

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

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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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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 miRDB¹² 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 \pm 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

Table 1 Univariate Cox analysis of 1,158 microRNAs

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

tests were two-sided and a P -value < 0.05 was considered statistically significant.

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 = $(0.494 * \text{Status of } hsa-mir-484) - (0.786 * \text{Status of } hsa-mir-16-2) - (0.620 * \text{Status of } hsa-mir-31)$. 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
<i>hsa-mir-31</i>	0.011486	-0.62045	Protective
<i>hsa-mir-16-2</i>	0.001398	-0.78621	Protective
<i>hsa-mir-484</i>	0.042246	0.493782	Increased risk

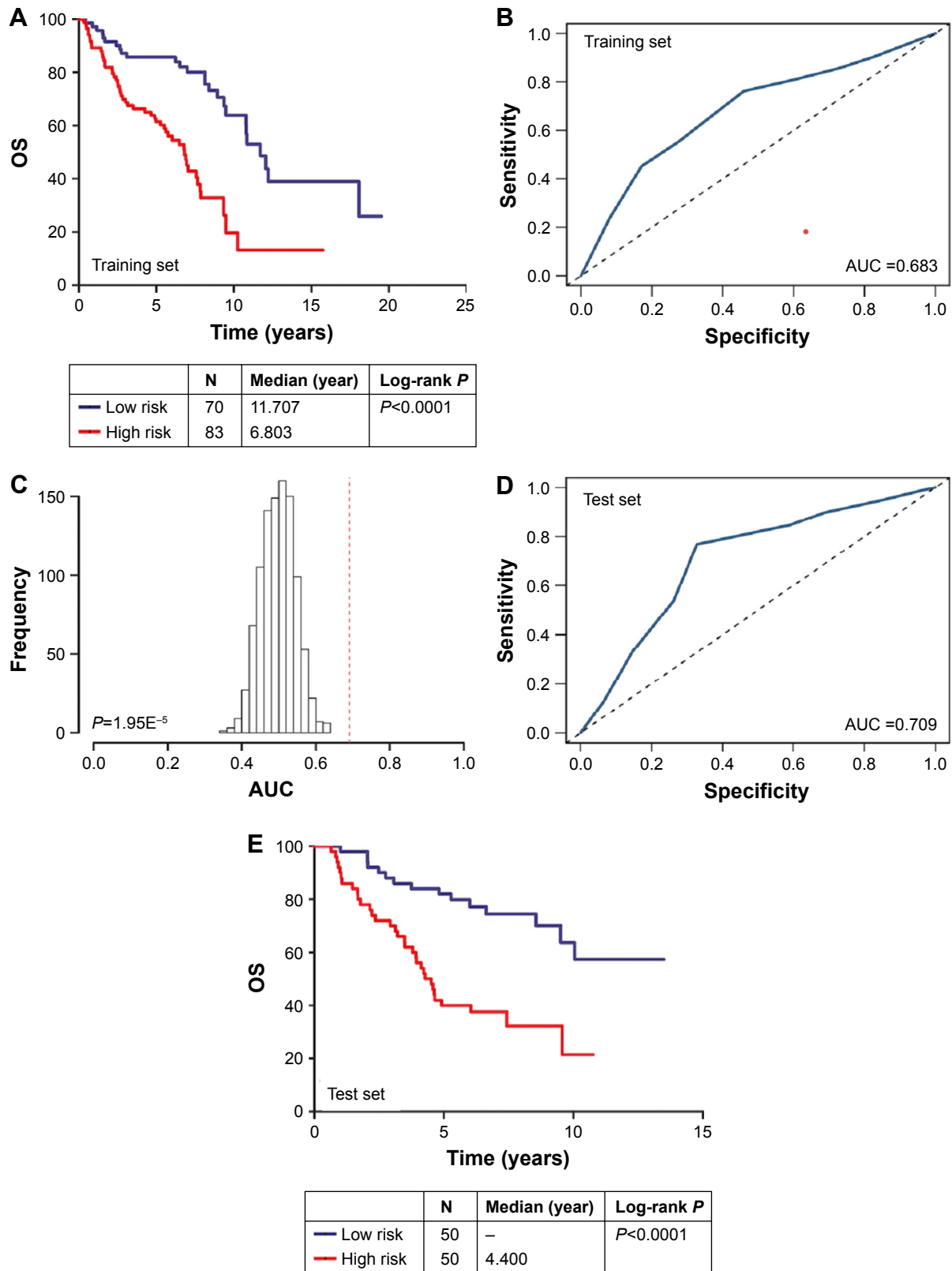


Figure 1 (A) Kaplan–Meier analysis of OS in the training set: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test ($P < 0.0001$); (B) ROC curve of the training set. (C) The permutation test found that the AUC of the random system 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.

