superfamily member 4
hsa-mir-31	*PPP1R12B*	protein phosphatase 1 regulatory subunit 12B
hsa-mir-31	*SLC39A14*	solute carrier family 39 member 14
hsa-mir-31	*AKAP7*	A-kinase anchoring protein 7
hsa-mir-31	*HOXC13*	homeobox C13

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-31	*RAB14*	RAB14, member RAS oncogene family
hsa-mir-31	*PPBP*	pro-platelet basic protein
hsa-mir-31	*KIAA1429*	KIAA1429
hsa-mir-31	*KRT6C*	keratin 6C
hsa-mir-31	*FTMT*	ferritin mitochondrial
hsa-mir-31	*IGSF11*	immunoglobulin superfamily member 11
hsa-mir-31	*RSBN1*	round spermatid basic protein 1
hsa-mir-31	*SEPHS1*	selenophosphate synthetase 1
hsa-mir-31	*PDZD2*	PDZ domain containing 2
hsa-mir-31	*TBXA2R*	thromboxane A2 receptor
hsa-mir-31	*LBH*	limb bud and heart development
hsa-mir-31	*PRKCE*	protein kinase C epsilon
hsa-mir-31	*SH2D1A*	SH2 domain containing 1A
hsa-mir-31	*GXYLT1*	glucoside xylosyltransferase 1
hsa-mir-31	*LATS2*	large tumor suppressor kinase 2
hsa-mir-31	*CAMK2D*	calcium/calmodulin dependent protein kinase II delta
hsa-mir-31	*SYDE2*	synapse defective Rho GTPase homolog 2
hsa-mir-31	*KIAA1024*	KIAA1024
hsa-mir-31	*ELAVL1*	ELAV like RNA binding protein 1
hsa-mir-31	*DCBLD2*	discoidin, CUB and LCCL domain containing 2
hsa-mir-31	*MAP4K5*	mitogen-activated protein kinase kinase kinase kinase 5
hsa-mir-31	*RGS4*	regulator of G protein signaling 4
hsa-mir-31	*MAP1B*	microtubule associated protein 1B
hsa-mir-31	*PPP1R9A*	protein phosphatase 1 regulatory subunit 9A
hsa-mir-31	*PAX9*	paired box 9
hsa-mir-31	*KANK1*	KN motif and ankyrin repeat domains 1
hsa-mir-31	*WNK1*	WNK lysine deficient protein kinase 1
hsa-mir-31	*WDR5*	WD repeat domain 5
hsa-mir-31	*SLC1A2*	solute carrier family 1 member 2
hsa-mir-31	*INSC*	inscuteable homolog (Drosophila)
hsa-mir-31	*NUP153*	nucleoporin 153
hsa-mir-31	*MBOAT2*	membrane bound O-acyltransferase domain containing 2
hsa-mir-31	*RNF144A*	ring finger protein 144A
hsa-mir-31	*MYO5A*	myosin VA
hsa-mir-31	*VPS26B*	VPS26, retromer complex component B
hsa-mir-31	*TNS1*	tensin 1
hsa-mir-31	*NR5A2*	nuclear receptor subfamily 5 group A member 2
hsa-mir-31	*SLC6A6*	solute carrier family 6 member 6
hsa-mir-31	*PPP2R2A*	protein phosphatase 2 regulatory subunit Balpha
hsa-mir-31	*MGAT1*	mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
hsa-mir-31	*RHOBTB1*	Rho related BTB domain containing 1
hsa-mir-31	*IL34*	interleukin 34
hsa-mir-31	*ZNF384*	zinc finger protein 384
hsa-mir-31	*RASA1*	RAS p21 protein activator 1
hsa-mir-31	*TMED10*	transmembrane p24 trafficking protein 10
hsa-mir-31	*ZFP30*	ZFP30 zinc finger protein
hsa-mir-31	*PSMB11*	proteasome subunit beta 11

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-31	*VAV3*	vav guanine nucleotide exchange factor 3
hsa-mir-31	*CRYBG3*	crystallin beta-gamma domain containing 3
hsa-mir-31	*PEX5*	peroxisomal biogenesis factor 5
hsa-mir-31	*RETREG1*	reticulophagy regulator 1
hsa-mir-31	*PPP3CA*	protein phosphatase 3 catalytic subunit alpha
hsa-mir-31	*NUMB*	NUMB, endocytic adaptor protein
hsa-mir-31	*PC*	pyruvate carboxylase
hsa-mir-31	*CEP85L*	centrosomal protein 85 like
hsa-mir-31	*YWHAE*	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon
hsa-mir-31	*BACH2*	BTB domain and CNC homolog 2
hsa-mir-31	*EIF5*	eukaryotic translation initiation factor 5
hsa-mir-31	*VEZT*	vezatin, adherens junctions transmembrane protein
hsa-mir-31	*TACC1*	transforming acidic coiled-coil containing protein 1
hsa-mir-31	*UBE2K*	ubiquitin conjugating enzyme E2 K
hsa-mir-31	*TM9SF3*	transmembrane 9 superfamily member 3
hsa-mir-31	*SGMS1*	sphingomyelin synthase 1
hsa-mir-31	*ARHGEF2*	Rho/Rac guanine nucleotide exchange factor 2
hsa-mir-31	*COPS2*	COP9 signalosome subunit 2
hsa-mir-31	*SPARC*	secreted protein acidic and cysteine rich
hsa-mir-31	*CACNB2*	calcium voltage-gated channel auxiliary subunit beta 2
hsa-mir-31	*ZSWIM6*	zinc finger SWIM-type containing 6
hsa-mir-31	*CLCN3*	chloride voltage-gated channel 3
hsa-mir-31	*AHCYL1*	adenosylhomocysteinase like 1
hsa-mir-31	*JAZF1*	JAZF zinc finger 1
hsa-mir-31	*RIMS3*	regulating synaptic membrane exocytosis 3
hsa-mir-31	*TESK2*	testis-specific kinase 2
hsa-mir-31	*HIF1AN*	hypoxia inducible factor 1 alpha subunit inhibitor
hsa-mir-31	*KCTD20*	potassium channel tetramerization domain containing 20
hsa-mir-31	*STX12*	syntaxin 12
hsa-mir-31	*OXSR1*	oxidative stress responsive 1
hsa-mir-31	*CLOCK*	clock circadian regulator
hsa-mir-31	*EDNRB*	endothelin receptor type B
hsa-mir-31	*ATF6*	activating transcription factor 6
hsa-mir-31	*VAPB*	VAMP associated protein B and C
hsa-mir-31	*BICRA*	BRD4 interacting chromatin remodeling complex associated protein
hsa-mir-31	*VPS53*	VPS53, GARP complex subunit
hsa-mir-31	*MBNL3*	muscleblind like splicing regulator 3
hsa-mir-31	*OSBP2*	oxysterol binding protein 2
hsa-mir-31	*MFAP3*	microfibrillar associated protein 3
hsa-mir-31	*CCNT1*	cyclin T1
hsa-mir-31	*ATP8A1*	ATPase phospholipid transporting 8A1
hsa-mir-31	*SIKE1*	suppressor of IKBKE 1
hsa-mir-31	*HERPUD2*	HERPUD family member 2
hsa-mir-31	*PTGFRN*	prostaglandin F2 receptor inhibitor
hsa-mir-31	*EPC1*	enhancer of polycomb homolog 1
hsa-mir-31	*GNA13*	G protein subunit alpha 13

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-31	*RPH3A*	rabphilin 3A
hsa-mir-31	*MAP3K1*	mitogen-activated protein kinase kinase kinase 1
hsa-mir-31	*CBL*	Cbl proto-oncogene
hsa-mir-31	*JMJD8*	jumonji domain containing 8
hsa-mir-31	*STK40*	serine/threonine kinase 40
hsa-mir-31	*FZD3*	frizzled class receptor 3
hsa-mir-31	*PPP6C*	protein phosphatase 6 catalytic subunit
hsa-mir-31	*SUPT16H*	SPT16 homolog, facilitates chromatin remodeling subunit
hsa-mir-31	*EBF3*	early B-cell factor 3
hsa-mir-484	*PRR14L*	proline rich 14 like
hsa-mir-484	*NFATC2*	nuclear factor of activated T-cells 2
hsa-mir-484	*PTPRF*	protein tyrosine phosphatase, receptor type F
hsa-mir-484	*HSPG2*	heparan sulfate proteoglycan 2
hsa-mir-484	*RSPO4*	R-spondin 4
hsa-mir-484	*PLCXD2*	phosphatidylinositol specific phospholipase C X domain containing 2
hsa-mir-484	*AGAP2*	ArfGAP with GTPase domain, ankyrin repeat and PH domain 2
hsa-mir-484	*DOLPP1*	dolichyldiphosphatase 1
hsa-mir-484	*M6PR*	mannose-6-phosphate receptor, cation dependent
hsa-mir-484	*CMPK1*	cytidine/uridine monophosphate kinase 1
hsa-mir-484	*SLC46A3*	solute carrier family 46 member 3
hsa-mir-484	*AP1G1*	adaptor related protein complex 1 gamma 1 subunit
hsa-mir-484	*TBC1D16*	TBC1 domain family member 16
hsa-mir-484	*THUMPD2*	THUMP domain containing 2
hsa-mir-484	*LDLRAD3*	low density lipoprotein receptor class A domain containing 3
hsa-mir-484	*FARP1*	FERM, ARH/RhoGEF and pleckstrin domain protein 1
hsa-mir-484	*PREB*	prolactin regulatory element binding
hsa-mir-484	*DND1*	DND microRNA-mediated repression inhibitor 1
hsa-mir-484	*ANAPC11*	anaphase promoting complex subunit 11
hsa-mir-484	*SEC24C*	SEC24 homolog C, COPII coat complex component
hsa-mir-484	*SLC1A4*	solute carrier family 1 member 4
hsa-mir-484	*UPF3A*	UPF3 regulator of nonsense transcripts homolog A (yeast)
hsa-mir-484	*TBL1X*	transducin beta like 1X-linked
hsa-mir-484	*CDS1*	CDP-diacylglycerol synthase 1
hsa-mir-484	*TAGLN2*	transgelin 2
hsa-mir-484	*CD4*	CD4 molecule
hsa-mir-484	*HR*	HR, lysine demethylase and nuclear receptor corepressor
hsa-mir-484	*RPL26*	ribosomal protein L26
hsa-mir-484	*TNNI1*	troponin I1, slow skeletal type
hsa-mir-484	*IPO9*	importin 9
hsa-mir-484	*COG2*	component of oligomeric golgi complex 2
hsa-mir-484	*MAP10*	microtubule associated protein 10
hsa-mir-484	*SPOCD1*	SPOC domain containing 1
hsa-mir-484	*HIC2*	HIC ZBTB transcriptional repressor 2
hsa-mir-484	*GUCD1*	guanylyl cyclase domain containing 1
hsa-mir-484	*SGMS2*	sphingomyelin synthase 2
hsa-mir-484	*MCTP1*	multiple C2 and transmembrane domain containing 1
hsa-mir-484	*ST6GAL1*	ST6 beta-galactoside alpha-2,6-sialyltransferase 1

