Review Paper:

Diagnostic and Prognostic Value of MicroRNA-30 Family in Breast Cancer: A Systematic Review and Meta-Analysis

Huynh Huu Luan[1,2,3], Duong Thi Chung Thuy[1,3], Nguyen Tu Linh[3], Nguyen Thi Ngoc Thanh[1,2,3] and Nguyen Thi Hue[1,2,3*]

1. Human Genetics Laboratory, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, VIETNAM
2. Department of Physiology and Animal Biotechnology, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, VIETNAM
3. Vietnam National University, Ho Chi Minh City, VIETNAM
*nthue@hcmus.edu.vn

Abstract

The diagnostic and prognostic role of the microRNA-30 (miR-30) family remains inconsistent in breast cancer (BC). This meta-analysis aimed to summarize the diagnostic and prognostic value of miR-30s in BC. A comprehensive search was performed through PubMed, BMC, Science Direct and Google Scholar. The QUADAS-2 and NOS tools were used to assess the quality of the included studies. The diagnostic accuracy of miR-30 family expression was measured using the pooled sensitivity, specificity, diagnostic odds ratio and positive/negative likelihood ratios while the pooled HR of survivals in BC patients was used to estimate the prognostic value. All statistical analyses were performed using R 4.1.3.

Twenty-two articles were eligible for meta-analysis. MiR-30s (-a-b-c) and (b-c-e) expression were suggested as promising BC and metastatic-BC diagnostic biomarkers respectively with areas under the SROC curve of 0.88. Especially, miR-30b served as a high diagnostic accuracy biomarker for early-stage BC (AUC = 0.92). Meanwhile, low-expression of miR-30s was associated with worse survivals in BC patients, with HRs for OS of 0.66 [0.51–0.85], DFS of 0.72 [0.62–0.83] and PFS of 0.61 [0.52–0.72]. In BC subtypes, decreased miR-30s expression predicted reduced DFS in HER2-positive (HR = 0.53 [0.37–0.77]) and TNBC (HR = 0.20 [0.11–0.37]), but was insignificant on OS of TNBC (p-value = 0.095) and DFS of luminal (p-value = 0.340). miR-30s expression was identified as BC, MBC and early-stage BC diagnostic biomarker and a valuable prognostic biomarker for survival in patients with BC.

Keywords: microRNA-30 family, biomarker, diagnosis, prognosis, breast cancer, meta-analysis.

Introduction

Breast cancer (BC) remains the leading cause of cancer-induced death in women worldwide, accounting for nearly one in six cancer-related women's deaths in 2020[35]. Based on hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) expression, breast cancer is usually classified as Luminal A (HR+/HER2-), Luminal B (HR+/HER2+), HER2-positive (HR-/HER2+), or TNBC (HR-/HER2-)[19]. The luminal A subtype is the most prevalent[30], while TNBC is implicated in the most aggressive clinical outcome[20]. Since early detection is critical in controlling disease and improving survival rates, early diagnostic strategies for BC are getting more attention. To date, clinical breast examination or imaging is still the standard screening method for breast cancer, but false-negative and false-positive results limit their application[7,25]. There is, therefore, a need for novel and more accurate detection strategies for breast cancer.

MicroRNAs (miRNA) have attracted much attention for their association with breast cancer pathophysiology and response to treatment[61,62]. MiRNA is a short, single-stranded non-coding RNA that regulates various physiological processes such as metabolism, apoptosis, cell growth and division[55,57,58]. Mounting evidence suggested a significant effect of miRNAs on breast cancer development and progression[52,54], indicating their expression as promising biomarkers for BC. In this regard, the mR-30 family members are mainly reported to be tumor suppressors, inhibiting breast cancer growth[56], epithelial-mesenchymal transition (EMT)[53] and anti-apoptosis[49].

There are five members and six distinct mature miRNAs of the miR-30 family including miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d and miR-30e, which share the same sequence of "GUAAACAU" in their seed region. These miRNAs are encoded by six genes located on three different chromosome regions: miR-30e and miR-30c-1 on 1p34.2, miR-30c-2 and miR-30a on 6q13 and miR-30b and miR-30d on 8q24.22[41].

Indeed, accumulating evidence[37,47,48,51] has confirmed the dysregulation of miR-30s members in BC patients and could serve as a potential diagnostic and prognostic biomarker, but has inconsistent findings. For instance, Tavakolpournegari et al[37] suggested that miR-30s members' dysregulation was correlated with survival in BC patients and subtype-specific miRNA signatures were involved in BC's prognosis and clinical treatment[21]. In contrast, others[5] confirmed no association between the miR-30s and BC patients' outcomes. In addition, inconsistencies in their application as BC

reliable detection was exhibited in several diagnostic studies[16,17,28]. Moreover, most of these studies assessed the abilities of individual members with a limited sample size. Therefore, the diagnostic and prognostic role of the miRNA-30 family in breast cancer needs to be validated using a quantitative method to combine data from multiple studies[28]. Here, we conducted a systematic review and meta-analysis to confirm the diagnostic and prognostic significance of the miR-30 family in breast cancer.

Literature search strategy, inclusion and exclusion criteria: We conducted a systematic literature search in the PubMed, BMC, ScienceDirect and Google Scholar databases up to January 2023 to identify studies that met our criteria, with a restriction on the English language. Our search strategy used the terms "miR-30" or "microRNA-30," or "hsa-miR-30," or "miR-30a," or "miR-30b" or "miR-30c" or "miR-30d" or "miR-30e" combined with "breast" or "mammary" and "cancer" or "tumor" or "neoplasm" or "carcinoma."

An article was eligible if it met the following criteria: (1) patients with breast cancer were confirmed by histopathological examination; (2) controls were healthy or metastasis breast cancer-free before; (3) focused on the association between miRNA-30 expression and diagnosis and prognosis for BC; (3) a miRNA profiling method with a cutoff value was available; (4) a clear description of the sensitivity, specificity and number of cases and controls was provided for the diagnosis or supplying the hazard ratio (HR) of observed survival in the number of BC patients with elevated versus decreased miRNA expression levels for prognosis.

The quality of the included studies used for the diagnosis was assessed by the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. By answering an eleven-question list in four domains (patient selection, index testing, reference standards and flow and timing), a study's risk bias was judged as "low," "unclear," or "high" when the answer was "yes," "unclear," and "no" respectively[40]. For prognostic studies, we used the Newcastle-Ottawa scale (NOS) to assess the quality based on three criteria: patient selection, study group comparability and outcome assessment[3]. The maximum score a study could reach was 9 and a cutoff of 6 was suggested as an acceptable quality[22].

Statistical analysis: The diagnostic accuracy was assessed using measurements, including sensitivity, specificity, diagnostic odds ratio (DOR), positive/negative likelihood ratio (PLR/NLR) and area under the curve (AUC). Of these indices, AUC and DOR were considered the global measures for diagnostic test accuracy[34]. For prognostic analysis, the overall HR with the corresponding 95% CI and *p*-value were estimated for assessing the association between the expression of the miR-30 family and the survival of BC patients, including overall survival (OS), progression-free survival (PFS) and disease-free survival (DFS). A *p*-value below 0.05 was considered statistically significant. An association of up-regulation of miRNA with worse survival was indicated if HR > 1, while an observed HR < 1 suggested that low miRNA levels were associated with poor survival.

The heterogeneity across the included studies was tested by Cochran's Q test and the I-squared (I2) statistic. If the *p-value* was less than 0.10 for the Q test or if the I2 value was greater than 50%, suggesting significant heterogeneity[18], a random-effects model was adopted for the analysis[11]; otherwise, a fixed-effects model was applied[29]. Sources of heterogeneity were addressed through sub-analyses based on member type, sample type, measurement method and ethnicity. Furthermore, the threshold effect was further evaluated for heterogeneity from diagnostic analysis using the Spearman correlation coefficient (r) between sensitivity and specificity, with $r \geq 0.6$ considered as a contribution of the diagnostic threshold to substantial heterogeneity[42].

To assess potential bias across studies, we used the trim-and-fill method and Egger's regression for the funnel plot asymmetry test. An asymmetric shape of the trim-and-fill plot and a *p*-value < 0.05 from Eager's test indicate the presence of publication bias among the included studies[33]. All statistical analyses were performed in this meta-analysis using R software (version 4.1.3, package meta, mada and metafor).

Study identification and characteristics: A total of 6,603 manuscripts were retrieved from the databases. Duplicate manuscripts and manuscripts with other language then English totalling 1,571 were removed. We excluded 4,334 articles after screening by title and abstract, of which 1,496 were reviews, meta-analysis articles, meetings, or case reports; 2,599 were about other diseases; and 239 used miRNAs other than miR-30 family members. Following a review of the 698 remaining full-text manuscripts, 676 were eliminated because they were not available in full-text, research was conducted on cell lines or animal models, or there was no case-control design or insufficient data. Finally, we enrolled 22 eligible articles[1,2,5,8-10,12-15,17,21,23,24,26,28,32,38,39,44,46,50] in the meta-analysis (Fig. 1).

Tables 1 and 2 illustrate the main characteristics of the 22 included articles, of which seven were for the diagnosis, fourteen were for the prognosis and one was used for both diagnostic and prognostic analysis. The QUADAS-2 result of the eight diagnostic studies was described indicating almost all the risk of bias was addressed in the index test domain. For the 15 prognostic studies, their acceptable quality was evaluated with a NOS score ranging from 7 to 9 (Table 2).

