

Upregulated miR-203a-3p and its potential molecular mechanism in breast cancer: A study based on bioinformatics analyses and a comprehensive meta-analysis

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Abstract. Breast cancer (BC) has been identified as the leading malignancy in women worldwide. However, the potential molecular mechanism of microRNA (miR)-203a-3p in BC remains to be elucidated. The present study evaluated the expression of miR-203a-3p in BC and adjacent normal tissue in several publically available datasets. The distinguishability of precursor miR-203a and miR-203a-3p in BC tissue and adjacent breast tissue was assessed using receiver operating characteristic (ROC) and summarized ROC (sROC) approaches. In addition, gene ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes pathway analysis and protein-protein interaction analysis were performed to determine the potential molecular mechanism of miR-203a-3p in BC. It was identified that the expression of precursor miR-203a was markedly upregulated in 1,077 BC tissue samples compared to 104 adjacent breast tissue samples from The Cancer Genome Atlas. Additionally, an increasing trend in miR-203a-3p expression was observed in 756 BC tissue samples compared with 76 adjacent breast tissue samples from the University of California Santa Cruz Xena project. In addition, a comprehensive meta-analysis suggested that the expression of miR-203a-3p was markedly increased in 2,444 BC tissue samples compared with 559 adjacent breast tissue samples. The area under the curve of the ROC and sROC revealed that miR-203a-3p expression was able to distinguish between BC tissue and adjacent breast tissue. However, miR-203a-3p exhibited no prognostic value in BC. The results of GO enrichment demonstrated that the miR-203a target genes were associated with 'plasma membrane integrity', 'cell surface receptor linked signal and transduction' and

'3',5'-cyclic nucleotide phosphodiesterase activity'. 'Purine metabolism' was identified as the pathway with the most enrichment of miR-203a-3p target genes in BC. The present study also identified insulin-like growth factor receptor (IGF1) as a hub gene associated with miR-203a in BC. In summary, miR-203a-3p may enhance the development and oncogenesis of BC, and IGF1 was defined as a hub gene of miR-203a-3p in BC.

Introduction

Breast cancer (BC) ranks as the most common malignancy in women worldwide and ranks as the second most common cause of cancer-associated mortality (1,2). The incidence of BC is increasing; the latest cancer statistics from the USA estimated that the expected numbers of new cancer cases and mortalities could reach 66,120 and 40,920, respectively, in 2018 (3). The human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) were established as the biomarkers of BC, and BC can be classified into four molecular subtypes depending on the expression of HER2, PR and ER: HER2(+), triple negative breast cancer, Luminal A and Luminal B. Currently, advanced therapeutic approaches have been applied in BC cases to improve the 5-year survival rate based on the above classification, including chemotherapy, surgical techniques and adjuvant radiotherapy (4-8). Nevertheless, the 5-year survival rate of BC patients with distant metastasis and tumor progression is only 26%. Additionally, only 1.9% of patients under 50 with BC received a BC diagnosis, but ~80% of BC patients over 50 received a BC diagnosis. Therefore, an improved understanding of potential treatment targets is imperative to improve the 5-year survival rate and diagnosis of patients with BC (9,10).

MicroRNAs (miRNAs/miRs) are small, non-coding RNAs of ~22 nucleotides. They regulate the expression of proteins by silencing the transcripts of target genes or inhibiting the translation of mRNA (11,12). Extensive studies have established that miRNAs are crucial in the diagnosis, proliferation, prognosis, invasion, apoptosis, migration and metastasis of cancer (13-17). For example, Liang *et al* suggested that miRNA-10b was a suppressor in BC growth, migration, proliferation and invasion (18). Du *et al* reported that miR-124

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inhibited the proliferation and migration of BC by targeting snail family transcriptional repressor 2 (19).

Located at 14q32.33 chromosome, miR-203a-3p may possess a vital role in cancer. It has been reported that miR-203a-3p can suppress hepatocellular carcinoma progression by targeting homeobox D3 through the EGFR signaling pathway (20). However, only one study has examined the role of miR-203a-3p in BC based on 109 BC cases and matched normal breast (21). Therefore, it is critical to establish the molecular mechanism of miR-203a-3p in BC with a large number of samples. The present study estimated the expression of precursor miR-203a and miR-203a-3p in BC tissue and adjacent breast tissue by combing data from The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and University of California Santa Cruz (UCSC) Xena projects. In addition, the potential molecular mechanisms of miR-203a-3p in BC were investigated through gene ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis and protein-protein interaction (PPI).