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*UBR2*	ubiquitin protein ligase E3 component n-recognin 2
hsa-mir-484	*NFIB*	nuclear factor I B
hsa-mir-484	*YTHDF3*	YTH N6-methyladenosine RNA binding protein 3
hsa-mir-484	*USP2*	ubiquitin specific peptidase 2
hsa-mir-484	*SEC31B*	SEC31 homolog B, COPII coat complex component
hsa-mir-484	*SH3PXD2A*	SH3 and PX domains 2A
hsa-mir-484	*SPTLC2*	serine palmitoyltransferase long chain base subunit 2
hsa-mir-484	*GLG1*	golgi glycoprotein 1
hsa-mir-484	*DCTN5*	dynactin subunit 5
hsa-mir-484	*SHANK1*	SH3 and multiple ankyrin repeat domains 1
hsa-mir-484	*S100PBP*	S100P binding protein
hsa-mir-484	*AMPD2*	adenosine monophosphate deaminase 2
hsa-mir-484	*NBPF14*	NBPF member 14
hsa-mir-484	*DACH2*	dachshund family transcription factor 2
hsa-mir-484	*ZNF341*	zinc finger protein 341
hsa-mir-484	*VAPB*	VAMP associated protein B and C
hsa-mir-484	*TRIOBP*	TRIO and F-actin binding protein
hsa-mir-484	*CCR9*	C-C motif chemokine receptor 9
hsa-mir-484	*TACR1*	tachykinin receptor 1
hsa-mir-484	*DCBLD2*	discoidin, CUB and LCCL domain containing 2
hsa-mir-484	*KALRN*	kalirin, RhoGEF kinase
hsa-mir-484	*OGDH*	oxoglutarate dehydrogenase
hsa-mir-484	*CYFIP2*	cytoplasmic FMR1 interacting protein 2
hsa-mir-484	*CYP3A43*	cytochrome P450 family 3 subfamily A member 43
hsa-mir-484	*TRPS1*	transcriptional repressor GATA binding 1
hsa-mir-484	*DCHS1*	dachsous cadherin-related 1
hsa-mir-484	*TARBP2*	TARBP2, RISC loading complex RNA binding subunit
hsa-mir-484	*NCAN*	neurocan
hsa-mir-484	*SERPINF2*	serpin family F member 2
hsa-mir-484	*EMC6*	ER membrane protein complex subunit 6
hsa-mir-484	*THPO*	thrombopoietin
hsa-mir-484	*TMEM184A*	transmembrane protein 184A
hsa-mir-484	*TRMT10B*	tRNA methyltransferase 10B
hsa-mir-484	*MLLT6*	MLLT6, PHD finger domain containing
hsa-mir-484	*ZBTB47*	zinc finger and BTB domain containing 47
hsa-mir-484	*TCEANC2*	transcription elongation factor A N-terminal and central domain containing 2
hsa-mir-484	*TEX261*	testis expressed 261
hsa-mir-484	*CLOCK*	clock circadian regulator
hsa-mir-484	*NR6A1*	nuclear receptor subfamily 6 group A member 1
hsa-mir-484	*MPRIP*	myosin phosphatase Rho interacting protein
hsa-mir-484	*TRIM66*	tripartite motif containing 66
hsa-mir-484	*MLXIP*	MLX interacting protein
hsa-mir-484	*EIF4G2*	eukaryotic translation initiation factor 4 gamma 2
hsa-mir-484	*SERTAD1*	SERTA domain containing 1
hsa-mir-484	*MBNL3*	muscleblind like splicing regulator 3
hsa-mir-484	*NEUROD4*	neuronal differentiation 4
hsa-mir-484	*DBNDD2*	dysbindin domain containing 2

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*PAX5*	paired box 5
hsa-mir-484	*IPO11*	importin 11
hsa-mir-484	*RFC5*	replication factor C subunit 5
hsa-mir-484	*GRB10*	growth factor receptor bound protein 10
hsa-mir-484	*RNF40*	ring finger protein 40
hsa-mir-484	*SORBS2*	sorbin and SH3 domain containing 2
hsa-mir-484	*CYB561D1*	cytochrome b561 family member D1
hsa-mir-484	*GAPVD1*	GTPase activating protein and VPS9 domains 1
hsa-mir-484	*SLC41A3*	solute carrier family 41 member 3
hsa-mir-484	*MAP2*	microtubule associated protein 2
hsa-mir-484	*POU2AF1*	POU class 2 associating factor 1
hsa-mir-484	*CREM*	cAMP responsive element modulator
hsa-mir-484	*HHIPL2*	HHIP like 2
hsa-mir-484	*NAGA*	alpha-N-acetylgalactosaminidase
hsa-mir-484	*RTN3*	reticulon 3
hsa-mir-484	*NPNT*	nephronectin
hsa-mir-484	*IL6R*	interleukin 6 receptor
hsa-mir-484	*RFFL*	ring finger and FYVE like domain containing E3 ubiquitin protein ligase
hsa-mir-484	*SLC25A45*	solute carrier family 25 member 45
hsa-mir-484	*WASF3*	WAS protein family member 3
hsa-mir-484	*OPN4*	opsin 4
hsa-mir-484	*FAM46B*	family with sequence similarity 46 member B
hsa-mir-484	*DBNL*	drebrin like
hsa-mir-484	*ADD2*	adducin 2
hsa-mir-484	*DPYSL3*	dihydropyrimidinase like 3
hsa-mir-484	*VTI1A*	vesicle transport through interaction with t-SNAREs 1A
hsa-mir-484	*CENPB*	centromere protein B
hsa-mir-484	*LRRC32*	leucine rich repeat containing 32
hsa-mir-484	*TOX4*	TOX high mobility group box family member 4
hsa-mir-484	*SNRNP200*	small nuclear ribonucleoprotein U5 subunit 200
hsa-mir-484	*PHF19*	PHD finger protein 19
hsa-mir-484	*FBXO31*	F-box protein 31
hsa-mir-484	*IL18BP*	interleukin 18 binding protein
hsa-mir-484	*SEMA4F*	ssemaphorin 4F
hsa-mir-484	*GTDC1*	glycosyltransferase like domain containing 1
hsa-mir-484	*COLQ*	collagen like tail subunit of asymmetric acetylcholinesterase
hsa-mir-484	*PRM1*	protamine 1
hsa-mir-484	*LMAN2L*	lectin, mannose binding 2 like
hsa-mir-484	*LPL*	lipoprotein lipase
hsa-mir-484	*WWC1*	WW and C2 domain containing 1
hsa-mir-484	*MAP3K11*	mitogen-activated protein kinase kinase kinase 11
hsa-mir-484	*ANGPT1*	angiopoietin 1
hsa-mir-484	*ZNF37A*	zinc finger protein 37A
hsa-mir-484	*SGSM2*	small G protein signaling modulator 2
hsa-mir-484	*EMX1*	empty spiracles homeobox 1
hsa-mir-484	*LENG9*	leukocyte receptor cluster member 9
hsa-mir-484	*FBXO11*	F-box protein 11