Diagnostic value of miR-30s in BC: Eight studies from six articles[1,12,17,28,44,46] containing 523 patients with BC and 344 healthy individuals were used to estimate the diagnostic value of miR-30s in BC. Three members were investigated: miR-30a, miR-30b and miR-30c (miR-30a-b-c) were found

to have dysregulated expression between BC patients and healthy controls. Due to substantial heterogeneity among the included studies (I2 = 70.3% and 63.6%, respectively) (Fig. 2), a random-effects model was applied in the analysis. A pooled sensitivity of 0.82 (95% CI: 0.73–0.8), specificity of 0.83 (95% CI: 0.72–0.91), PLR of 3.74 (95% CI: 2.48–5.62), NLR of 0.26 (95% CI: 0.18–0.38), DOR of 21.06 (95% CI: 7.33–60.52) and AUC of 0.88 (95% CI: 0.83–0.93) (Table 3), along with being close to the top left corner of the SROC curve indicated that miR-30a-b-c had very good diagnostic accuracy in distinguishing BC patients from healthy controls.

Notably, five studies from two articles [1; 28] identified miR-30b as a biomarker for early BC. Fitting the fixed-effect model in the analysis with a sample size of 194 patients with early stages (≤ II stages) and 275 healthy controls (Fig. 2), miR-30b showed excellent diagnostic performance with an AUC of 0.92 (95% CI: 0.87–0.97) (sensitivity = 0.81, specificity = 0.78). A pooled PLR, NLR and DOR were 3.76 (95% CI: 2.65–5.34), 0.25 (95% CI: 0.18–0.33) and 16.42 (95% CI: 8.97–30.07), respectively (Table 3), demonstrating that miR-30b could discriminate early BC from healthy with moderate accuracy.

To investigate the diagnostic potential of miR-30s in MBC, we performed a meta-analysis including 139 patients with MBC and 165 MBC-free patients from three studies[10,12,13] (Fig. 2). In the results (Table 3), the heterogeneity was high in the specificity and DOR data (I^2 = 88.7% and 87.0%, respectively), (p-values < 0.01) and miR-30s (b-c-e) showed very good diagnostic performance (AUC = 0.88) in MBC with a sensitivity of 0.86 (95% CI: (0.70–0.94) and specificity of 0.77 (95% CI: 0.47–0.92) (Supplementary Fig. 2). Additionally, miR-30b-c-e could be used as a very good diagnostic accuracy biomarker for MBC with a pooled PLR of 3.69 (95% CI: 1.31–10.35), NLR of 0.20 (95% CI: 0.06–0.64) and DOR of 22.98 (95% CI: 1.85–284.74) (Table 3).

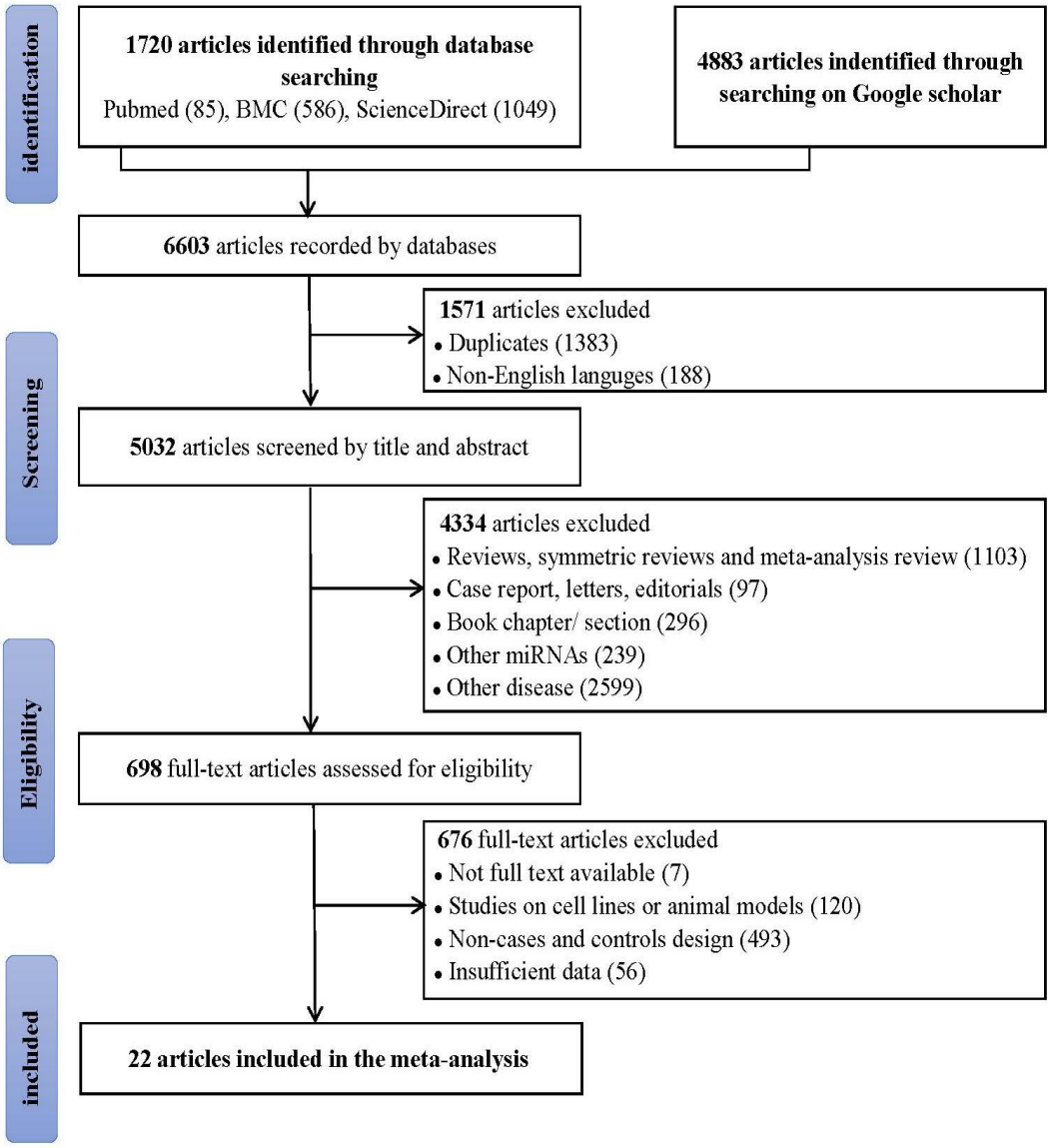


Fig. 1: A flowchart of literature search and study selection in the meta-analysis

Table 1
Characteristics of eligible diagnostic studies in the meta-analysis

Author, year [ref]	Country	miRNA	Sample Type	Study participants	Patient/ Control	Tumor grades	Test method	Reference gene	Cutoff	TP	FN	FP	TN
Zhang et al[46]	China	miR-30b	Blood	BC vs. Healthy	15/13	I-IV	RT-qPCR	miR-16	2.042	12	3	0	13
Zheng et al[44]	China	miR-30a	Plasma	BC vs. Healthy	100/64	I-IV	RT-qPCR	miR-16	0.0036	74	26	22	42
Hamdi et al[17]	Tunisia	miR-30b	Serum	BC vs. Healthy	20/20	II-III	RT-qPCR	RNU-48	-16.8	15	5	7	13
Adam-Artigues et al[1]	Spain	miR-30b	Tissue	BC vs. Healthy	112/40	I-IV	RT-qPCR	miR-16/ RNU-38B	NR	93	19	8	32
			Plasma	BC vs. Healthy	38/40	I-IV			NR	23	15	4	36
			Plasma	BC vs. Healthy	83/83	I-IV			NR	65	18	23	60
			Tissue	Early BC vs. Healthy	83/40	I-II			NR	71	12	8	32
			Plasma	Early BC vs. Healthy	51/83	I-II			NR	39	12	23	60
			Tissue	Early BC vs. Healthy	19/40	I			NR	14	5	3	37
			Plasma	Early BC vs. Healthy	21/83	I			NR	17	4	22	61
Luo et al[28]	China	miR-30b	Serum	BC vs. Healthy	80/29	I-IV	RT-qPCR	Cel-miR-356	NR	70	10	5	24
				Early BC vs. Healthy	20/29	I-II			NR	17	3	4	25
Elhelbawy et al[12]	Egypt	miR-30c	Blood	BC vs. Healthy	75/55	I-III	RT-qPCR	RNU6	≤20.6	73	2	2	53
				MBC vs. non-MBC	22/53	I-III			≤1.05	21	1	3	50
D'aiuto, et al[10]	Italy	miR-30e	Tissue	MBC vs. non-MBC	92/92	NR	Micro array		13.39	70	22	42	50
Estevão-Pereira et al[13]	Portugal	miR-30b	Plasma	MBC vs. non-MBC	25/20	I-IV	RT-qPCR	SNORD38B	4611	22	3	6	14

BC breast cancer, MBC metastasis breast cancer, TP true positive, FP: false positive, FN false negative, TN true negative, NR not reported

Supplementary Fig. 1: Risk of bias and applicability concerns graph about each domain for each included study for diagnostic value of miR-30s in breast cancer

Investigation of heterogeneity in the diagnostic value of miR-30 family for BC and MBC: Due to significant heterogeneity in the diagnostic value of miR-30s for BC and MBC, we investigated the possible cause of heterogeneity by Spearman's test and sub-analysis. As a result, a correlation coefficient of 0.429 and a *p*-value of 0.289 confirmed no heterogeneity derived from the threshold effect in the diagnostic value of miR-30s for BC. However, the threshold effect may be a potential source of heterogeneity in the diagnostic analysis for MBC (r = 1, *p*-value <0.001).