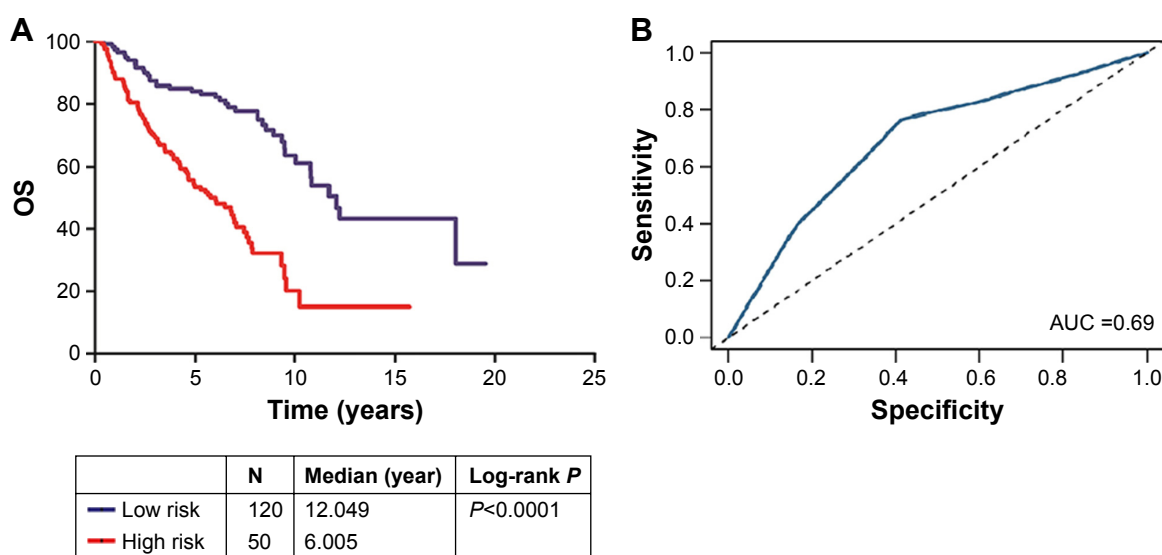


Figure 2 (A) Kaplan–Meier analysis of OS in the total set; (B) the ROC curve of the total set. AUC was 0.69.