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*HNF1A*	HNF1 homeobox A
hsa-mir-484	*SPATA2L*	spermatogenesis associated 2 like
hsa-mir-484	*TXNRD3*	thioredoxin reductase 3
hsa-mir-484	*CPSF4*	cleavage and polyadenylation specific factor 4
hsa-mir-484	*NEO1*	neogenin 1
hsa-mir-484	*TCF7*	transcription factor 7 (T-cell specific, HMG-box)
hsa-mir-484	*HOXA5*	homeobox A5
hsa-mir-484	*MTF2*	metal response element binding transcription factor 2
hsa-mir-484	*PIK3CD*	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta
hsa-mir-484	*NCOA2*	nuclear receptor coactivator 2
hsa-mir-484	*RIN1*	Ras and Rab interactor 1
hsa-mir-484	*TRIM71*	tripartite motif containing 71
hsa-mir-484	*DDX31*	DEAD-box helicase 31
hsa-mir-484	*ACBD5*	acyl-CoA binding domain containing 5
hsa-mir-484	*ABR*	active BCR-related
hsa-mir-484	*GPR63*	G protein-coupled receptor 63
hsa-mir-484	*RARG*	retinoic acid receptor gamma
hsa-mir-484	*YAP1*	Yes associated protein 1
hsa-mir-484	*RANBP17*	RAN binding protein 17
hsa-mir-484	*POLD4*	DNA polymerase delta 4, accessory subunit
hsa-mir-484	*FAM160B2*	family with sequence similarity 160 member B2
hsa-mir-484	*LYSMD1*	LysM domain containing 1
hsa-mir-484	*PPARD*	peroxisome proliferator activated receptor delta
hsa-mir-484	*COL20A1*	collagen type XX alpha 1 chain
hsa-mir-484	*SCP2*	sterol carrier protein 2
hsa-mir-484	*IL20RB*	interleukin 20 receptor subunit beta
hsa-mir-484	*TMC8*	transmembrane channel like 8
hsa-mir-484	*SOX5*	SRY-box 5
hsa-mir-484	*MAPKAPK3*	mitogen-activated protein kinase-activated protein kinase 3
hsa-mir-484	*ZNF667*	zinc finger protein 667
hsa-mir-484	*GRAMD1C*	GRAM domain containing 1C
hsa-mir-484	*CRTC2*	CREB regulated transcription coactivator 2
hsa-mir-484	*SERF1B*	small EDRK-rich factor 1B
hsa-mir-484	*FLVCR2*	feline leukemia virus subgroup C cellular receptor family member 2
hsa-mir-484	*TRIM74*	tripartite motif containing 74
hsa-mir-484	*STAG3L3*	stromal antigen 3-like 3 (pseudogene)
hsa-mir-484	*PKNOX1*	PBX/knotted 1 homeobox 1
hsa-mir-484	*SOX21*	SRY-box 21
hsa-mir-484	*GLDN*	gliomedin
hsa-mir-484	*HOXC8*	homeobox C8
hsa-mir-484	*FFAR2*	free fatty acid receptor 2
hsa-mir-484	*SH2D1B*	SH2 domain containing 1B
hsa-mir-484	*KDM4A*	lysine demethylase 4A
hsa-mir-484	*BCL7B*	BCL tumor suppressor 7B
hsa-mir-484	*PCDH19*	protocadherin 19
hsa-mir-484	*SERF1A*	small EDRK-rich factor 1A
hsa-mir-484	*EIF3J*	eukaryotic translation initiation factor 3 subunit J

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*NGRN*	neugrin, neurite outgrowth associated
hsa-mir-484	*C3ORF62*	chromosome 3 open reading frame 62
hsa-mir-484	*MYCBP2*	MYC binding protein 2, E3 ubiquitin protein ligase
hsa-mir-484	*PDE11A*	phosphodiesterase 11A
hsa-mir-484	*AXIN2*	axin 2
hsa-mir-484	*BRD9*	bromodomain containing 9
hsa-mir-484	*CLCN4*	chloride voltage-gated channel 4
hsa-mir-484	*FCF1*	FCF1 rRNA-processing protein
hsa-mir-484	*SUSD5*	sushi domain containing 5
hsa-mir-484	*SP6*	Sp6 transcription factor
hsa-mir-484	*LAMB3*	laminin subunit beta 3
hsa-mir-484	*MFRP*	membrane frizzled-related protein
hsa-mir-484	*THRSP*	thyroid hormone responsive
hsa-mir-484	*MED8*	mediator complex subunit 8
hsa-mir-484	*CCDC142*	coiled-coil domain containing 142
hsa-mir-484	*FOXH1*	forkhead box H1
hsa-mir-484	*LGI4*	leucine rich repeat LGI family member 4
hsa-mir-484	*CHD8*	chromodomain helicase DNA binding protein 8
hsa-mir-484	*VLDLR*	very low density lipoprotein receptor
hsa-mir-484	*PGGHG*	protein-glucosylgalactosylhydroxylysine glucosidase
hsa-mir-484	*CSRNP1*	cysteine and serine rich nuclear protein 1
hsa-mir-484	*N4BP2L2*	NEDD4 binding protein 2 like 2
hsa-mir-484	*CYB5B*	cytochrome b5 type B
hsa-mir-484	*PROM2*	prominin 2
hsa-mir-484	*CNTFR*	ciliary neurotrophic factor receptor
hsa-mir-484	*SEMA4D*	semaphorin 4D
hsa-mir-484	*DOK4*	docking protein 4
hsa-mir-484	*TOMM5*	translocase of outer mitochondrial membrane 5
hsa-mir-484	*DKK2*	dickkopf WNT signaling pathway inhibitor 2
hsa-mir-484	*DACH1*	dachshund family transcription factor 1
hsa-mir-484	*CLEC6A*	C-type lectin domain containing 6A
hsa-mir-484	*TTC39A*	tetratricopeptide repeat domain 39A
hsa-mir-484	*TGFBRAP1*	transforming growth factor beta receptor associated protein 1
hsa-mir-484	*VCP*	valosin containing protein
hsa-mir-484	*F2RL3*	F2R like thrombin/trypsin receptor 3
hsa-mir-484	*SNN*	stannin
hsa-mir-484	*ARL15*	ADP ribosylation factor like GTPase 15
hsa-mir-484	*CNKSR3*	CNKSR family member 3
hsa-mir-484	*IGBP1*	immunoglobulin binding protein 1
hsa-mir-484	*TINF2*	TERF1 interacting nuclear factor 2
hsa-mir-484	*SMYD4*	SET and MYND domain containing 4
hsa-mir-484	*ACVR1B*	activin A receptor type 1B
hsa-mir-484	*IL21R*	interleukin 21 receptor
hsa-mir-484	*DACT3*	disheveled binding antagonist of beta catenin 3
hsa-mir-484	*PDGFA*	platelet derived growth factor subunit A
hsa-mir-484	*NUP62*	nucleoporin 62
hsa-mir-484	*TAF1L*	TATA-box binding protein associated factor 1 like

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*CDH1*	cadherin 1
hsa-mir-484	*MRFAP1L1*	Morf4 family associated protein 1 like 1
hsa-mir-484	*NDUFA2*	NADH:ubiquinone oxidoreductase subunit A2
hsa-mir-484	*CCNL1*	cyclin L1
hsa-mir-484	*COL25A1*	collagen type XXV alpha 1 chain
hsa-mir-484	*HERC3*	HECT and RLD domain containing E3 ubiquitin protein ligase 3
hsa-mir-484	*TRIM73*	tripartite motif containing 73
hsa-mir-484	*C9ORF62*	chromosome 9 open reading frame 62
hsa-mir-484	*SMUG1*	single-strand-selective monofunctional uracil-DNA glycosylase 1
hsa-mir-484	*PYGO2*	pygopus family PHD finger 2
hsa-mir-484	*PEX6*	peroxisomal biogenesis factor 6
hsa-mir-484	*CTAGE1*	cutaneous T-cell lymphoma-associated antigen 1
hsa-mir-484	*IGLON5*	IgLON family member 5
hsa-mir-484	*ESR2*	estrogen receptor 2
hsa-mir-484	*LIN28B*	lin-28 homolog B
hsa-mir-484	*CTTNBP2NL*	CTTNBP2 N-terminal like
hsa-mir-484	*GJD4*	gap junction protein delta 4
hsa-mir-484	*SREBF2*	sterol regulatory element binding transcription factor 2
hsa-mir-484	*TSTD2*	thiosulfate sulfurtransferase like domain containing 2
hsa-mir-484	*GIGYF1*	GRB10 interacting GYF protein 1
hsa-mir-484	*RETREG1*	reticulophagy regulator 1
hsa-mir-484	*SLC6A1*	solute carrier family 6 member 1
hsa-mir-484	*GTF3C4*	general transcription factor IIIC subunit 4
hsa-mir-484	*TMIE*	transmembrane inner ear
hsa-mir-484	*HIPK1*	homeodomain interacting protein kinase 1
hsa-mir-484	*HIVEP2*	human immunodeficiency virus type I enhancer binding protein 2
hsa-mir-484	*ANAPC7*	anaphase promoting complex subunit 7
hsa-mir-484	*THBD*	thrombomodulin
hsa-mir-484	*PTGER4*	prostaglandin E receptor 4
hsa-mir-484	*HOXA11*	homeobox A11
hsa-mir-484	*RHOBTB1*	Rho related BTB domain containing 1
hsa-mir-484	*IFNAR1*	interferon alpha and beta receptor subunit 1
hsa-mir-484	*JPT1*	Jupiter microtubule associated homolog 1
hsa-mir-484	*FGF1*	fibroblast growth factor 1
hsa-mir-484	*PTPRE*	protein tyrosine phosphatase, receptor type E
hsa-mir-484	*DPYSL2*	dihydropyrimidinase like 2
hsa-mir-484	*SORBS1*	sorbin and SH3 domain containing 1
hsa-mir-484	*ZSWIM6*	zinc finger SWIM-type containing 6
hsa-mir-484	*NUP54*	nucleoporin 54
hsa-mir-484	*RIMS2*	regulating synaptic membrane exocytosis 2
hsa-mir-484	*STEAP3*	STEAP3 metalloreductase
hsa-mir-484	*ABLIM2*	actin binding LIM protein family member 2
hsa-mir-484	*TNRC6C*	trinucleotide repeat containing 6C
hsa-mir-484	*TNFSF9*	TNF superfamily member 9
hsa-mir-484	*PIKFYVE*	phosphoinositide kinase, FYVE-type zinc finger containing
hsa-mir-484	*CPLX3*	complexin 3
hsa-mir-484	*PEA15*	phosphoprotein enriched in astrocytes 15