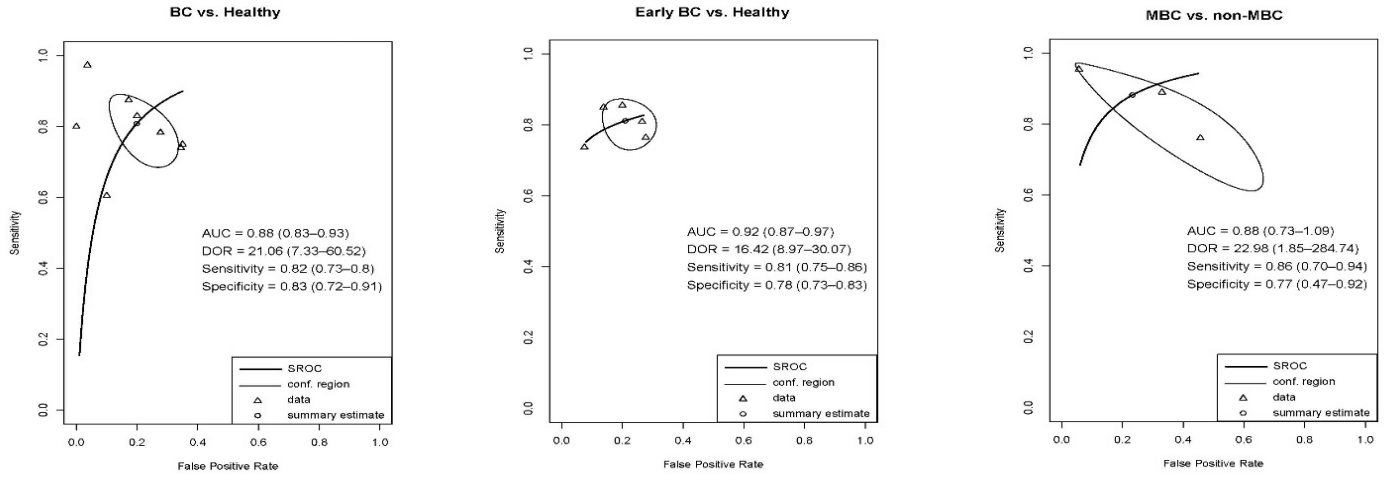
We used DOR and AUC to measure the sub-analyses based on ethnicity, sample type, miRNA type and measurement method. As the meta-regression results (Table 4), the Asian population was a possible cause of heterogeneity in the diagnostic value of miR-30s for BC (*p*-value = 0.003) whereas the Caucasian population may contribute to heterogeneity in the diagnostic value of miR-30s for MBC (*p*-value <0.001).

Prognostic value of miR-30 family in general BC: The prognostic value of the expression level of miR-30s in general BC was investigated across nine articles[8,9,15,21,23,26,32,39,50] (n = 6,346) performed with OS, DFS and PFS data. A substantial heterogeneity was observed in the analysis and the pooled HR revealed that the decreased regulation of miR-30 was associated with a worse prognosis in patients with BC (HR = 0.68, 95% CI: 0.52–0.72, p-value < 0.001) (Fig. 3).

Table 2
Characteristics of eligible prognostic studies in the meta-analysis

Author, year [ref]	Country	miRNA	Sample Type	Subtype	Patients	Tumor grades	Test method	Cutoff	Survival	NOS
Cheng et al[8]	Taiwan	miR-30a	Tissue	All	221	I-III	Microarray	> 2 FC	DFS/OS	9/9
Croset et al[9]	France	miR-30a/b/c/d/e	Tissue	All	109	I-III	RT-qPCR	Median	DFS	9/9
Gong et al[15]	China	miR-30a/b/c/d/e	Tissue	All	303	I-III	RT-qPCR	Median	DFS	9/9
Jamshidi et al[21]	Finland	miR-30d	Tissue	All	1238	I-III	ISH	Median	DFS/ OS	9/9
Wang et al[39]	China	miR-30a	Tissue	All	69	I-III	RT-qPCR	Median	OS	9/9
Zhou et al[50]	China	miR-30a	Tissue	All	1262	I-III	NGS	Median	OS	7/9
Lin et al[26]	China	miR-30c	Tissue	All	1262	I-III	ISH	Median	OS	7/9
Kawaguchi et al[23]	USA	miR-30a	Tissue	All	103	I-IV	NGS	Median	OS/ DFS	7/9
Rodriguez-Gonzalez et al[32]	Netherland	miR-30a/ miR-30c	Tissue	All	246	I-III	RT-qPCR	Median	PFS	9/9
Amorim, et al[2]	Portugal	miR-30b/ miR-30c	Tissue	Luminal	149	I-III	RT-qPCR	Median	DFS	9/9
Kim et al[24]	Korean	miR-30a	Tissue	Luminal	176	I-III	RT-qPCR	16.46	DFS	9/9
D'Aiuto et al[10]	Italy	miR-30e-3p	Tissue	Luminal HER2+	1027	I-III	Microarray	Median	DFS	7/9
Block et al[5]	Denmark	miR-30e-3p	Tissue	HER2+	465	I-III	Microarray	Median	DFS/ OS	7/9
Gasparini et al[14]	US	miR-30e	Tissue	TNBC	160	I-III	Microarray	Median	OS	9/9
Turashvili et al[38]	Canada	miR-30a/ miR-30c	Tissue	TNBC	51	II-III	NGS	Median	OS/ DFS	9/9

DFS disease-free survival, OS overall survival, PFS progression-free survival, NGS next-generation sequencing, ISH in-situ hybridization, FC fold change.



Supplementary Fig. 2: SROC plots of diagnostic value of miR-30a-b-c in BC, miR-30b in early BC and miR-30b-c-e in MBC

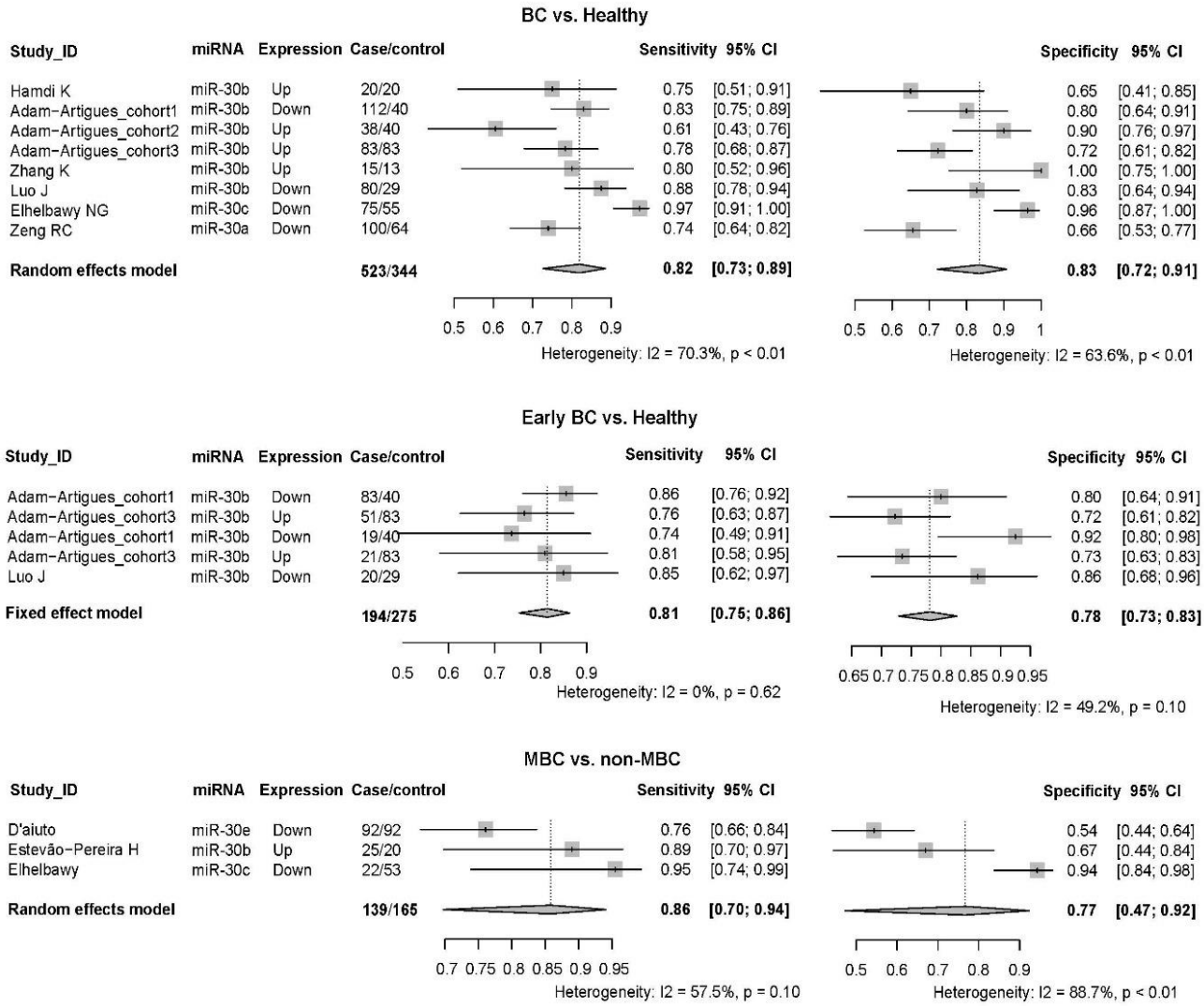


Fig. 2: Forest plots of sensitivity and specificity for miR-30s in diagnosing BC, early BC and MBC

Table 3
The results of diagnostic accuracy of miR-30s in breast cancer

Study participants	miRNA profile	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
BC vs. Healthy	miR-30a-b-c	0.82 (0.73–0.89)	0.83 (0.72–0.91)	3.74 (2.48–5.62)	0.26 (0.18–0.38)	21.06 (7.33–60.52)	0.88 (0.83–0.93)
Heterogeneity I^2 (*p*-value)		70.3% (<0.01)	63.6% (<0.01)	31.8% (0.17)	35.4% (0.15)	77.7% (<0.01)	7.8% (0.25)
Early BC vs. Healthy	miR-30b	0.81 (0.75–0.86)	0.78 (0.73–0.83)	3.76 (2.65–5.34)	0.25 (0.18–0.33)	16.42 (8.97–30.07)	0.92 (0.87–0.97)
Heterogeneity I^2 (*p*-value)		0% (0.62)	49.2% (0.10)	14.0% (0.33)	0% (0.57)	21.2% (0.28)	4.9% (0.18)
MBC vs. non-MBC	miR-30b-c-e	0.86 (0.70–0.94)	0.77 (0.47–0.92)	3.69 (1.31–10.35)	0.20 (0.06–0.64)	22.98 (1.85–284.74)	0.88 (0.73–1.09)
Heterogeneity I^2 (*p*-value)		57.5% (0.10)	88.7% (<0.01)	38.8% (0.20)	3.6% (0.35)	87.0% (<0.01)	3.0% (0.08)