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

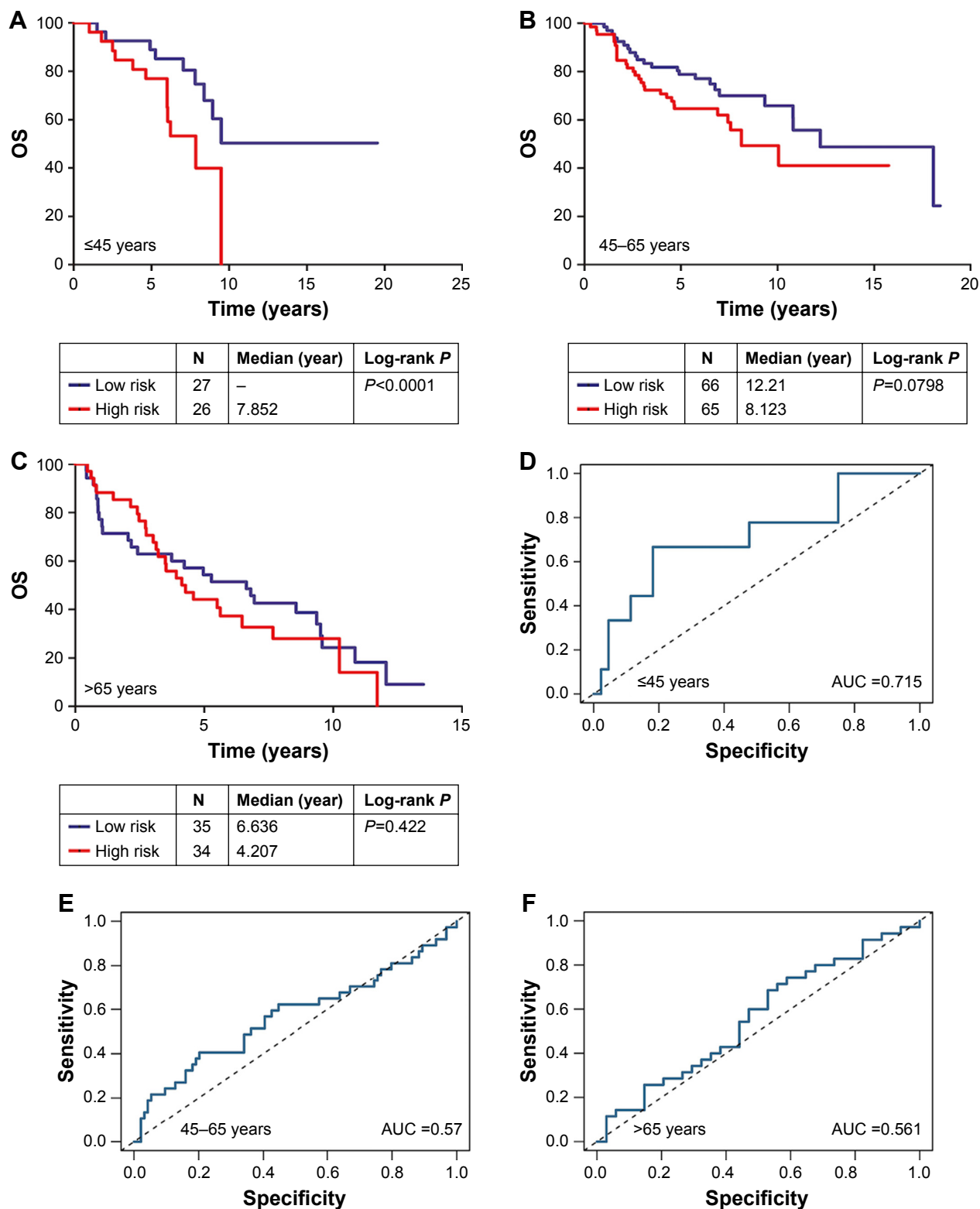


Figure 3 (A) Kaplan–Meier analysis of OS in the ≤ 45 -year age group: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test ($P < 0.0001$); (B) the ROC curve AUC was 0.715. (C) Kaplan–Meier analysis of OS in the 46–65-year age group: 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.

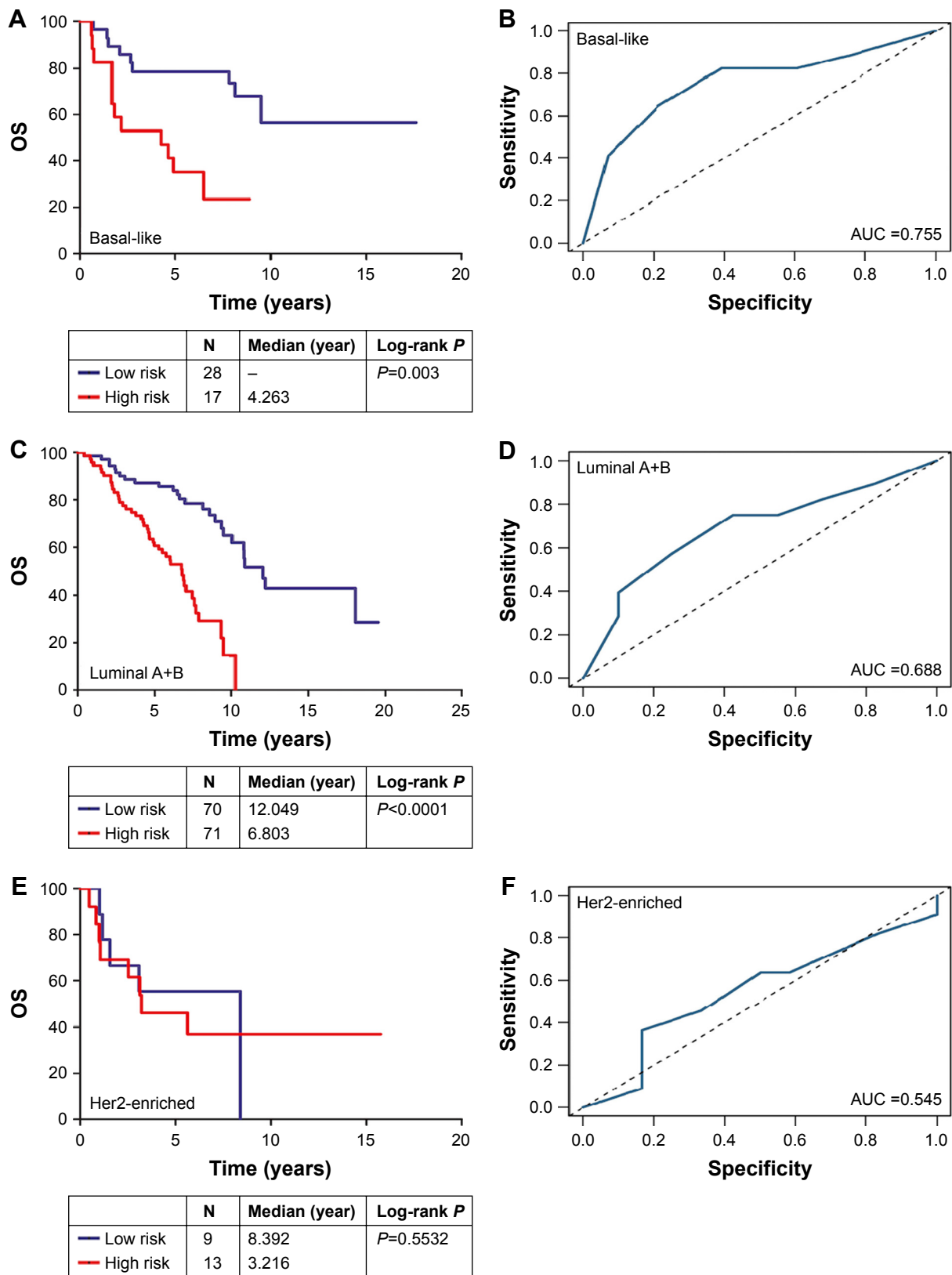


Figure 4 (A) Kaplan–Meier analysis of OS in the basal-like carcinoma group: OS rates between the high-risk group and low-risk group showed statistically significant differences using the log-rank test ($P=0.003$); (B) the ROC curve AUC was 0.755. (C) Kaplan–Meier analysis of OS in the luminal carcinoma 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.