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*KIAA1549*	KIAA1549
hsa-mir-484	*SLC20A2*	solute carrier family 20 member 2
hsa-mir-484	*CDK9*	cyclin dependent kinase 9
hsa-mir-484	*MAPKAPK2*	mitogen-activated protein kinase-activated protein kinase 2
hsa-mir-484	*CSF1*	colony stimulating factor 1
hsa-mir-484	*PITPNA*	phosphatidylinositol transfer protein alpha
hsa-mir-484	*CSRNP2*	cysteine and serine rich nuclear protein 2
hsa-mir-484	*NFATC4*	nuclear factor of activated T-cells 4
hsa-mir-484	*AVL9*	AVL9 cell migration associated
hsa-mir-484	*POT1*	protection of telomeres 1
hsa-mir-484	*HLA-DOB*	major histocompatibility complex, class II, DO beta
hsa-mir-484	*DAG1*	dystroglycan 1
hsa-mir-484	*STX5*	syntaxin 5
hsa-mir-484	*PRPF4B*	pre-mRNA processing factor 4B
hsa-mir-484	*STRN*	striatin
hsa-mir-484	*CRTC3*	CREB regulated transcription coactivator 3
hsa-mir-484	*B3GNT9*	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9
hsa-mir-484	*WFS1*	wolframin ER transmembrane glycoprotein
hsa-mir-484	*SLC17A9*	solute carrier family 17 member 9
hsa-mir-484	*TRIM33*	tripartite motif containing 33
hsa-mir-484	*KCNJ14*	potassium voltage-gated channel subfamily J member 14
hsa-mir-484	*TSPAN17*	tetraspanin 17
hsa-mir-484	*ELMO2*	engulfment and cell motility 2
hsa-mir-484	*RAPGEF3*	Rap guanine nucleotide exchange factor 3
hsa-mir-484	*GTPBP10*	GTP binding protein 10
hsa-mir-484	*TSGA10*	testis specific 10
hsa-mir-484	*ZFYVE1*	zinc finger FYVE-type containing 1
hsa-mir-484	*ADAM33*	ADAM metallopeptidase domain 33
hsa-mir-484	*MINK1*	misshapen like kinase 1
hsa-mir-484	*NAF1*	nuclear assembly factor 1 ribonucleoprotein
hsa-mir-484	*VKORC1*	vitamin K epoxide reductase complex subunit 1
hsa-mir-484	*TNR*	tenascin R
hsa-mir-484	*PNRC1*	proline rich nuclear receptor coactivator 1
hsa-mir-484	*PRRT2*	proline rich transmembrane protein 2
hsa-mir-484	*SAMD4B*	sterile alpha motif domain containing 4B
hsa-mir-484	*GOSR2*	golgi SNAP receptor complex member 2
hsa-mir-484	*TMEM130*	transmembrane protein 130
hsa-mir-484	*FAM71E2*	family with sequence similarity 71 member E2
hsa-mir-484	*DCLK3*	doublecortin like kinase 3
hsa-mir-484	*TMEM56*	transmembrane protein 56
hsa-mir-484	*TRAT1*	T-cell receptor associated transmembrane adaptor 1
hsa-mir-484	*ALPK3*	alpha kinase 3
hsa-mir-484	*GRPEL2*	GrpE like 2, mitochondrial
hsa-mir-484	*RIPOR2*	RHO family interacting cell polarization regulator 2
hsa-mir-484	*MAN1A2*	mannosidase alpha class 1A member 2
hsa-mir-484	*STC1*	stanniocalcin 1
hsa-mir-484	*ZMIZ1*	zinc finger MIZ-type containing 1

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-484	*TCHP*	trichoplein keratin filament binding
hsa-mir-484	*BSDC1*	BSD domain containing 1
hsa-mir-484	*TOX2*	TOX high mobility group box family member 2
hsa-mir-484	*FLOT1*	flotillin 1
hsa-mir-484	*GRM1*	glutamate metabotropic receptor 1
hsa-mir-484	*BMP1*	bone morphogenetic protein 1
hsa-mir-484	*WDR3*	WD repeat domain 3
hsa-mir-484	*HK2*	hexokinase 2
hsa-mir-484	*PCDHA9*	protocadherin alpha 9
hsa-mir-484	*XKR9*	XK related 9
hsa-mir-484	*CYB5RL*	cytochrome b5 reductase like
hsa-mir-484	*SUSD2*	sushi domain containing 2
hsa-mir-484	*RBM24*	RNA binding motif protein 24
hsa-mir-484	*DLG2*	discs large MAGUK scaffold protein 2
hsa-mir-484	*DENND5A*	DENN domain containing 5A
hsa-mir-484	*SAP130*	Sin3A associated protein 130
hsa-mir-16-2	*CCNB2*	cyclin B2
hsa-mir-16-2	*C22ORF29*	chromosome 22 open reading frame 29
hsa-mir-16-2	*CMTM7*	CKLF like MARVEL transmembrane domain containing 7
hsa-mir-16-2	*PRKG1*	protein kinase, cGMP-dependent, type 1
hsa-mir-16-2	*PTER*	phosphotriesterase related
hsa-mir-16-2	*FAM49B*	family with sequence similarity 49 member B
hsa-mir-16-2	*TSHZ1*	teashirt zinc finger homeobox 1
hsa-mir-16-2	*KIAA2022*	KIAA2022
hsa-mir-16-2	*PRDM15*	PR/SET domain 15
hsa-mir-16-2	*KAT6A*	lysine acetyltransferase 6A
hsa-mir-16-2	*KCTD15*	potassium channel tetramerization domain containing 15
hsa-mir-16-2	*DIP2B*	disco interacting protein 2 homolog B
hsa-mir-16-2	*NEGR1*	neuronal growth regulator 1
hsa-mir-16-2	*ACTN1*	actinin alpha 1
hsa-mir-16-2	*ZBTB44*	zinc finger and BTB domain containing 44
hsa-mir-16-2	*ABTB2*	ankyrin repeat and BTB domain containing 2
hsa-mir-16-2	*CNR1*	cannabinoid receptor 1
hsa-mir-16-2	*PCDH11Y*	protocadherin 11 Y-linked
hsa-mir-16-2	*RAB1A*	RAB1A, member RAS oncogene family
hsa-mir-16-2	*RAB6B*	RAB6B, member RAS oncogene family
hsa-mir-16-2	*FAM135A*	family with sequence similarity 135 member A
hsa-mir-16-2	*ANKRD44*	ankyrin repeat domain 44
hsa-mir-16-2	*CFL2*	cofilin 2
hsa-mir-16-2	*PHLPP1*	PH domain and leucine rich repeat protein phosphatase 1
hsa-mir-16-2	*STAG2*	stromal antigen 2
hsa-mir-16-2	*LMNB1*	lamin B1
hsa-mir-16-2	*SHANK2*	SH3 and multiple ankyrin repeat domains 2
hsa-mir-16-2	*TANC2*	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2
hsa-mir-16-2	*MAP3K5*	mitogen-activated protein kinase kinase kinase 5
hsa-mir-16-2	*ELOA*	elongin A
hsa-mir-16-2	*SNRK*	SNF related kinase

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-16-2	*CLIC4*	chloride intracellular channel 4
hsa-mir-16-2	*DGKB*	diacylglycerol kinase beta
hsa-mir-16-2	*TENM1*	teneurin transmembrane protein 1
hsa-mir-16-2	*AMOTL2*	angiomotin like 2
hsa-mir-16-2	*PBRM1*	polybromo 1
hsa-mir-16-2	*ANKRD12*	ankyrin repeat domain 12
hsa-mir-16-2	*ZNF260*	zinc finger protein 260
hsa-mir-16-2	*GLS*	glutaminase
hsa-mir-16-2	*GRHL2*	grainyhead like transcription factor 2
hsa-mir-16-2	*KDM2A*	lysine demethylase 2A
hsa-mir-16-2	*GDPD1*	glycerophosphodiester phosphodiesterase domain containing 1
hsa-mir-16-2	*PTPN12*	protein tyrosine phosphatase, non-receptor type 12
hsa-mir-16-2	*SBNO1*	strawberry notch homolog 1
hsa-mir-16-2	*MPPED2*	metallophosphoesterase domain containing 2
hsa-mir-16-2	*IL13RA1*	interleukin 13 receptor subunit alpha 1
hsa-mir-16-2	*CASP3*	caspase 3
hsa-mir-16-2	*SYVN1*	synoviolin 1
hsa-mir-16-2	*USP16*	ubiquitin specific peptidase 16
hsa-mir-16-2	*FAM120C*	family with sequence similarity 120C
hsa-mir-16-2	*TMBIM4*	transmembrane BAX inhibitor motif containing 4
hsa-mir-16-2	*INTU*	inturned planar cell polarity protein
hsa-mir-16-2	*RAB6A*	RAB6A, member RAS oncogene family
hsa-mir-16-2	*PABPC4L*	poly(A) binding protein cytoplasmic 4 like
hsa-mir-16-2	*CPEB2*	cytoplasmic polyadenylation element binding protein 2
hsa-mir-16-2	*FAM126B*	family with sequence similarity 126 member B
hsa-mir-16-2	*CNTN4*	contactin 4
hsa-mir-16-2	*SEC24A*	SEC24 homolog A, COPII coat complex component
hsa-mir-16-2	*TLK1*	tousled like kinase 1
hsa-mir-16-2	*RNF6*	ring finger protein 6
hsa-mir-16-2	*SPOPL*	speckle type BTB/POZ protein like
hsa-mir-16-2	*RAD21*	RAD21 cohesin complex component
hsa-mir-16-2	*AMOTL1*	angiomotin like 1
hsa-mir-16-2	*CHML*	CHM like, Rab escort protein 2
hsa-mir-16-2	*RAP1A*	RAP1A, member of RAS oncogene family
hsa-mir-16-2	*CADM2*	cell adhesion molecule 2
hsa-mir-16-2	*CDK17*	cyclin dependent kinase 17
hsa-mir-16-2	*SGIP1*	SH3 domain GRB2 like endophilin interacting protein 1
hsa-mir-16-2	*FRS2*	fibroblast growth factor receptor substrate 2
hsa-mir-16-2	*HSPA5*	heat shock protein family A (Hsp70) member 5
hsa-mir-16-2	*PAPD7*	poly(A) RNA polymerase D7, non-canonical
hsa-mir-16-2	*TSHZ3*	teashirt zinc finger homeobox 3
hsa-mir-16-2	*PLAGL1*	PLAG1 like zinc finger 1
hsa-mir-16-2	*ACER3*	alkaline ceramidase 3
hsa-mir-16-2	*RCN2*	reticulocalbin 2
hsa-mir-16-2	*CYP26B1*	cytochrome P450 family 26 subfamily B member 1
hsa-mir-16-2	*BTG3*	BTG anti-proliferation factor 3
hsa-mir-16-2	*ZNF770*	zinc finger protein 770