PLR positive likelihood ratio, NLR negative likelihood ratio, DOR diagnostic odds ratio, AUC area under the curve, CI confidence interval

Table 4
The results of subgroup analysis for the diagnostic value of miR-30s in BC and MBC measuring DOR and AUC

Subgroup		BC vs. healthy			MBC vs. non-MBC		
		DOR (95% CI)	**AUC (95%CI)**	**Regression**	**DOR (95% CI)**	**AUC (95%CI)**	**Regression**
MiRNA	MiR-30b	14.44 (8.50–24.54)	0.88 (0.84–0.92)	0.200	16.43 (3.452–78.29)	0.95	
	MiR-30c	967.25 (132.00–109.44)	0.99		350.00 (34.40–3560.95)	0.98	
	MiR-30a	5.43 (2.75–10.75)	0.521		NA	NA	
	MiR-30e	NA	NA		3.79 (2.02–7.12)	0.74	
Sample type	Plasma	7.85 (4.80–12.85)	0.82 (0.75–0.92)	0.142	16.43 (3.45–78.29)	0.95	
	Serum	14.19 (2.44–82.42)	0.82 (0.75–0.92)		NA	NA	
	Tissue	19.58 (7.81–49.06)	0.90		3.79 (2.02–7.12)	0.74	
	Blood	414.39 (46.90–3661.75)	0.99 (0.94–1.04)		350.00 (34.40–3560.95)	0.98	
Ethnicity	Asian	18.09 (3.61–90.68)	0.88 (0.31–1.00)	0.003	NA	NA	
	Caucasian	23.45 (4.77–115.30)	0.88 (0.80–0.99)	0.907	22.98 (1.85–284.74)	0.88 (0.73–1.09)	<0.001
Measurements	Taqman	11.42 (7.08–18.42)	0.86 (0.79–0.95)	0.999	16.43 (3.452–78.29)	0.95	
	SYBR-dye	70.05 (2.95–1663.65)	0.90 (0.74–1.14)	0.611	350.00 (34.40–3560.95)	0.98	
	ROX dye	33.60 (10.44–108.19)	0.93		NA	NA	
	Microarray	NA	NA		3.79 (2.02–7.12)	0.74	

DOR diagnostic odds ratio, AUC area under the curve, CI confidence interval, NA not available

Five studies[21,23,26,39,50] (n = 3,147) reported effects of miR-30a, miR-30c and miR-30d (miR-30a-c-d) expression on OS. The analysis exhibited substantial heterogeneity (I2 = 82.69, p-value < 0.001). The results indicated a significant correlation between low miR-30a-c-d expression and poor OS in BC patients (HR = 0.66. 95% CI: 0.51–0.85, p-value = 0.002) (Fig. 3).

The impact of miR-30s expression on DFS was assessed by 18 studies from five articles[8,9,15,21,23] (n = 2,835). A random-effects model was applied in the analysis due to moderate heterogeneity (I^2 = 56.43%, *p*-value < 0.001) and the pooled HR suggested that the down expression of miR-30s was correlated with the worsening of DFS in patients with BC (HR = 0.72, 95% CI: 0.62 – 0.83, *p*-value < 0.001) (Fig. 3).

An internal meta-analysis was conducted from six studies in one paper[32], recording the correlation between miR-30a and miR-30c with PFS (n = 364). The pooled results showed that high expression had better PFS for miR-30a and miR-30c (HR = 0.61, 95% CI: 0.52–0.72, *p*-value < 0.001) by fitting a fixed-effect model (Fig. 3).

Prognostic value of miR-30 in BC subtypes: In order to investigate the association between miR-30s expression and survival in BC subtypes, we assessed the predictive ability of miR-30s for luminal DFS (n = 1,288), HER2-positive DFS (n = 370), TNBC OS (n = 262) and DFS (n = 153) based on six articles[2,5,10,14,24,38]. The heterogeneity of HR data for DFS of luminal and OS of TNBC was significant (I^2 > 50%, p-value < 0.1) (Fig. 4). Therefore, the random effects were applied to estimate the pooled HRs in these prognostic analyses; other analyses (DFS in HER2-positive and TNBC) used the fixed effect as a fitting model. In luminal, the overall HR was 0.57 (95% CI: 0.18–1.82) and the *p-value* was 0.340 (Fig. 4), indicating that the effect of miR-30 expression was insignificant on DFS.

In contrast, a significant correlation between miR-30e-3p down-expression and worse DFS was revealed in HER2-positive patients (HR = 0.53, 95% CI: 0.37–0.77, p-value = 0.0009) (Fig. 4). Likewise, for TNBC, we found that decreased expression of miR-30 has significantly interfered with reduced DFS patients (HR = 0.20, 95% CI: 0.11–0.37, p-value < 0.001) but was not associated with OS (HR = 0.41, 95% CI: 0.14–1.17, p-value = 0.095) (Fig. 4).

Investigation of heterogeneity in the prognostic analyses of miR-30s for BC and subtypes: The heterogeneity of miR-30 for OS and DFS in general BC, TNBC and Luminal was significant (I^2 > 50%, *p*-value < 0.1).

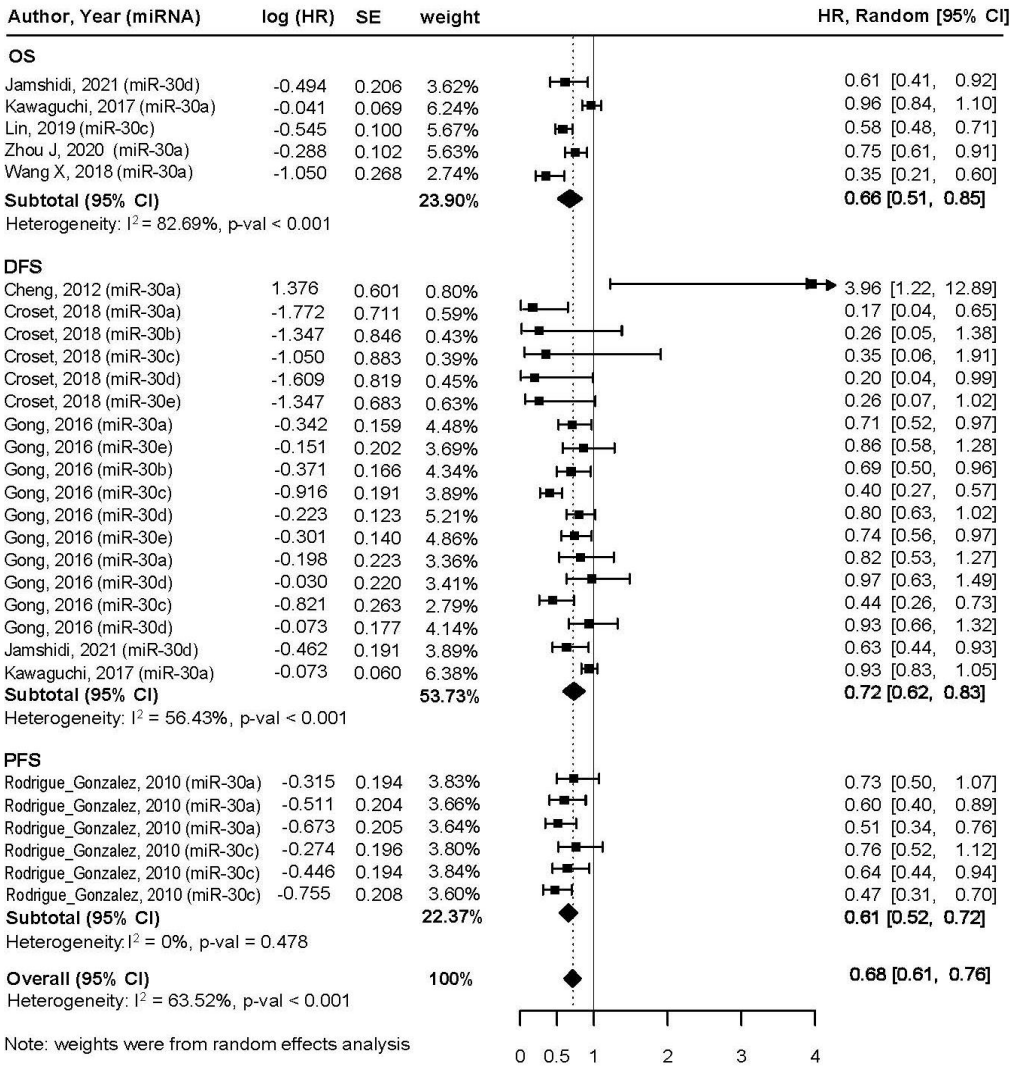


Fig. 3: Forest plots of the HRs for miR-30s expression levels in OS, DFS and PFS of general breast cancer patients

Therefore, the meta-regression was performed to explore the heterogeneity sources based on different publication years, ethnicities, sample sizes, miRNA types and measurement methods. As a result, (Supplementary Table 1), no potential source was found in comparisons for the OS of BC and the OS of TNBC. However, differences in miRNA type and ethnicity may contribute to the heterogeneity of prognostic analyses for DFS of BC and Luminal respectively (*p*-value < 0.05).

Publication bias and sensitivity analysis in the diagnostic and prognostic value of the miRNA-30 family for BC: We examined publication bias using trim and fill funnel plots and Egger's regression test. The trim-and-fill funnel plots' sharps were symmetrical for the diagnostic and prognostic analyses (Supplementary Figs. 3A and B). The *p*-values yielded from Egger's test were 0.475 for the diagnostic and 0.054 for the prognosis, suggesting that no publication bias exists among these studies.