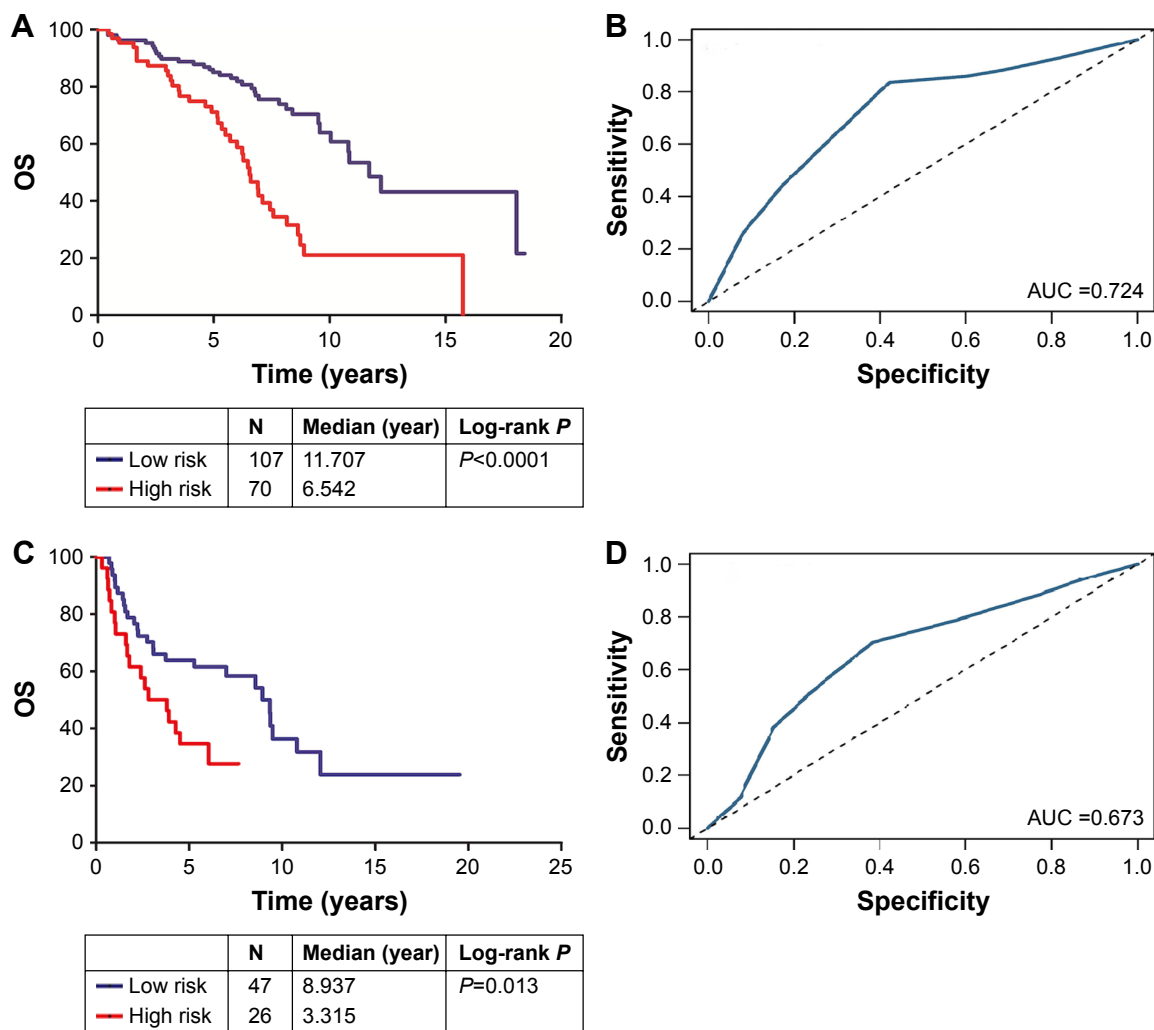


Figure 5 (A) Kaplan–Meier analysis of OS in stage I and II groups: OS rates between the high-risk group and low-risk group showed statistically significant differences ($P<0.0001$); (B) the ROC curve AUC was 0.724. (C) Kaplan–Meier analysis of OS in stage III and IV groups: OS rates between the high-risk 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
<i>hsa-mir-16-2</i>	237	0.556	0.379–0.817	0.003
<i>hsa-mir-484</i>	237	1.560	1.043–2.332	0.030
<i>hsa-mir-31</i>	237	0.486	0.333–0.711	0.000
<i>hsa-mir-877</i>	237	1.476	0.968–2.251	0.071
<i>hsa-mir-937</i>	237	1.223	0.815–1.837	0.331
<i>hsa-let-7b</i>	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
<i>hsa-mir-31</i>	<i>NR2C2</i>	nuclear receptor subfamily 2 group C member 2
<i>hsa-mir-31</i>	<i>MLXIP</i>	MLX interacting protein
<i>hsa-mir-31</i>	<i>STAU2</i>	staufen double-stranded RNA binding protein 2
<i>hsa-mir-31</i>	<i>ATF7IP</i>	activating transcription factor 7 interacting protein
<i>hsa-mir-31</i>	<i>PRKAA2</i>	protein kinase AMP-activated catalytic subunit alpha 2
<i>hsa-mir-31</i>	<i>ZNF16</i>	zinc finger protein 16
<i>hsa-mir-31</i>	<i>RHBDL3</i>	rhomboid like 3
<i>hsa-mir-31</i>	<i>GPRC5A</i>	G protein-coupled receptor class C group 5 member A
<i>hsa-mir-31</i>	<i>ARID1A</i>	AT-rich interaction domain 1A
<i>hsa-mir-31</i>	<i>KHDRBS3</i>	KH RNA binding domain containing, signal transduction associated 3
<i>hsa-mir-31</i>	<i>UCN2</i>	urocortin 2
<i>hsa-mir-31</i>	<i>CTNND2</i>	catenin delta 2
<i>hsa-mir-31</i>	<i>KLF13</i>	Kruppel like factor 13
<i>hsa-mir-31</i>	<i>IQSEC2</i>	IQ motif and Sec7 domain 2
<i>hsa-mir-31</i>	<i>RAB6B</i>	RAB6B, member RAS oncogene family
<i>hsa-mir-31</i>	<i>TFRC</i>	transferrin receptor
<i>hsa-mir-31</i>	<i>SLC24A3</i>	solute carrier family 24 member 3
<i>hsa-mir-31</i>	<i>KCNN3</i>	potassium calcium-activated channel subfamily N member 3
<i>hsa-mir-31</i>	<i>APBB2</i>	amyloid beta precursor protein binding family B member 2
<i>hsa-mir-31</i>	<i>TACC2</i>	transforming acidic coiled-coil containing protein 2
<i>hsa-mir-31</i>	<i>NDRG3</i>	NDRG family member 3
<i>hsa-mir-31</i>	<i>DICER1</i>	dicer 1, ribonuclease III
<i>hsa-mir-31</i>	<i>SPRED1</i>	sprouty related EVH1 domain containing 1
<i>hsa-mir-31</i>	<i>NFAT5</i>	nuclear factor of activated T-cells 5
<i>hsa-mir-31</i>	<i>BAHD1</i>	bromo adjacent homology domain containing 1
<i>hsa-mir-31</i>	<i>RTL9</i>	retrotransposon Gag like 9
<i>hsa-mir-31</i>	<i>KLF7</i>	Kruppel like factor 7
<i>hsa-mir-31</i>	<i>PRSS8</i>	protease, serine 8
<i>hsa-mir-31</i>	<i>PIK3C2A</i>	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha
<i>hsa-mir-31</i>	<i>FNDC5</i>	fibronectin type III domain containing 5
<i>hsa-mir-31</i>	<i>ZNHIT6</i>	zinc finger HIT-type containing 6
<i>hsa-mir-31</i>	<i>BTBD11</i>	BTB domain containing 11
<i>hsa-mir-31</i>	<i>PHF8</i>	PHD finger protein 8
<i>hsa-mir-31</i>	<i>ZNF662</i>	zinc finger protein 662
<i>hsa-mir-31</i>	<i>TMPRSS11F</i>	transmembrane protease, serine 11F
<i>hsa-mir-31</i>	<i>CCNC</i>	cyclin C
<i>hsa-mir-31</i>	<i>FZD4</i>	frizzled class receptor 4
<i>hsa-mir-31</i>	<i>SATB2</i>	SATB homeobox 2
<i>hsa-mir-31</i>	<i>SLC43A2</i>	solute carrier family 43 member 2
<i>hsa-mir-31</i>	<i>RSF1</i>	remodeling and spacing factor 1
<i>hsa-mir-31</i>	<i>RAP2B</i>	RAP2B, member of RAS oncogene family
<i>hsa-mir-31</i>	<i>FMNL3</i>	formin like 3
<i>hsa-mir-31</i>	<i>TM9SF4</i>	transmembrane 9 superfamily member 4