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-16-2	*AEBP2*	AE binding protein 2
hsa-mir-16-2	*HNRNPLL*	heterogeneous nuclear ribonucleoprotein L like
hsa-mir-16-2	*FMNL2*	formin like 2
hsa-mir-16-2	*SP3*	Sp3 transcription factor
hsa-mir-16-2	*FGL2*	fibrinogen like 2
hsa-mir-16-2	*PTPN13*	protein tyrosine phosphatase, non-receptor type 13
hsa-mir-16-2	*BCL11B*	B-cell CLL/lymphoma 11B
hsa-mir-16-2	*LLGL1*	LLGL1, scribble cell polarity complex component
hsa-mir-16-2	*DPP10*	dipeptidyl peptidase like 10
hsa-mir-16-2	*ZSWIM6*	zinc finger SWIM-type containing 6
hsa-mir-16-2	*GRIA2*	glutamate ionotropic receptor AMPA type subunit 2
hsa-mir-16-2	*GALNT1*	polypeptide N-acetylgalactosaminyltransferase 1
hsa-mir-16-2	*PDE10A*	phosphodiesterase 10A
hsa-mir-16-2	*HIF1A*	hypoxia inducible factor 1 alpha subunit
hsa-mir-16-2	*PRRX1*	paired related homeobox 1
hsa-mir-16-2	*DSTYK*	dual serine/threonine and tyrosine protein kinase
hsa-mir-16-2	*KAT6B*	lysine acetyltransferase 6B
hsa-mir-16-2	*PCGF3*	polycomb group ring finger 3
hsa-mir-16-2	*EMB*	embigin
hsa-mir-16-2	*TMLHE*	trimethyllysine hydroxylase, epsilon
hsa-mir-16-2	*TMEM161B*	transmembrane protein 161B
hsa-mir-16-2	*EIF1AX*	eukaryotic translation initiation factor 1A, X-linked
hsa-mir-16-2	*ADCYAP1*	adenylate cyclase activating polypeptide 1
hsa-mir-16-2	*NAT2*	N-acetyltransferase 2
hsa-mir-16-2	*PEX5L*	peroxisomal biogenesis factor 5 like
hsa-mir-16-2	*AGL*	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase
hsa-mir-16-2	*COL11A1*	collagen type XI alpha 1 chain
hsa-mir-16-2	*RBFOX1*	RNA binding protein, fox-1 homolog 1
hsa-mir-16-2	*CAV2*	caveolin 2
hsa-mir-16-2	*TDG*	thymine DNA glycosylase
hsa-mir-16-2	*IYD*	iodotyrosine deiodinase
hsa-mir-16-2	*FRK*	fyn related Src family tyrosine kinase
hsa-mir-16-2	*CLOCK*	clock circadian regulator
hsa-mir-16-2	*MEX3B*	mex-3 RNA binding family member B
hsa-mir-16-2	*SATB1*	SATB homeobox 1
hsa-mir-16-2	*DPY19L4*	dpy-19 like 4 (C. elegans)
hsa-mir-16-2	*ZNF254*	zinc finger protein 254
hsa-mir-16-2	*CREB1*	cAMP responsive element binding protein 1
hsa-mir-16-2	*ANKRD26*	ankyrin repeat domain 26
hsa-mir-16-2	*VDAC1*	voltage dependent anion channel 1
hsa-mir-16-2	*LRIG1*	leucine rich repeats and immunoglobulin like domains 1
hsa-mir-16-2	*INPP1*	inositol polyphosphate-1-phosphatase
hsa-mir-16-2	*ZFP36*	ZFP36 ring finger protein
hsa-mir-16-2	*HORMAD1*	HORMA domain containing 1
hsa-mir-16-2	*TBC1D12*	TBC1 domain family member 12
hsa-mir-16-2	*C1ORF21*	chromosome 1 open reading frame 21
hsa-mir-16-2	*PAIP2*	poly(A) binding protein interacting protein 2

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-16-2	*HNRNPUL2*	heterogeneous nuclear ribonucleoprotein U like 2
hsa-mir-16-2	*STX12*	syntaxin 12
hsa-mir-16-2	*RORA*	RAR related orphan receptor A
hsa-mir-16-2	*TTC39B*	tetratricopeptide repeat domain 39B
hsa-mir-16-2	*ARAP2*	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2
hsa-mir-16-2	*IGSF11*	immunoglobulin superfamily member 11
hsa-mir-16-2	*MTF2*	metal response element binding transcription factor 2
hsa-mir-16-2	*CPEB3*	cytoplasmic polyadenylation element binding protein 3
hsa-mir-16-2	*ZNF615*	zinc finger protein 615
hsa-mir-16-2	*MIER3*	MIER family member 3
hsa-mir-16-2	*AHCTF1*	AT-hook containing transcription factor 1
hsa-mir-16-2	*ZNF280D*	zinc finger protein 280D
hsa-mir-16-2	*UBE2V2*	ubiquitin conjugating enzyme E2 V2
hsa-mir-16-2	*SCN2A*	sodium voltage-gated channel alpha subunit 2
hsa-mir-16-2	*PTAR1*	protein prenyltransferase alpha subunit repeat containing 1
hsa-mir-16-2	*EYA4*	EYA transcriptional coactivator and phosphatase 4
hsa-mir-16-2	*KRTAP4-5*	keratin associated protein 4–5
hsa-mir-16-2	*LPAR1*	lysophosphatidic acid receptor 1
hsa-mir-16-2	*TAOK3*	TAO kinase 3
hsa-mir-16-2	*AFF2*	AF4/FMR2 family member 2
hsa-mir-16-2	*NYAP2*	neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adaptor 2
hsa-mir-16-2	*DLL1*	delta like canonical Notch ligand 1
hsa-mir-16-2	*RNF44*	ring finger protein 44
hsa-mir-16-2	*SEPSECS*	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase
hsa-mir-16-2	*CD226*	CD226 molecule
hsa-mir-16-2	*HAND2*	heart and neural crest derivatives expressed 2
hsa-mir-16-2	*ST13*	ST13, Hsp70 interacting protein
hsa-mir-16-2	*ICK*	intestinal cell kinase
hsa-mir-16-2	*ZNF117*	zinc finger protein 117
hsa-mir-16-2	*OAZ1*	ornithine decarboxylase antizyme 1
hsa-mir-16-2	*ATP11B*	ATPase phospholipid transporting 11B (putative)
hsa-mir-16-2	*HSDL1*	hydroxysteroid dehydrogenase like 1
hsa-mir-16-2	*MME*	membrane metalloendopeptidase
hsa-mir-16-2	*PURA*	purine rich element binding protein A
hsa-mir-16-2	*RGS4*	regulator of G protein signaling 4
hsa-mir-16-2	*AUH*	AU RNA binding methylglutaconyl-CoA hydratase
hsa-mir-16-2	*SOAT1*	sterol O-acyltransferase 1
hsa-mir-16-2	*TBX18*	T-box 18
hsa-mir-16-2	*HS6ST2*	heparan sulfate 6-O-sulfotransferase 2
hsa-mir-16-2	*ZNF569*	zinc finger protein 569
hsa-mir-16-2	*AZIN1*	antizyme inhibitor 1
hsa-mir-16-2	*IRF6*	interferon regulatory factor 6
hsa-mir-16-2	*RGS5*	regulator of G-protein signaling 5
hsa-mir-16-2	*ANKIB1*	ankyrin repeat and IBR domain containing 1
hsa-mir-16-2	*TPP2*	tripeptidyl peptidase 2
hsa-mir-16-2	*SCARB2*	scavenger receptor class B member 2
hsa-mir-16-2	*KIAA1107*	KIAA1107