We performed a sensitivity analysis to assess the stability of our results. As shown in supplementary tables 2 and 3, there was no significant change in the overall result or heterogeneity between studies in the diagnostic and prognostic analyses, indicating that our findings were consistent.

Discussion

Early diagnosis and state-of-the-art treatment are the most important strategies to improve BC patients' survival rates[4]. Recently, miRNAs have become potential biomarkers for BC because their altered expression has been implicated in tumor growth, progression and metastasis[59,60,62]. Among many miRNAs, the miR-30 family has been identified as a tumor suppressor[45,47] and has signatures associated with diagnosing, prognosis and responding to treatment in BC[1,36,48]. In this study, we aimed to validate the diagnostic and prognostic significance of the miR-30 family in breast cancer through a systematic review and meta-analysis. To date, numerous studies have provided valuable information on diagnostic and prognostic biomarkers for BC. In the diagnostic data, we found that miR-30a, miR-30b and miR-30c were identified as BC diagnostic biomarkers, while miR-30b, miR-30c and miR-30e were MBC diagnostic biomarkers and miR-30b was used for early BC diagnosis.

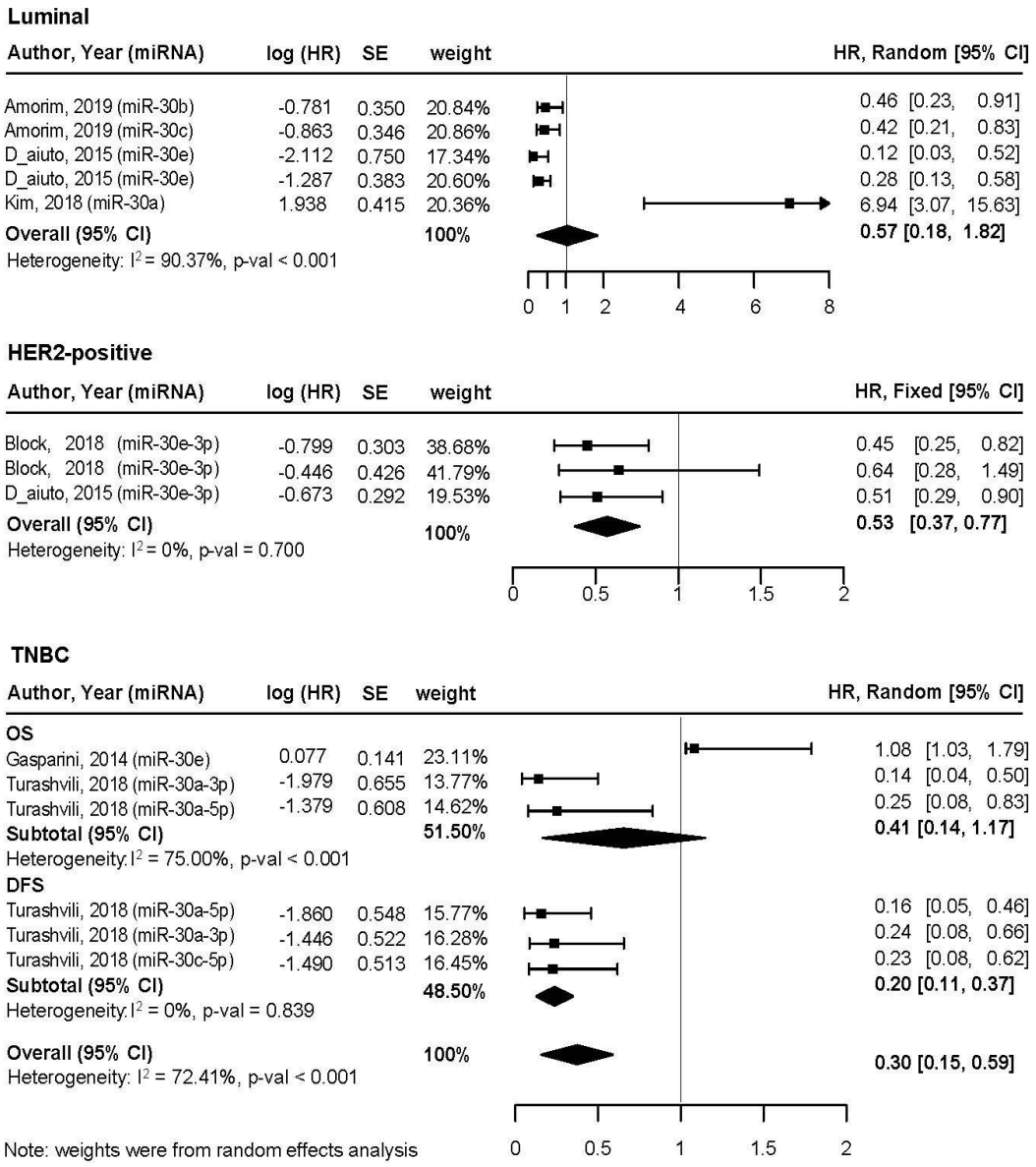
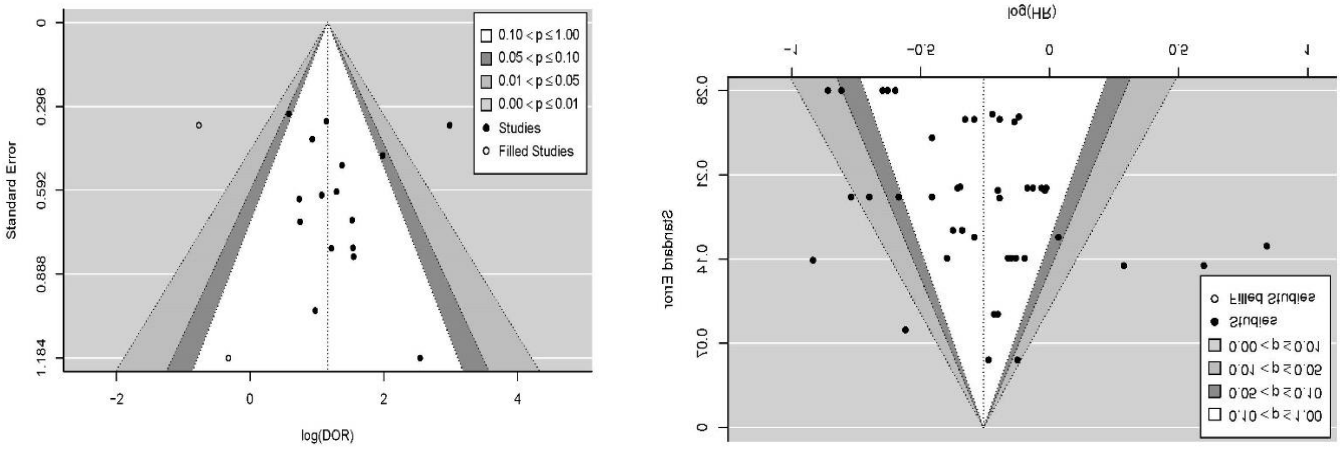


Fig. 4: Forest plots of the HRs for miR-30s expression levels in DFS and OS of patients with Luminal, HER2 and TNBC subtypes



(A) **(B)**

Supplementary Fig. 3: Funnel plots of publication bias regarding the diagnostic (A) and prognostic value (B) of miR-30s in BC

Supplementary Table 1
The results of heterogeneity test in the prognostic value of miR-30s for BC and subtypes

Comparisons	**Coef.**	**Std. Err.**	***t-value***	**p-value**	**95% CI**
OS of BC					
Publication year	-0.058	0.119	-0.491	0.657	-0.437 to 0.320
Ethnic	0.335	0.279	1.201	0.316	-0.553 to 1.223
Sample size	0	0.0003	-0.029	0.979	-0.001 to 0.001
Measurements	0.924	0.361	2.558	0.125	-0.630 to 2.478
MiRNA type	-0.079	0.137	-0.579	0.603	-0.515 to 0.357
DFS of BC					
Publication year	-0.111	0.066	-1.677	0.113	-0.251 to 0.029
Ethnic	-0.226	0.248	-0.913	0.375	-0.752 to 0.299
Sample size	-0.001	0.001	-0.553	0.589	-0.003 to 0.002
Measurements	1.838	0.920	1.999	0.065	-0.134 to 3.811
MiRNA type	-0.779	0.226	-3.449	0.004	-1.267 to -0.291
DFS of Luminal					
Publication year	0.333	0.383	0.871	0.448	-0.885 to 1.551
Ethnic	3.397	0.379	8.971	0.012	1.768 to 5.026
Sample size	-0.002	0.004	-0.522	0.638	-0.015 to 0.011
Measurements	-0.438	1.555	-0.281	0.797	-5.385 to 4.510
MiRNA type	-0.698	0.277	-2.517	0.086	-1.581 to 0.185
OS of TNBC					
Publication year	-0.434	0.079	-5.522	0.114	-1.431 to 0.564
Ethnic	-1.734	0.314	-5.522	0.114	-5.723 to 2.256
Sample size	0.016	0.003	5.522	0.114	-0.021 to 0.053
Measurements	1.734	0.314	5.522	0.114	-2.256 to 5.723
MiRNA type	-1.734	0.314	-5.522	0.114	-5.723 to 2.256

Supplementary Table 2
The results of sensitivity analysis for diagnostic value of miR-30s in breast cancer