<i>hsa-mir-31</i>	<i>PPP1R12B</i>	protein phosphatase 1 regulatory subunit 12B
<i>hsa-mir-31</i>	<i>SLC39A14</i>	solute carrier family 39 member 14
<i>hsa-mir-31</i>	<i>AKAP7</i>	A-kinase anchoring protein 7
<i>hsa-mir-31</i>	<i>HOXC13</i>	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	TACCI	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	OXSRI	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	VP53	VP53, 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	GNAI3	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	APIG1	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	TBLIX	transducin beta like IX-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 11, 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-recogin 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	S100BP	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	VT11A	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	semaphorin 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	WWC 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	<i>HNF1A</i>	HNF1 homeobox A
hsa-mir-484	<i>SPATA2L</i>	spermatogenesis associated 2 like
hsa-mir-484	<i>TXNRD3</i>	thioredoxin reductase 3
hsa-mir-484	<i>CPSF4</i>	cleavage and polyadenylation specific factor 4
hsa-mir-484	<i>NEO1</i>	neogenin 1
hsa-mir-484	<i>TCF7</i>	transcription factor 7 (T-cell specific, HMG-box)
hsa-mir-484	<i>HOXA5</i>	homeobox A5
hsa-mir-484	<i>MTF2</i>	metal response element binding transcription factor 2
hsa-mir-484	<i>PIK3CD</i>	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta
hsa-mir-484	<i>NCOA2</i>	nuclear receptor coactivator 2
hsa-mir-484	<i>RIN1</i>	Ras and Rab interactor 1
hsa-mir-484	<i>TRIM71</i>	tripartite motif containing 71
hsa-mir-484	<i>DDX31</i>	DEAD-box helicase 31
hsa-mir-484	<i>ACBD5</i>	acyl-CoA binding domain containing 5
hsa-mir-484	<i>ABR</i>	active BCR-related
hsa-mir-484	<i>GPR63</i>	G protein-coupled receptor 63
hsa-mir-484	<i>RARG</i>	retinoic acid receptor gamma
hsa-mir-484	<i>YAP1</i>	Yes associated protein 1
hsa-mir-484	<i>RANBP17</i>	RAN binding protein 17
hsa-mir-484	<i>POLD4</i>	DNA polymerase delta 4, accessory subunit
hsa-mir-484	<i>FAM160B2</i>	family with sequence similarity 160 member B2
hsa-mir-484	<i>LYSMD1</i>	LysM domain containing 1
hsa-mir-484	<i>PPARD</i>	peroxisome proliferator activated receptor delta
hsa-mir-484	<i>COL20A1</i>	collagen type XX alpha 1 chain
hsa-mir-484	<i>SCP2</i>	sterol carrier protein 2
hsa-mir-484	<i>IL20RB</i>	interleukin 20 receptor subunit beta
hsa-mir-484	<i>TMC8</i>	transmembrane channel like 8
hsa-mir-484	<i>SOX5</i>	SRY-box 5
hsa-mir-484	<i>MAPKAPK3</i>	mitogen-activated protein kinase-activated protein kinase 3
hsa-mir-484	<i>ZNF667</i>	zinc finger protein 667
hsa-mir-484	<i>GRAMD1C</i>	GRAM domain containing 1C
hsa-mir-484	<i>CRTC2</i>	CREB regulated transcription coactivator 2
hsa-mir-484	<i>SERF1B</i>	small EDRK-rich factor 1B
hsa-mir-484	<i>FLVCR2</i>	feline leukemia virus subgroup C cellular receptor family member 2
hsa-mir-484	<i>TRIM74</i>	tripartite motif containing 74
hsa-mir-484	<i>STAG3L3</i>	stromal antigen 3-like 3 (pseudogene)
hsa-mir-484	<i>PKNOX1</i>	PBX/knotted 1 homeobox 1
hsa-mir-484	<i>SOX21</i>	SRY-box 21
hsa-mir-484	<i>GLDN</i>	gliomedin
hsa-mir-484	<i>HOXC8</i>	homeobox C8
hsa-mir-484	<i>FFAR2</i>	free fatty acid receptor 2
hsa-mir-484	<i>SH2D1B</i>	SH2 domain containing 1B
hsa-mir-484	<i>KDM4A</i>	lysine demethylase 4A
hsa-mir-484	<i>BCL7B</i>	BCL tumor suppressor 7B