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-16-2	*ZNF624*	zinc finger protein 624
hsa-mir-16-2	*BLOC1S2*	biogenesis of lysosomal organelles complex 1 subunit 2
hsa-mir-16-2	*CHIC1*	cysteine rich hydrophobic domain 1
hsa-mir-16-2	*TUBB2B*	tubulin beta 2B class IIb
hsa-mir-16-2	*ZNF681*	zinc finger protein 681
hsa-mir-16-2	*ZNF236*	zinc finger protein 236
hsa-mir-16-2	*B2M*	beta-2-microglobulin
hsa-mir-16-2	*PRKAA1*	protein kinase AMP-activated catalytic subunit alpha 1
hsa-mir-16-2	*CUL2*	cullin 2
hsa-mir-16-2	*NAB1*	NGFI-A binding protein 1
hsa-mir-16-2	*CAMK1D*	calcium/calmodulin dependent protein kinase ID
hsa-mir-16-2	*SLC2A13*	solute carrier family 2 member 13
hsa-mir-16-2	*FGF14*	fibroblast growth factor 14
hsa-mir-16-2	*KL*	klotho
hsa-mir-16-2	*HS2ST1*	heparan sulfate 2-O-sulfotransferase 1
hsa-mir-16-2	*ARID2*	AT-rich interaction domain 2
hsa-mir-16-2	*KIAA0408*	KIAA0408
hsa-mir-16-2	*STRBP*	spermatid perinuclear RNA binding protein
hsa-mir-16-2	*CLIP4*	CAP-Gly domain containing linker protein family member 4
hsa-mir-16-2	*DSC3*	desmocollin 3
hsa-mir-16-2	*SLC9C2*	solute carrier family 9 member C2 (putative)
hsa-mir-16-2	*RC3H1*	ring finger and CCCH-type domains 1
hsa-mir-16-2	*ATF3*	activating transcription factor 3
hsa-mir-16-2	*TAF5L*	TATA-box binding protein associated factor 5 like
hsa-mir-16-2	*HNRNPR*	heterogeneous nuclear ribonucleoprotein R
hsa-mir-16-2	*SSX2IP*	SSX family member 2 interacting protein
hsa-mir-16-2	*RAI2*	retinoic acid induced 2
hsa-mir-16-2	*RPS6KA3*	ribosomal protein S6 kinase A3
hsa-mir-16-2	*CYBB*	cytochrome b-245 beta chain
hsa-mir-16-2	*NKRF*	NFKB repressing factor
hsa-mir-16-2	*ARHGEF6*	Rac/Cdc42 guanine nucleotide exchange factor 6
hsa-mir-16-2	*ARFGEF2*	ADP ribosylation factor guanine nucleotide exchange factor 2
hsa-mir-16-2	*USP25*	ubiquitin specific peptidase 25
hsa-mir-16-2	*UBE2E2*	ubiquitin conjugating enzyme E2 E2
hsa-mir-16-2	*UBP1*	upstream binding protein 1 (LBP-1a)
hsa-mir-16-2	*ZNF512*	zinc finger protein 512
hsa-mir-16-2	*STRN*	striatin
hsa-mir-16-2	*BCL11A*	B-cell CLL/lymphoma 11A
hsa-mir-16-2	*MAP3K2*	mitogen-activated protein kinase kinase kinase 2
hsa-mir-16-2	*GSTCD*	glutathione S-transferase C-terminal domain containing
hsa-mir-16-2	*TRPC3*	transient receptor potential cation channel subfamily C member 3
hsa-mir-16-2	*RAPGEF2*	Rap guanine nucleotide exchange factor 2
hsa-mir-16-2	*CLCN3*	chloride voltage-gated channel 3
hsa-mir-16-2	*CDH12*	cadherin 12
hsa-mir-16-2	*DNAJC21*	DnaJ heat shock protein family (Hsp40) member C21
hsa-mir-16-2	*SNX18*	sorting nexin 18
hsa-mir-16-2	*ZBTB38*	zinc finger and BTB domain containing 38

(*Continued*)

Table 6 (*Continued*)

MicroRNA	Target gene	Annotation
hsa-mir-16-2	*CCDC50*	coiled-coil domain containing 50
hsa-mir-16-2	*RBPJ*	recombination signal binding protein for immunoglobulin kappa J region
hsa-mir-16-2	*USP46*	ubiquitin specific peptidase 46
hsa-mir-16-2	*MOB1B*	MOB kinase activator 1B
hsa-mir-16-2	*PARM1*	prostate androgen-regulated mucin-like protein 1
hsa-mir-16-2	*CNKSR3*	CNKSR family member 3
hsa-mir-16-2	*CDK13*	cyclin dependent kinase 13
hsa-mir-16-2	*PCDHA6*	protocadherin alpha 6
hsa-mir-16-2	*PCDHAC1*	protocadherin alpha subfamily C, 1
hsa-mir-16-2	*RBM27*	RNA binding motif protein 27
hsa-mir-16-2	*USP49*	ubiquitin specific peptidase 49
hsa-mir-16-2	*SAMD9L*	sterile alpha motif domain containing 9 like
hsa-mir-16-2	*PEG10*	paternally expressed 10
hsa-mir-16-2	*SMARCA2*	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
hsa-mir-16-2	*ZNF483*	zinc finger protein 483
hsa-mir-16-2	*ASTN2*	astrotactin 2
hsa-mir-16-2	*FOXP2*	forkhead box P2
hsa-mir-16-2	*CALU*	calumenin
hsa-mir-16-2	*NUP205*	nucleoporin 205
hsa-mir-16-2	*TMEM178B*	transmembrane protein 178B
hsa-mir-16-2	*DCAF4L2*	DDB1 and CUL4 associated factor 4 like 2
hsa-mir-16-2	*FBXO32*	F-box protein 32
hsa-mir-16-2	*KBTBD3*	kelch repeat and BTB domain containing 3
hsa-mir-16-2	*MAB21L1*	mab-21 like 1
hsa-mir-16-2	*RGCC*	regulator of cell cycle
hsa-mir-16-2	*NALCN*	sodium leak channel, non-selective
hsa-mir-16-2	*TEX30*	testis expressed 30
hsa-mir-16-2	*RASSF8*	Ras association domain family member 8
hsa-mir-16-2	*C12ORF66*	chromosome 12 open reading frame 66
hsa-mir-16-2	*DYRK2*	dual specificity tyrosine phosphorylation regulated kinase 2
hsa-mir-16-2	*TRPM7*	transient receptor potential cation channel subfamily M member 7
hsa-mir-16-2	*WDR72*	WD repeat domain 72
hsa-mir-16-2	*IREB2*	iron responsive element binding protein 2
hsa-mir-16-2	*ZNF790*	zinc finger protein 790
hsa-mir-16-2	*ZNF558*	zinc finger protein 558

The microRNA *hsa-mir-16-2* plays a tumor suppressor role by inducing cell cycle arrest, DNA damage repair, and apoptosis.[33–35] Of the three microRNAs, *hsa-mir-31* is the most studied. Previous studies show that *hsa-mir-31* is a major contributor to breast cancer progression and metastasis by regulating metastasis-related genes, including RhoA, Radexin,[36] WAVE3,[37] RDX, SATB2,[38,39] FOXP3,[40] GNA13,[41] and several integrin subunits,[42] all involved in key steps in the invasion–metastasis cascade. In addition, *hsa-mir-31* expression level is high in early-stage breast cancer tissues, diminishes as the tumor progresses to more advanced stages, and is even sometimes undetectable in metastatic tumors.[36,37] Loss of *hsa-mir-31* expression is accompanied by increased expression of its target genes, allowing the tumor to become more invasive and ultimately metastasize.[37] In summary, these three microRNAs are involved in chemoresistance, cell cycle arrest, and metastasis, and therefore, they can theoretically predict the prognosis of breast cancer.

Of note, our analysis indicates that our prognostic signature performed especially well in young patients (age ≤45 years) with basal-like breast carcinoma. To our knowledge,

Table 7 Gene Ontology annotation analysis

MicroRNA	Category	ID	Term	*P*-value	Fold enrichment
hsa-mir-16-2	Biological process	GO:0032774	RNA biosynthetic process	3.31E-02	1.82
		GO:0010556	Regulation of macromolecule biosynthetic process	4.31E-05	1.77
		GO:2000112	Regulation of cellular macromolecule biosynthetic process	2.76E-04	1.74
		GO:0051252	Regulation of RNA metabolic process	8.69E-04	1.73
		GO:1903506	Regulation of nucleic acid-templated transcription	3.70E-03	1.72
		GO:2001141	Regulation of RNA biosynthetic process	4.09E-03	1.71
		GO:0006355	Regulation of transcription, DNA-templated	5.83E-03	1.71
		GO:0009889	Regulation of biosynthetic process	2.13E-04	1.7
		GO:0019219	Regulation of nucleobase-containing compound metabolic process	7.37E-04	1.69
		GO:0031326	Regulation of cellular biosynthetic process	4.32E-04	1.69
		GO:0048523	Negative regulation of cellular process	6.20E-04	1.68
		GO:0048519	Negative regulation of biological process	1.52E-03	1.61
		GO:0051171	Regulation of nitrogen compound metabolic process	1.02E-04	1.58
		GO:0060255	Regulation of macromolecule metabolic process	7.20E-05	1.57
		GO:0010468	Regulation of gene expression	2.39E-02	1.57
		GO:0080090	Regulation of primary metabolic process	1.22E-04	1.57
		GO:0031323	Regulation of cellular metabolic process	2.27E-04	1.55
		GO:0048856	Anatomical structure development	3.73E-02	1.51
		GO:0019222	Regulation of metabolic process	7.13E-04	1.5
	Cellular component	GO:0005634	Nucleus	4.54E-06	1.52
	Molecular function	GO:0003700	Transcription factor activity, sequence-specific DNA binding	1.11E-02	2.29
		GO:0001071	Nucleic acid binding transcription factor activity	1.13E-02	2.29
		GO:0003677	DNA binding	1.48E-02	1.83
		GO:0046872	Metal ion binding	1.88E-05	1.77
		GO:0043169	Cation binding	5.41E-05	1.73
		GO:0043167	Ion binding	5.96E-04	1.51
hsa-mir-31	Biological process	GO:0042325	Regulation of phosphorylation	4.93E-02	2.57
		GO:0031325	Positive regulation of cellular metabolic process	7.67E-03	2.07
		GO:0051173	Positive regulation of nitrogen compound metabolic process	4.79E-02	2.01
		GO:0009893	Positive regulation of metabolic process	2.07E-02	1.98
		GO:0048522	Positive regulation of cellular process	3.44E-05	1.93
		GO:0048518	Positive regulation of biological process	2.12E-06	1.92
		GO:0051171	Regulation of nitrogen compound metabolic process	5.65E-03	1.67
		GO:0060255	Regulation of macromolecule metabolic process	3.60E-03	1.67
		GO:0031323	Regulation of cellular metabolic process	4.80E-03	1.66
		GO:0080090	Regulation of primary metabolic process	7.25E-03	1.65
		GO:0019222	Regulation of metabolic process	4.29E-03	1.62
hsa-mir-484	Biological process	GO:0048666	Neuron development	3.68E-02	2.48
		GO:0010557	Positive regulation of macromolecule biosynthetic process	1.00E-03	2.08
		GO:0031328	Positive regulation of cellular biosynthetic process	2.42E-03	2
		GO:0010628	Positive regulation of gene expression	3.84E-03	1.99
		GO:0051254	Positive regulation of RNA metabolic process	4.90E-02	1.98
		GO:0009891	Positive regulation of biosynthetic process	4.15E-03	1.97
		GO:0045935	Positive regulation of nucleobase-containing compound metabolic process	9.25E-03	1.96
		GO:0010604	Positive regulation of macromolecule metabolic process	4.02E-05	1.84