Study eliminated	**Sensitivity**		**Specificity**		**AUC**
	Overall [95% CI]	**Heterogeneity *p*-value, I2 (%)**	**Overall [95% CI]**	**Heterogeneity *p*-value, I2 (%)**	**Overall [95% CI]**
MiR-30a-b-c in breast cancer					
None	0.82 [0.73; 0.89]	<0.01 70.3%	0.83 [0.72; 0.91]	<0.01 63.6%	0.88 [0.83; 0.93]
Hamdi, 2014	0.83 [0.72; 0.90]	<0.01 74.3%	0.85 [0.74; 0.92]	<0.01 66.7%	0.88 [0.83; 0.94]
Adam-Artigue, 2021	0.86 [0.74; 0.93]	<0.01 72.8%	0.86 [0.66; 0.95]	<0.01 71.0%	0.89 [0.81; 0.99]
Zhang, 2017	0.82 [0.72; 0.89]	< 0.01 74.5%	0.81 [0.70; 0.89]	<0.01 68.8%	0.87 [0.82; 0.94]
Luo J, 2014	0.81 [0.70; 0.89]	<0.01 69.4%	0.84 [0.70; 0.92]	<0.01 67.2%	0.87 [0.81; 0.94]
Elhelbawy, 2021	0.78 [0.72; 0.84]	0.04 54.3%	0.79 [0.69; 0.86]	0.12 39.3%	0.86 [0.82; 0.90]
Zheng RC, 2013	0.83 [0.73; 0.90]	<0.01 72.3%	0.86 [0.75; 0.92]	0.02 59.5%	0.90 [0.85; 0.95]
MiR-30b in early breast cancer					
None	0.81 [0.75; 0.86]	0.62 0%	0.78 [0.73; 0.83]	0.10 49.2%	0.92 [0.87; 0.97]
Adam-Artigue, 2021	0.85 [0.62; 0.95]	NA	0.86 [0.69; 0.95]	NA	0.93
Luo J, 2014	0.81 [0.75; 0.86]	0.49 0%	0.77 [0.72; 0.82]	0.09 53.0%	0.89 [0.82; 0.97]

MiR-30b-c-e in Metastasis breast cancer					
None	0.86 [0.70; 0.94]	0.10 57.5%	0.77 [0.47; 0.92]	<0.01 88.7%	0.88 [0.73; 1.09]
Elhelbawy, 2021	0.79 [0.71; 0.85]	0.19 46.2%	0.57 [0.47; 0.65]	0.30 5.3%	0.81
D'aiuto, 2015	0.92 [0.80; 0.97]	0.43 0%	0.86 [0.57; 0.96]	<0.01 86.9%	0.98
Estevão-Pereira H, 2019	0.85 [0.60; 0.96]	0.07 68.9%	0.81 [0.39; 0.97]	<0.01 94.3%	0.86

Supplementary Table 3
The results of sensitivity analysis for prognostic value of miR-30s for breast cancer

Study eliminated	**HR [95% CI]**	**Heterogeneity**	
I2 (%)		*p-value*	
OS of general BC			
None	0.66 [0.51; 0.85]	82.69	<0.001
Jamshidi, 2021	0.66 [0.49; 0.90]	87.90	0.004
Kawaguchi, 2017	0.61 [0.50; 0.74]	45.31	<0.001
Lin, 2019	0.68 [0.49; 0.94]	83.39	<0.001
Zhou J, 2020	0.63 [0.45; 0.87]	84.92	<0.001
Wang X, 2018	0.73 [0.59; 0.90]	75.88	<0.001
DFS of general BC			
None	0.77 [0.62; 0.83]	56.43	<0.001
Croset, 2018	0.70 [0.61; 0.81]	54.96	<0.001
Gong, 2016	0.76 [0.66; 0.87]	58.45	<0.001
Jamshidi, 2021	0.54 [0.28; 0.98]	83.87	<0.001
Kawaguchi, 2017	0.72 [0.61; 0.85]	59.35	<0.001
Cheng, 2012	0.69 [0.60; 0.81]	43.32	<0.001
DFS of Luminal subtype			
None	0.57 [0.18; 1.82]	90.37	<0.001
D_aiuto, 2015	0.65 [0.09; 4.74]	92.32	<0.001
Amorim, 2019	1.08 [0.25; 0.64]	91.79	<0.001
Kim, 2018	0.35 [0.24; 0.52]	0	0.349
DFS of HER2-positive subtype			
None	0.53 [0.37–0.77]	0	0.700
Block, 2018	0.51 [0.29; 0.90]	0	1
D_aiuto, 2015	0.47 [0.29; 0.76]	0	0.811
OS of TNBC			
None	0.41 [0.14; 1.17]	75.00	<0.001
J Gasparini, 2014	0.19 [0.07; 0.46]	0	0.805
Turashvili, 2018	1.08 [1.03; 1.79]	0	1

After pooled analysis from 523 patients with BC and 344 healthy controls, the diagnosis of BC using dysregulation of miR-30s (-a, -b and -c) showed high accuracy in terms of test sensitivity and specificity (0.82 and 0.83 respectively). Two measurement indices of the overall performance of the diagnostic test, the pooled AUC and DOR of miR-30a-b-c were 0.88 and 21.06 respectively, indicating a high efficacy in diagnosing BC from healthy.

Similarly, miR-30s (-b-c-e) had a high capability to accurately discriminate MBC from non-MBC with an AUC of 0.88 and DOR of 22.98. The pooled sensitivity and specificity of miR-30s for MBC were 0.86 and 0.77 respectively, indicating a lower underdiagnosis rate but a higher misdiagnosis rate than those distinguishing BC from healthy. The miR-30s, however, possessed SROC curves close to the top left corner, confirming their very good diagnostic performance for both BC and MBC.

In addition, by estimating the diagnostic measurements in 194 patients with I-II stage BC and 375 healthy individuals, miR-30b was proven to be an excellent performance biomarker for early-stage BC detection (AUC = 0.92) with a sensitivity of 0.81 and a specificity of 0.78. The DOR of 30b expression was 16.42, implying that individuals who tested positive for dysregulated miR-30b have 16.42 times higher chance of BC than those testing a negative result. These results, therefore, suggest potential clinical values of miR-30s as BC, MBC and early BC biomarkers. With regard to prognostic value, 15 articles investigated miR-30s as BC

prognosis biomarkers and subtype-specific biomarkers. The results, providing 3,147 patients with OS, 2,835 patients with DFS and 364 patients with PFS, suggested that the downregulation of miR-30s was associated with poor OS (HR = 0.66. 95% CI: 0.51–0.85, p-value = 0.002), DFS (HR = 0.72, 95% CI: 0.62–0.83, p-value < 0.001) and PFS (HR = 0.61, 95% CI: 0.52–0.72, p-value < 0.001) in breast cancer.

A similar finding was demonstrated by a recent report[9]; a low miR-30s was associated with an increased histological grade and lymph node metastases of breast cancer. The authors also found that overexpression of miR-30s promoted the anti-invasion and migration properties of BC cells, indicating miR-30s expression potential as a protective prognostic marker for breast cancer. Regarding the implication of miR-30 family expression and survival outcomes in BC subtypes, the prognostic meta-analyses were performed in luminal, HER2-positive and TNBC. Interestingly, a positive correlation between miR-30 expression and DFS was also found in HER2-positive (HR = 0.53, 95% CI: 0.37–0.77, p-value = 0.0009) and TNBC (HR = 0.20, 95% CI: 0.11–0.37, p-value < 0.001), indicating that the reduced miR-30 expression may be an abridged prognostic factor for DFS in these subtypes. However, the effect of specific miR-30 expression was insignificant on the DFS of the lumina (HR = 0.57, 95% CI: 0.18–1.82, p-value = 0.340) and OS of the TNBC (HR = 0.41, 95% CI: 0.14–1.17, p-value = 0.095).

The impact of the miR-30 family on diagnosis and prognosis may be explained by their tumor-suppressive role in multiple pathways (Fig. 5). The miR-30 family expression could reduce breast tumor proliferation and progression by suppressing the target genes, such as AVEN, FOXD1[31], Ubc9, ITGB3[43] and ITGA5[9]. An inhibitory effect of the miR-30 family on BC metastasis and invasion was suggested through interfering EMT process by targeting CDH11, ITGB3, ITGA5[9] and Ubc9[43]. MiR-30a plays as a tumor suppressor in BC tumorigenesis by targeting MDTH, VIM and Eya2 and the downregulation of these oncogenes by miR-30a could block EMT progression. Inhibition of notch intracellular domain (NICD) translocation of miR-30a by directly targeting Notch1 or DLL4[45] leads to suppression of BC angiogenesis and metastasis. miR-30b, miR-30d and miR-30e proved to negatively control PI3K/AKT signaling pathway via binding the 3'-UTR of *Derlin*[51], CREB[37] and IRS1[27] respectively, thereby inhibiting proliferation, migration and invasion in BC progression.

miR-30c overexpression could block EMT progression by downregulating VIM and TWF1[6] as well as block KRAS/MAPK signaling by KRAS suppression[36]. Moreover, functional experiments *in vivo* also reported that interference of miR-30 family expression significantly increased BC tumorigenesis and migration[6,36,51]. Consequently, mechanistic evidence supports our findings that a decrease in miR-30s level in breast cancer, as a tumor suppressor, is associated with poor prognosis and is suitable as a diagnostic biomarker.

This meta-analysis, however, has several limitations. First, there was significant heterogeneity in some diagnostic analyses. Different members, sample types and measurement methods used in RT-qPCR profiling may be potential cause of heterogeneity. Secondly, although the miRNA profile was related to the pathological grade of the tumor, different reference genes and cutoff values were used to normalize miRNA expression profiling in RT-qPCR, which may influence the variation in results. Subgroup analyses based on these parameters were limited due to the deficient published data. Thirdly, some HRs and 95% CI collected from the survival curve, which were not multivariate-adjusted HRs, might produce minor inaccuracies.

Finally, relatively small studies included some analyses and two internal meta-analyses (association between miR-30s expression and PFS of general BC and DFS of TNBC patients) that combine multiple studies within a single paper, which may reduce the statistical power of the diagnostic and prognostic outcomes.