hsa-mir-484	<i>PCDH19</i>	protocadherin 19
hsa-mir-484	<i>SERF1A</i>	small EDRK-rich factor 1A
hsa-mir-484	<i>EIF3J</i>	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	PDE1IA	phosphodiesterase 1IA
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	CY5B	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 1 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	SAPI30	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	angiomin 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	angiomin 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	INPPI	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	C10RF21	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 I 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	NABI	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
<i>hsa-mir-16-2</i>	<i>CCDC50</i>	coiled-coil domain containing 50
<i>hsa-mir-16-2</i>	<i>RBPJ</i>	recombination signal binding protein for immunoglobulin kappa J region
<i>hsa-mir-16-2</i>	<i>USP46</i>	ubiquitin specific peptidase 46
<i>hsa-mir-16-2</i>	<i>MOB1B</i>	MOB kinase activator 1B
<i>hsa-mir-16-2</i>	<i>PARM1</i>	prostate androgen-regulated mucin-like protein 1
<i>hsa-mir-16-2</i>	<i>CNKSR3</i>	CNKSR family member 3
<i>hsa-mir-16-2</i>	<i>CDK13</i>	cyclin dependent kinase 13
<i>hsa-mir-16-2</i>	<i>PCDHA6</i>	protocadherin alpha 6
<i>hsa-mir-16-2</i>	<i>PCDHAC1</i>	protocadherin alpha subfamily C, 1
<i>hsa-mir-16-2</i>	<i>RBM27</i>	RNA binding motif protein 27
<i>hsa-mir-16-2</i>	<i>USP49</i>	ubiquitin specific peptidase 49
<i>hsa-mir-16-2</i>	<i>SAMD9L</i>	sterile alpha motif domain containing 9 like
<i>hsa-mir-16-2</i>	<i>PEG10</i>	paternally expressed 10
<i>hsa-mir-16-2</i>	<i>SMARCA2</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
<i>hsa-mir-16-2</i>	<i>ZNF483</i>	zinc finger protein 483
<i>hsa-mir-16-2</i>	<i>ASTN2</i>	astrotactin 2
<i>hsa-mir-16-2</i>	<i>FOXP2</i>	forkhead box P2
<i>hsa-mir-16-2</i>	<i>CALU</i>	calumenin
<i>hsa-mir-16-2</i>	<i>NUP205</i>	nucleoporin 205
<i>hsa-mir-16-2</i>	<i>TMEM178B</i>	transmembrane protein 178B
<i>hsa-mir-16-2</i>	<i>DCAF4L2</i>	DDB1 and CUL4 associated factor 4 like 2
<i>hsa-mir-16-2</i>	<i>FBXO32</i>	F-box protein 32
<i>hsa-mir-16-2</i>	<i>KBTBD3</i>	kelch repeat and BTB domain containing 3
<i>hsa-mir-16-2</i>	<i>MAB21L1</i>	mab-21 like 1
<i>hsa-mir-16-2</i>	<i>RGCC</i>	regulator of cell cycle
<i>hsa-mir-16-2</i>	<i>NALCN</i>	sodium leak channel, non-selective
<i>hsa-mir-16-2</i>	<i>TEX30</i>	testis expressed 30
<i>hsa-mir-16-2</i>	<i>RASSF8</i>	Ras association domain family member 8
<i>hsa-mir-16-2</i>	<i>C12ORF66</i>	chromosome 12 open reading frame 66
<i>hsa-mir-16-2</i>	<i>DYRK2</i>	dual specificity tyrosine phosphorylation regulated kinase 2
<i>hsa-mir-16-2</i>	<i>TRPM7</i>	transient receptor potential cation channel subfamily M member 7
<i>hsa-mir-16-2</i>	<i>WDR72</i>	WD repeat domain 72
<i>hsa-mir-16-2</i>	<i>IREB2</i>	iron responsive element binding protein 2
<i>hsa-mir-16-2</i>	<i>ZNF790</i>	zinc finger protein 790
<i>hsa-mir-16-2</i>	<i>ZNF558</i>	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
	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
	hsa-mir-31	Apoptosis signaling pathway	PANTHER	P00006	4	108
CCKR signaling map		PANTHER	P06959	5	176	0.036514088
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
	Ionotropic 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
<i>hsa-mir-484</i>	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