(*Continued*)

Table 7 (*Continued*)

MicroRNA	Category	ID	Term	*P*-value	Fold enrichment
		GO:0051173	Positive regulation of nitrogen compound metabolic process	4.58E-04	1.79
		GO:0031325	Positive regulation of cellular metabolic process	2.55E-04	1.79
		GO:0009893	Positive regulation of metabolic process	8.18E-05	1.78
		GO:0009892	Negative regulation of metabolic process	1.73E-03	1.77
		GO:0031324	Negative regulation of cellular metabolic process	8.96E-03	1.77
		GO:0010605	Negative regulation of macromolecule metabolic process	4.78E-02	1.71
		GO:0048869	Cellular developmental process	3.51E-02	1.57
		GO:0048731	System development	8.96E-03	1.55
		GO:0048523	Negative regulation of cellular process	5.89E-03	1.54
		GO:0048522	Positive regulation of cellular process	1.07E-03	1.54
		GO:0007275	Multicellular organism development	2.81E-03	1.53
	Cellular component	GO:0005667	Transcription factor complex	8.69E-03	3.49
		GO:0043234	Protein complex	6.53E-04	1.68
		GO:0032991	Macromolecular complex	3.60E-05	1.56
	Molecular function	GO:0043565	Sequence-specific DNA binding	1.34E-02	2.23

Table 8 KEGG and PANTHER analyses

MicroRNA	Term	Database	ID	Input number	Background number	*P*-value
hsa-mir-16-2	Circadian rhythm	KEGG pathway	hsa04710	4	30	0.000365555
	MAPK signaling pathway	KEGG pathway	hsa04010	8	257	0.005579127
	Gap junction	KEGG pathway	hsa04540	4	88	0.014007203
	ALS	KEGG pathway	hsa05014	3	51	0.017107832
	Progesterone-mediated oocyte maturation	KEGG pathway	hsa04914	4	97	0.019095593
	Glycosaminoglycan biosynthesis – heparan sulfate/heparin	KEGG pathway	hsa00534	2	25	0.029980769
	Long-term potentiation	KEGG pathway	hsa04720	3	66	0.032404978
	Renal cell carcinoma	KEGG pathway	hsa05211	3	69	0.036094866
	Dorsoventral axis formation	KEGG pathway	hsa04320	2	28	0.036434645
	Oocyte meiosis	KEGG pathway	hsa04114	4	120	0.036784673
	Neurotrophin signaling pathway	KEGG pathway	hsa04722	4	122	0.038652503
	Thyroid hormone synthesis	KEGG pathway	hsa04918	3	71	0.03866969
	Antigen processing and presentation	KEGG pathway	hsa04612	3	71	0.03866969
	RNA degradation	KEGG pathway	hsa03018	3	77	0.046937327
	FAS signaling pathway	PANTHER	P00020	3	31	0.004779418
	Integrin signaling pathway	PANTHER	P00034	6	166	0.008045548
	Cadherin signaling pathway	PANTHER	P00012	5	154	0.022562161
	FGF signaling pathway	PANTHER	P00021	4	103	0.023047641
	Heterotrimeric G-protein signaling pathway – Gi alpha and Gs alpha-mediated pathway	PANTHER	P00026	5	157	0.02421771
	Apoptosis signaling pathway	PANTHER	P00006	4	108	0.026693923
	CCKR signaling map	PANTHER	P06959	5	176	0.036514088
hsa-mir-31	Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	PANTHER	P00027	4	121	0.037711913
	Hippo signaling pathway	KEGG pathway	hsa04390	5	153	0.00249984
	Oxytocin signaling pathway	KEGG pathway	hsa04921	5	160	0.003013067

(*Continued*)

Table 8 (*Continued*)

MicroRNA	Term	Database	ID	Input number	Background number	*P*-value
	Melanogenesis	KEGG pathway	hsa04916	4	100	0.003392532
	Sphingolipid signaling pathway	KEGG pathway	hsa04071	4	123	0.006872453
	AMPK signaling pathway	KEGG pathway	hsa04152	4	125	0.007255462
	Dopaminergic synapse	KEGG pathway	hsa04728	4	129	0.008063183
	Proteoglycans in cancer	KEGG pathway	hsa05205	5	208	0.008756401
	Ubiquitin-mediated proteolysis	KEGG pathway	hsa04120	4	137	0.009850899
	Wnt signaling pathway	KEGG pathway	hsa04310	4	142	0.011089117
	Circadian rhythm	KEGG pathway	hsa04710	2	30	0.015281252
	cGMP-PKG signaling pathway	KEGG pathway	hsa04022	4	173	0.021002596
	Axon guidance	KEGG pathway	hsa04360	4	178	0.022982044
	Calcium signaling pathway	KEGG pathway	hsa04020	4	179	0.023391098
	Glucagon signaling pathway	KEGG pathway	hsa04922	3	102	0.024384407
	T-cell receptor signaling pathway	KEGG pathway	hsa04660	3	107	0.02748699
	Insulin resistance	KEGG pathway	hsa04931	3	111	0.030113491
	Oocyte meiosis	KEGG pathway	hsa04114	3	120	0.036489064
	Neurotrophin signaling pathway	KEGG pathway	hsa04722	3	122	0.037992724
	Vascular smooth muscle contraction	KEGG pathway	hsa04270	3	123	0.038756301
	ALS	KEGG pathway	hsa05014	2	51	0.039184796
	Natural killer cell-mediated cytotoxicity	KEGG pathway	hsa04650	3	130	0.044318798
	Basal cell carcinoma	KEGG pathway	hsa05217	2	55	0.044700392
	FGF signaling pathway	PANTHER	P00021	5	103	0.000457823
	EGF receptor signaling pathway	PANTHER	P00018	5	114	0.000712265
	Angiogenesis	PANTHER	P00005	5	161	0.003092145
	Endothelin signaling pathway	PANTHER	P00019	3	79	0.012699626
	T-cell activation	PANTHER	P00053	3	79	0.012699626
	CCKR signaling map	PANTHER	P06959	4	176	0.022177133
	Apoptosis signaling pathway	PANTHER	P00006	3	108	0.028131602
	Alzheimer disease – presenilin pathway	PANTHER	P00004	3	112	0.030790107
	Lonotropic glutamate receptor pathway	PANTHER	P00037	2	46	0.03269137
	Wnt signaling pathway	PANTHER	P00057	5	295	0.032927779
	Inflammation mediated by chemokine and cytokine signaling pathway	PANTHER	P00031	4	202	0.034033451
	Oxytocin receptor mediated signaling pathway	PANTHER	P04391	2	55	0.044700392
	Thyrotropin-releasing hormone receptor signaling pathway	PANTHER	P04394	2	57	0.047559619
hsa-mir-484	Wnt signaling pathway	KEGG pathway	hsa04310	8	142	0.000852343
	HTLV-I infection	KEGG pathway	hsa05166	11	259	0.000948874
	Cytokine–cytokine receptor interaction	KEGG pathway	hsa04060	11	265	0.001132924
	Adherens junction	KEGG pathway	hsa04520	5	74	0.003864995
	Hippo signaling pathway	KEGG pathway	hsa04390	7	153	0.005339916
	Jak-STAT signaling pathway	KEGG pathway	hsa04630	7	160	0.006710057
	Endometrial cancer	KEGG pathway	hsa05213	4	54	0.00710104
	Hippo signaling pathway – multiple species	KEGG pathway	hsa04392	3	28	0.007685262
	Axon guidance	KEGG pathway	hsa04360	7	178	0.011418848
	VEGF signaling pathway	KEGG pathway	hsa04370	4	64	0.012312511
	CAMs	KEGG pathway	hsa04514	6	143	0.014087791
	SNARE interactions in vesicular transport	KEGG pathway	hsa04130	3	36	0.014469204

(*Continued*)

Table 8 (*Continued*)

MicroRNA	Term	Database	ID	Input number	Background number	*P*-value
	PPAR signaling pathway	KEGG pathway	hsa03320	4	73	0.018678443
	Melanoma	KEGG pathway	hsa05218	4	73	0.018678443
	PI3K-Akt signaling pathway	KEGG pathway	hsa04151	10	343	0.018921484
	Protein processing in endoplasmic reticulum	KEGG pathway	hsa04141	6	167	0.027083189
	ECM-receptor interaction	KEGG pathway	hsa04512	4	83	0.027778622
	RNA transport	KEGG pathway	hsa03013	6	171	0.029828233
	N-glycan biosynthesis	KEGG pathway	hsa00510	3	49	0.030909326
	Hematopoietic cell lineage	KEGG pathway	hsa04640	4	86	0.030942
	Mismatch repair	KEGG pathway	hsa03430	2	23	0.042292382
	Insulin signaling pathway	KEGG pathway	hsa04910	5	141	0.043874311
	Pathways in cancer	KEGG pathway	hsa05200	10	399	0.044847988
	Acute myeloid leukemia	KEGG pathway	hsa05221	3	59	0.048121632
	Angiogenesis	PANTHER	P00005	7	161	0.006925275
	Wnt signaling pathway	PANTHER	P00057	10	295	0.007391165
	Pyrimidine metabolism	PANTHER	P02771	2	10	0.01040345
	Axon guidance mediated by netrin	PANTHER	P00009	3	32	0.010767727
	Blood coagulation	PANTHER	P00011	3	38	0.016557181
	Axon guidance mediated by semaphorins	PANTHER	P00007	2	19	0.030634121