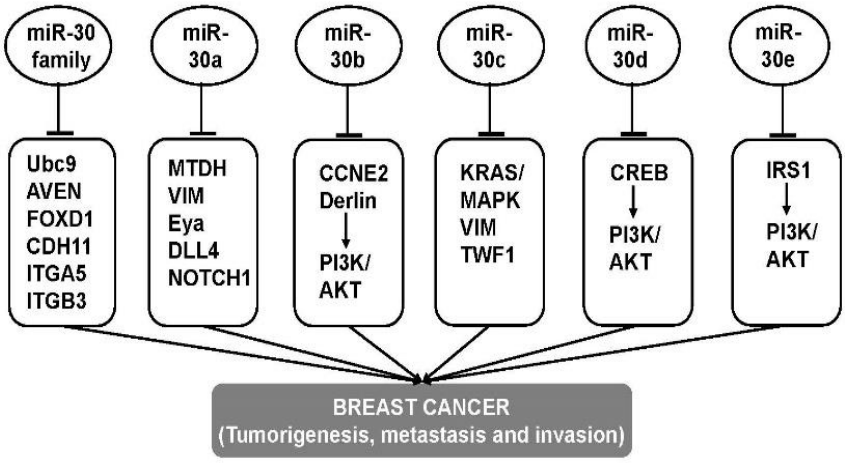


Fig. 5: The participation of miR-30s members in breast cancer tumorigenesis, metastasis and invasion

Conclusion

This systematic review and meta-analysis identified the miR-30 family as a promising diagnostic and prognostic biomarker for breast cancer. Combining miR-30a, miR-30b and miR-30c has very good diagnostic accuracy in breast cancer while miR-30b is able to detect early-stage breast cancer. In addition, miR-30b, miR-30c and miR-30e serve as metastasis breast cancer biomarkers. Furthermore, a low level of miR-30s is significantly associated with poor prognosis of patients with breast cancer. Well-designed studies on a larger scale are needed to validate our results further.

Acknowledgement

This research is funded by University of Science, VNU-HCM under grant number SH-CNSH 2022-09

References

1. Adam-Artigues A., Garrido-Cano I., Simón S., Ortega B., Moragón S., Lameirinhas A., Constâncio V., Salta S., Burgués O., Bermejo B., Henrique R., Lluch A., Jerónimo C., Eroles P. and Cejalvo J.M., Circulating miR-30b-5p levels in plasma as a novel potential biomarker for early detection of breast cancer, *ESMO Open*, **6(1)**, 100039 **(2021)**

2. Amorim M., Lobo J., Fontes-Sousa M., Estevão-Pereira H., Salta S., Lopes P., Coimbra N., Antunes L., Palma de Sousa S., Henrique R. and Jerónimo C., Predictive and Prognostic Value of Selected MicroRNAs in Luminal Breast Cancer, *Front Genet*, **10**, 815 **(2019)**

3. Bae J.M., A suggestion for quality assessment in systematic reviews of observational studies in nutritional epidemiology, *Epidemiol Health*, **38**, e2016014 **(2016)**

4. Birnbaum J.K., Duggan C., Anderson B.O. and Etzioni R., Early detection and treatment strategies for breast cancer in low-income and upper middle-income countries: a modelling study, *Lancet Glob Health*, **6(8)**, e885-e893 **(2018)**

5. Block I., Burton M., Sørensen K.P., Andersen L., Larsen M.J., Bak M., Cold S., Thomassen M., Tan Q. and Kruse T.A., Association of miR-548c-5p, miR-7-5p, miR-210-3p, miR-128-3p with recurrence in systemically untreated breast cancer, *Oncotarget*, **9(10)**, 9030-9042 **(2018)**

6. Bockhorn J., Dalton R., Nwachukwu C., Huang S., Prat A., Yee K., Chang Y.F., Huo D., Wen Y., Swanson K.E., Qiu T., Lu J., Park S.Y., Dolan M.E., Perou C.M., Olopade O.I., Clarke M.F., Greene G.L. and Liu H., MicroRNA-30c inhibits human breast tumour chemotherapy resistance by regulating TWF1 and IL-11, *Nat Commun*, **4**, 1393 **(2013)**

7. Brodersen J. and Siersma V.D., Long-term psychosocial consequences of false-positive screening mammography, *Ann Fam Med*, **11(2)**, 106-115 **(2013)**

8. Cheng C.W., Wang H.W., Chang C.W., Chu H.W., Chen C.Y., Yu J.C., Chao J.I., Liu H.F., Ding S.L. and Shen C.Y., MicroRNA-30a inhibits cell migration and invasion by downregulating vimentin expression and is a potential prognostic marker in breast cancer, *Breast Cancer Res Treat*, **134(3)**, 1081-1093 **(2012)**

9. Croset M. and Pantano F., miRNA-30 Family Members Inhibit Breast Cancer Invasion, Osteomimicry and Bone Destruction by Directly Targeting Multiple Bone Metastasis-Associated Genes, *Cancer Res*, **78(18)**, 5259-5273 **(2018)**

10. D'Aiuto F., Callari M., Dugo M., Merlino G., Musella V., Miodini P., Paolini B., Cappelletti V. and Daidone M.G., miR-30e* is an independent subtype-specific prognostic marker in breast cancer, *Br J Cancer*, **113(2)**, 290-298 **(2015)**

11. DerSimonian R. and Laird N., Meta-analysis in clinical trials, *Control Clin Trials*, **7(3)**, 177-188 **(1986)**

12. Elhelbawy N.G., Zaid I.F., Khalifa A.A., Gohar S.F. and Fouda E.A., miRNA-148a and miRNA-30c expressions as potential biomarkers in breast cancer patients, *Biochem Biophys Rep*, **27**, 101060 **(2021)**

13. Estevão-Pereira H., Lobo J., Salta S., Amorim M., Lopes P., Cantante M., Reis B., Antunes L., Castro F., Palma de Sousa S., Gonçalves C.S., Costa B.M., Henrique R. and Jerónimo C., Overexpression of circulating MiR-30b-5p identifies advanced breast cancer, *J Transl Med*, **17(1)**, 435 **(2019)**

14. Gasparini P., Cascione L., Fassan M., Lovat F., Guler G., Balci S., Irkkan C., Morrison C., Croce C.M., Shapiro C.L. and Huebner K., microRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers, *Oncotarget*, **5(5)**, 1174-1184 **(2014)**

15. Gong C., Tan W., Chen K., You N., Zhu S., Liang G., Xie X., Li Q., Zeng Y., Ouyang N., Li Z., Zeng M., Zhuang S., Lau W.Y., Liu Q., Yin D., Wang X., Su F. and Song E., Prognostic Value of a BCSC-associated MicroRNA Signature in Hormone Receptor-Positive HER2-Negative Breast Cancer, *EBioMedicine*, **11**, 199-209 **(2016)**

16. Hafez M.M., Hassan Z.K., Zekri A.R., Gaber A.A., Al Rejaie S.S., Sayed-Ahmed M.M. and Al Shabanah O., MicroRNAs and metastasis-related gene expression in Egyptian breast cancer patients, *Asian Pac J Cancer Prev*, **13(2)**, 591-598 **(2012)**

17. Hamdi K., Goerlitz D., Stambouli N., Islam M., Baroudi O., Neili B., Benayed F., Chivi S., Loffredo C., Jillson I.A., Benammar Elgaaied A., Blancato J.K. and Marrakchi R., miRNAs in Sera of Tunisian patients discriminate between inflammatory breast cancer and non-inflammatory breast cancer, *Springerplus*, **3**, 636 **(2014)**

18. Higgins J.P., Thompson S.G., Deeks J.J. and Altman D.G., Measuring inconsistency in meta-analyses, *Bmj*, **327(7414)**, 557-560 **(2003)**

19. Howlader N., Altekruse S.F., Li C.I., Chen V.W., Clarke C.A., Ries L.A. and Cronin K.A., US incidence of breast cancer subtypes defined by joint hormone receptor and HER2 status, *J Natl Cancer Inst*, **106(5)**, dju055 **(2014)**

20. Howlader N., Cronin K.A., Kurian A.W. and Andridge R., Differences in Breast Cancer Survival by Molecular Subtypes in the United States, *Cancer Epidemiol Biomarkers Prev*, **27(6)**, 619-626 **(2018)**

21. Jamshidi M., Fagerholm R. and Muranen T.A., High miR-30 Expression Associates with Improved Breast Cancer Patient

Survival and Treatment Outcome, *Cancers (Basel)*, **13(12)**, 2907 **(2021)**

22. Jennifer M., Gierisch C.B., Abigail Shapiro, Jennifer R. McDuffie, Natasha Cunningham, Daniel Bradford, Jennifer Strauss, Marie Callahan, May Chen, Adam Hemminger, Andrzej Kosinski and John W. Williams, Health Disparities in Quality Indicators of Healthcare Among Adults with Mental Illness, Department of Veterans Affairs (US) [Internet] **(2014)**

23. Kawaguchi T., Yan L., Qi Q., Peng X., Gabriel E.M., Young J., Liu S. and Takabe K., Overexpression of suppressive microRNAs, miR-30a and miR-200c are associated with improved survival of breast cancer patients, *Sci Rep*, **7(1)**, 15945 **(2017)**

24. Kim C., Go E.J. and Kim A., Recurrence prediction using microRNA expression in hormone receptor positive breast cancer during tamoxifen treatment, *Biomarkers*, **23(8)**, 804-811 **(2018)**

25. Le M.T., Mothersill C.E., Seymour C.B. and McNeill F.E., Is the false-positive rate in mammography in North America too high?, *Br J Radiol*, **89(1065)**, 20160045 **(2016)**

26. Lin S., Yu L., Song X., Bi J., Jiang L., Wang Y., He M., Xiao Q., Sun M., Olopade O.I., Zhao L. and Wei M., Intrinsic adriamycin resistance in p53-mutated breast cancer is related to the miR-30c/FANCF/REV1-mediated DNA damage response, *Cell Death Dis*, **10(9)**, 666 **(2019)**

27. Liu M.M., Li Z., Han X.D., Shi J.H., Tu D.Y., Song W., Zhang J., Qiu X.L., Ren Y. and Zhen L.L., MiR-30e inhibits tumor growth and chemoresistance via targeting IRS1 in Breast Cancer, *Sci Rep*, **7(1)**, 15929 **(2017)**