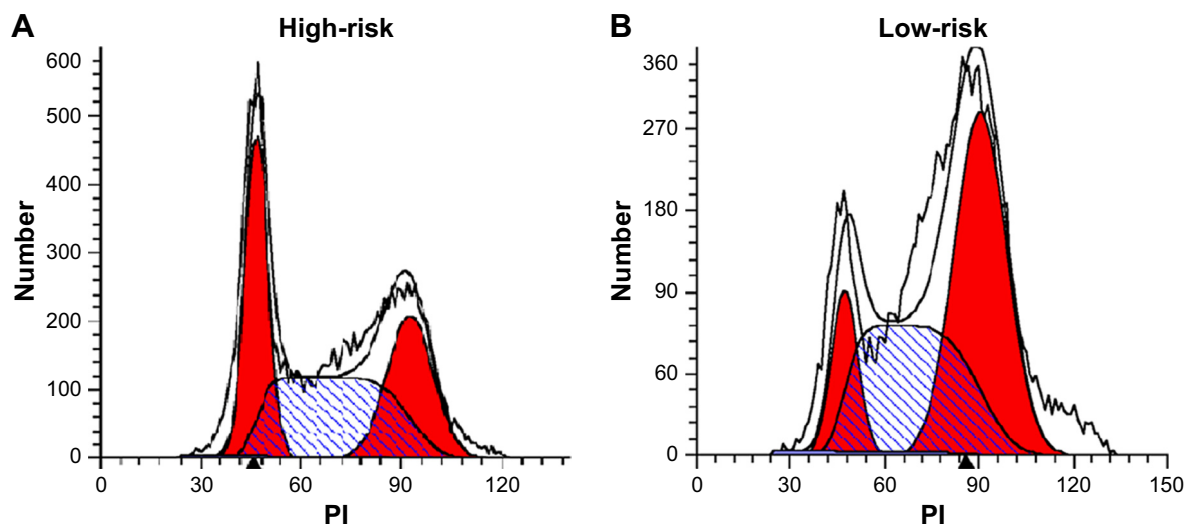


Figure 6 (Continued)