Abbreviations: ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; CAM, cell adhesion molecule; CCKR, cholecystokinin receptor; cGMP-PKG, cyclic guanosine monophosphate-dependent protein kinase G; ECM, extracellular matrix; EGF, epidermal growth factor; FAS, fatty acid synthase; FGF, fibroblast growth factor; HTLV-I, human T-cell lymphotropic virus I; Jak-STAT, janus kinase–STAT; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; PPAR, peroxisome-proliferator-activated receptor; VEGF, vascular endothelial growth factor.

triple-negative breast cancer is characterized by the lack of hormone receptors (ER and PR) and HER2 expression, a common basal-like subtype, and a high propensity for distant site metastases.[43] Furthermore, effective targeted therapies beyond chemotherapy and radiotherapy are absent for triple-negative breast cancer, leading to poor clinical outcomes and a high mortality rate.[44,45] These features make our signature even more valuable. We propose that high-risk patients, as determined by the calculations derived from our model, should be treated more aggressively and have a shorter follow-up interval.

Moreover, our experimental results also verified our signature. In the low-risk group, cell proliferative ability was inhibited, and S and G2/M phase cell counts were significantly increased, indicating that the cell cycle was arrested at the G2/M phase. In the high-risk group, cell proliferative

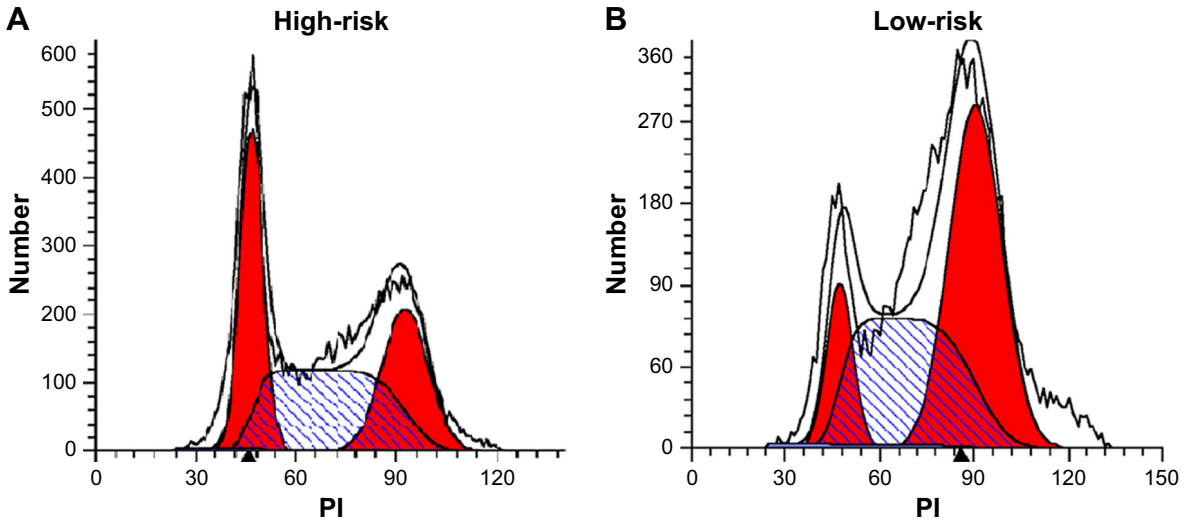


Figure 6 (*Continued*)

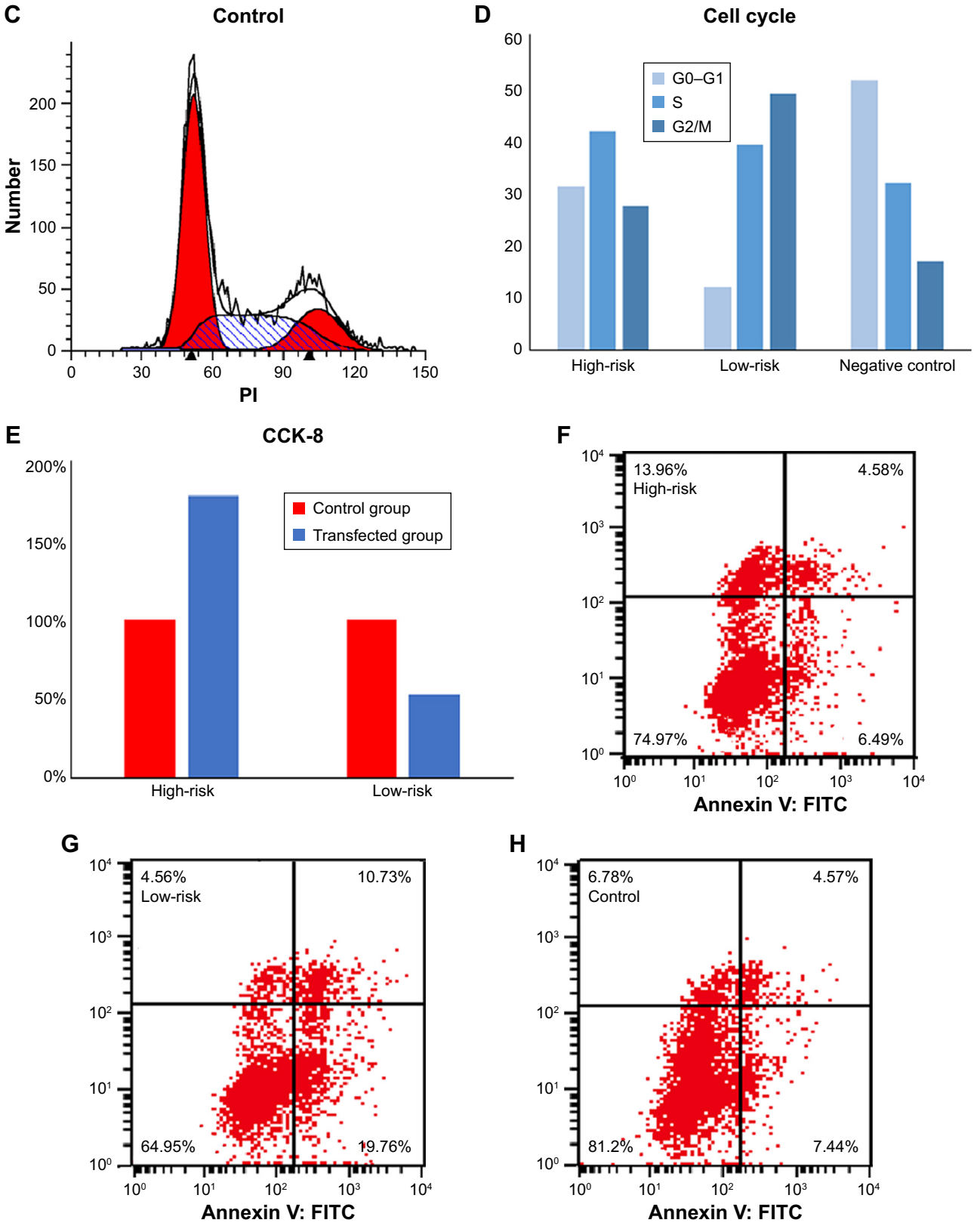


Figure 6 (**A**–**D**) Flow cytometry analysis of the cell cycle revealed that low-risk group cells were arrested at S and G2/M phase, while the cell cycle was activated in the high-risk group compared to the control group. (**E**) The cell viability of the high-risk group was significantly increased compared to the control group, while the viability of the low-risk group was decreased. (**F–H**) Flow cytometry analysis of apoptosis revealed that the apoptosis rate was 11.07% in the high-risk group, 30.49% in the low-risk group, and 12.01% in the control group.

Abbreviations: CCK-8, Cell-Counting Kit-8; FITC, fluorescein isothiocyanate; PI, propidium iodide.

ability was significantly increased combined with low cell counts in S and G2/M phase, indicating that the cells were proliferating rapidly. We also conducted an apoptosis assay in which the cell apoptosis rate was significantly increased in the low-risk group compared to the control group. Meanwhile, there was no significant difference between the high-risk group and the control group. This was not consistent with our prediction, and we propose that perhaps this signature could not significantly affect the apoptosis of breast cancer cells. Combined together, these results suggest that our signature was associated with the viability and cell cycle of breast cancer cells.

Limitations

We must acknowledge some limitations of our study. Since we excluded patients with insufficient data for analysis (such as RNA sequencing data, histological data, and follow-up data), there could be an influence of selection bias on our final results. Despite this, our microRNA signature demonstrated performance stability. As it is well accepted that microRNAs can be secreted and/or released to the local microenvironment and into the circulation,[46] it may be possible to use blood or tissue samples to detect the expression level of these three microRNAs as a reference to guide the treatment of breast cancer patients.

Conclusion

We recommend more aggressive therapy and appropriate shorter follow-up intervals for patients in the high-risk group.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Acknowledgment

This work was supported by the National Natural Science Foundation of China: Jie Ming (grant no. 81672611) and Hui Guo (grant no. 81602350).

Disclosure

The authors report no conflicts of interest in this work.

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