28. Luo J., Zhao Q., Zhang W., Zhang Z., Gao J., Zhang C., Li Y. and Tian Y., A novel panel of microRNAs provides a sensitive and specific tool for the diagnosis of breast cancer, *Mol Med Rep*, **10(2)**, 785-791 **(2014)**

29. Mantel N. and Haenszel W., Statistical aspects of the analysis of data from retrospective studies of disease, *J Natl Cancer Inst*, **22(4)**, 719-748 **(1959)**

30. Orrantia-Borunda E., Anchondo-Nuñez P., Acuña-Aguilar L.E., Gómez-Valles F.O. and Ramírez-Valdespino C.A., Subtypes of Breast Cancer, In Mayrovitz H.N., ed., Breast Cancer, Exon Publications, https://doi.org/10.36255/exon-publications-breast-cancer-subtypes **(2022)**

31. Ouzounova M., Vuong T., Ancey P.B., Ferrand M., Durand G., Le-Calvez Kelm F., Croce C., Matar C., Herceg Z. and Hernandez-Vargas H., MicroRNA miR-30 family regulates non-attachment growth of breast cancer cells, *BMC Genomics*, **14**, 139 **(2013)**

32. Rodríguez-González F.G., Sieuwerts A.M., Smid M., Look M.P., Meijer-van Gelder M.E., de Weerd V., Sleijfer S., Martens J.W. and Foekens J.A., MicroRNA-30c expression level is an independent predictor of clinical benefit of endocrine therapy in advanced estrogen receptor positive breast cancer, *Breast Cancer Res Treat*, **127(1)**, 43-51 **(2011)**

33. Shi L. and Lin L., The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses, *Medicine (Baltimore)*, **98(23)**, e15987 **(2019)**

34. Šimundić A.M., Measures of Diagnostic Accuracy: Basic Definitions, *Ejifcc*, **19(4)**, 203-211 **(2009)**

35. Sung H., Ferlay J. and Siegel R.L., Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, *CA Cancer J Clin*, **71(3)**, 209-249 **(2021)**

36. Tanic M., Yanowsky K., Rodriguez-Antona C., Andrés R., Márquez-Rodas I., Osorio A., Benitez J. and Martinez-Delgado B., Deregulated miRNAs in hereditary breast cancer revealed a role for miR-30c in regulating KRAS oncogene, *PLoS One*, **7(6)**, e38847 **(2012)**

37. Tavakolpournegari A., Hashemi M., Zare Karizi S., Matin Ahmadi A., Bidooki S.H. and Banaei G., Expression Patterns of miR181a and miR30d in Patients with Breast Cancer, *Iran J Public Health*, **51(7)**, 1594-1601 **(2022)**

38. Turashvili G., Lightbody E.D., Tyryshkin K., SenGupta S.K., Elliott B.E., Madarnas Y., Ghaffari A., Day A. and Nicol C.J.B., Novel prognostic and predictive microRNA targets for triple-negative breast cancer, *Faseb J*, **32(11)**, 5937–5954 **(2018)**

39. Wang X., Qiu H., Tang R., Song H., Pan H., Feng Z. and Chen L., miR-30a inhibits epithelial-mesenchymal transition and metastasis in triple-negative breast cancer by targeting ROR1, *Oncol Rep*, **39(6)**, 2635-2643 **(2018)**

40. Whiting P.F., Rutjes A.W., Westwood M.E., Mallett S., Deeks J.J., Reitsma J.B., Leeflang M.M., Sterne J.A. and Bossuyt P.M., QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies, *Ann Intern Med*, **155(8)**, 529-536 **(2011)**

41. Yang S.J., Yang S.Y., Wang D.D., Chen X., Shen H.Y., Zhang X.H., Zhong S.L., Tang J.H. and Zhao J.H., The miR-30 family: Versatile players in breast cancer, *Tumour Biol*, **39(3)**, 1010428317692204 **(2017)**

42. Young Ho L., Overview of the Process of Conducting Meta-analyses of the Diagnostic Test Accuracy, *J. Rheum. Dis.*, **25(1)**, 3-10 **(2018)**

43. Yu F., Deng H., Yao H., Liu Q., Su F. and Song E., Mir-30 reduction maintains self-renewal and inhibits apoptosis in breast tumor-initiating cells, *Oncogene*, **29(29)**, 4194-4204 **(2010)**

44. Zeng R.C., Zhang W., Yan X.Q., Ye Z.Q., Chen E.D., Huang D.P., Zhang X.H. and Huang G.L., Down-regulation of miRNA-30a in human plasma is a novel marker for breast cancer, *Med Oncol*, **30(1)**, 477 **(2013)**

45. Zhang H.D., Jiang L.H., Sun D.W., Li J. and Tang J.H., miR-30a inhibits the biological function of breast cancer cells by targeting Notch1, *Int J Mol Med*, **40(4)**, 1235-1242 **(2017)**

46. Zhang K., Wang Y.W., Wang Y.Y., Song Y., Zhu J., Si P.C. and Ma R., Identification of microRNA biomarkers in the blood of breast cancer patients based on microRNA profiling, *Gene*, **619**, 10-20 **(2017)**

47. Zhang N., Wang X., Huo Q., Sun M., Cai C., Liu Z., Hu G. and Yang Q., MicroRNA-30a suppresses breast tumor growth and metastasis by targeting metadherin, *Oncogene*, **33(24)**, 3119-3128 **(2014)**

48. Zhao Q., Yuan X., Zheng L. and Xue M., miR-30d-5p: A Non-Coding RNA With Potential Diagnostic, Prognostic and Therapeutic Applications, *Front Cell Dev Biol*, **10**, 829435 **(2022)**

49. Zheng S.R., Guo G.L., Zhang W., Huang G.L., Hu X.Q., Zhu J., Huang Q.D., You J. and Zhang X.H., Clinical significance of miR-155 expression in breast cancer and effects of miR-155 ASO on cell viability and apoptosis, *Oncol Rep*, **27(4)**, 1149-1155 **(2012)**

50. Zhou J., Wang L., Liu S., Zhou W., Jiang Y., Du J. and Dai J., Genetic Variations in miR-30 Family Member Regulatory Regions Are Associated with Breast Cancer Risk in a Chinese Population, *Biomed Res Int*, **2020**, 8781348 **(2020)**

51. Zhou J., Xiang A.Z., Guo J.F. and Cui H.D., miR-30b suppresses the progression of breast cancer through inhibition of the PI3K/Akt signaling pathway by targeting Derlin-1, *Transl Cancer Res*, **8(1)**, 180-190 **(2019)**

52. Zhou S.W., Su B.B., Zhou Y., Feng Y.Q., Guo Y., Wang Y.X., Qi P. and Xu S., Aberrant miR-215 expression is associated with clinical outcome in breast cancer patients, *Med Oncol*, **31(11)**, 259 **(2014)**

53. Zhou W., Ye X.L., Xu J., Cao M.G., Fang Z.Y., Li L.Y., Guan G.H., Liu Q., Qian Y.H. and Xie D., The lncRNA H19 mediates breast cancer cell plasticity during EMT and MET plasticity by differentially sponging miR-200b/c and let-7b, *Sci Signal*, **10(483)**, doi: 10.1126/scisignal.aak9557 **(2017)**

54. Zhu D., Lv W., Zhou X., He Y., Yao H., Yu Y., Zhang G. and Zhang Q., Long non-coding RNA TMPO-AS1 promotes tumor progression via sponging miR-140-5p in breast cancer, *Exp Ther Med*, **21(1)**, 17 **(2021)**

55. Zhu Q., Zhang X., Zai H.Y., Jiang W., Zhang K.J., He Y.Q. and Hu Y., circSLC8A1 sponges miR-671 to regulate breast cancer tumorigenesis via PTEN/PI3k/Akt pathway, *Genomics*, **113(1 Pt 1)**, 398-410 **(2021)**

56. Zhu X., Qiu J. and Zhang T., MicroRNA-188-5p promotes apoptosis and inhibits cell proliferation of breast cancer cells via the MAPK signaling pathway by targeting Rap2c, *J Cell Physiol*, **235(3)**, 2389-2402 **(2020)**

57. Zhu Z., Wang S., Zhu J., Yang Q., Dong H. and Huang J., MicroRNA-544 down-regulates both Bcl6 and Stat3 to inhibit tumor growth of human triple negative breast cancer, *Biol Chem*, **397(10)**, 1087-1095 **(2016)**

58. Zou Q., Tang Q., Pan Y., Wang X., Dong X., Liang Z. and Huang D., MicroRNA-22 inhibits cell growth and metastasis in breast cancer via targeting of SIRT1, *Exp Ther Med*, **14(2)**, 1009-1016 **(2017)**

59. Zou Q., Yi W., Huang J., Fu F., Chen G. and Zhong D., MicroRNA-375 targets PAX6 and inhibits the viability, migration and invasion of human breast cancer MCF-7 cells, *Exp Ther Med*, **22(4)**, 1169 **(2021)**

60. Zou Q., Zhou E., Xu F., Zhang D., Yi W. and Yao J., A TP73-AS1/miR-200a/ZEB1 regulating loop promotes breast cancer cell invasion and migration, *J Cell Biochem*, **119(2)**, 2189-2199 **(2018)**

61. Zou R., Loke S.Y., Tang Y.C., Too H.P. and Zhou L., Development and validation of a circulating microRNA panel for the early detection of breast cancer, *Br J Cancer*, **126(3)**, 472-481 **(2022)**

62. Zuo Z., Ye F., Liu Z., Huang J. and Gong Y., MicroRNA-153 inhibits cell proliferation, migration, invasion and epithelial-mesenchymal transition in breast cancer via direct targeting of RUNX2, *Exp Ther Med*, **17(6)**, 4693-4702 **(2019)**.

(Received 04th September 2023, revised 28th January 2025, accepted 25th February 2025)