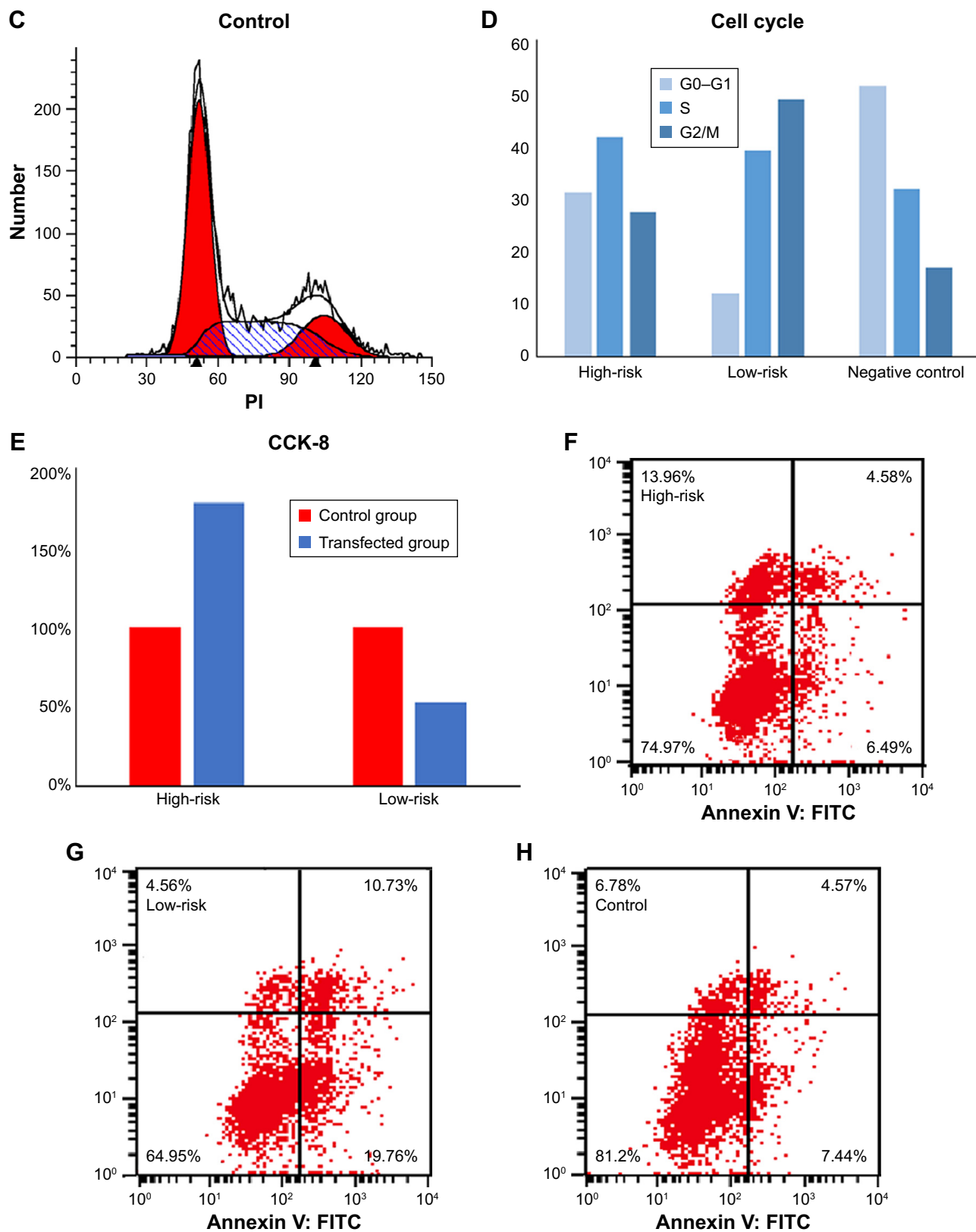


Figure 6 (A–D) Flow cytometry analysis of the cell cycle revealed that low-risk group cells were arrested at S and G2/M phase, while the cell cycle was activated in the high-risk group compared to the control group. **(E)** The cell viability of the high-risk group was significantly increased compared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

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Disclosure

The authors report no conflicts of interest in this work.

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