Materials and methods

Expression of miRNA in TCGA and UCSC Xena projects. The TCGA data with level 3 miRNA-Seq profiles and full annotation of clinical parameters were acquired from TCGA (<http://cancergenome.nih.gov/>) (22). Additionally, the expression of miR-203a-3p was downloaded from the UCSC Xena project (<http://xena.ucsc.edu/>) (23).

Selection of BC microarrays from GEO data. The GEO (<https://www.ncbi.nlm.nih.gov/geo/>) (24) was used to download BC-associated microarrays with the following prerequisites: (Breast OR mammary) AND (carcinoma OR tumor OR tumor OR neoplas* OR adenocarcinoma OR malignant* OR cancer). Microarrays were selected using the following criteria: The microarrays should include BC tissue and adjacent breast tissue, and the expression of miR-203a-3p in the two types of tissue should be provided. A gene expression profile named GSE50697 was screened to identify the differentially expressed genes (DEGs).

Selection of prospective DEGs and target genes of miR-203a-3p in BC. The prospective target genes of miR-203a-3p were obtained from miRWalk2.0 databases (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>) (25), which included 12 online prediction tools: miRDB, miRNAMap, RNAhybrid, miRBridge, miRMap, PICTAR2, PITA, MicroT4, TargetScan, miRWalk2.0, miRanda and RNA22. Prospective target genes were selected if they appeared at least four times in the above 12 online prediction tools to augment the accuracy of the prediction. Gene Expression Profiling Interactive Analysis (<http://gepia.cancer-pku.cn/index.html>) (26) was performed to acquire the DEGs from TCGA with $P < 0.05$ and \log_2 fold change > 1 . DEGs from GEO were achieved by using GEO2R (ncbi.nlm.nih.gov/geo/geo2r/) to analyze GSE50697 with $P < 0.05$ and \log_2 fold change < -1 .

Bioinformatics analyses. Venn diagrams were created to obtain the intersection of prospective target genes, such as DEGs

from GEO and DEGs from TCGA, and to identify the potential target genes of miR-203a-3p in BC (27). Subsequently, GO and KEGG pathway analyses were used to confirm the potential mechanism of miR-203a-3p in BC (28,29). PPI analysis was also undertaken using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.1 database (<https://string-db.org/>) (30-32) to generate an association between the possible target genes and hub genes were selected by counting the number of edges and nodes.

Statistical analyses. Student's t-test was used to evaluate statistically significant differences between two groups. Simultaneously, one way analysis variance and Dunnett's test were carried out to estimate statistically significant differences between multiple groups. The receiver operating characteristic (ROC) curve was adopted to assess the distinguishability of precursor miR-203a and miR-203a-3p between BC tissue and adjacent breast tissue. The Kaplan-Meier survival analysis was undertaken to evaluate the prognostic value of precursor miR-203a in BC. The log-rank test was used to compare high and low precursor miR-203a expression groups. STATA version 12.0 (StataCorp LP, College Station, TX, USA) was used to perform the statistical analyses of the meta-analysis in the present study. The standard mean difference (SMD) with a random effects model was used to measure the expression of miR-203a-3p in BC tissue and adjacent breast tissue. To identify the heterogeneity of the studies, a heterogeneity test was performed and the level of I^2 calculated simultaneously. An influence analysis was also conducted to ensure the source of heterogeneity. Concurrently, a funnel plot asymmetry test was undertaken to assess the publication bias, with $P < 0.05$ indicating significant publication bias. The distinguishability of miR-203a-3p in BC tissue and adjacent breast tissue was estimated using a summarized ROC (sROC) approach, with an area under the curve (AUC) > 0.7 indicating an ability to distinguish miR-203a-3p in BC. Spearman's correlation analysis was used to verify the correlation between miR-203a-3p and target genes based on TCGA data. $r > 0$ and $r < 0$ indicated a positive and negative correlation, respectively.

Results

Clinical value of precursor miR-203a and miR-203a-3p in BC, using TCGA and UCSC Xena data. The expression of precursor miR-203a was markedly elevated in 1,077 BC tissue cases compared to 104 adjacent breast tissue cases according to TCGA project data (13.45 ± 1.97 vs. 11.69 ± 1.72 , $P < 0.001$; Fig. 1A). Subsequently, the expression of miR-203a-3p was substantially upregulated in 756 BC tissue cases compared to 76 adjacent breast tissue cases in UCSC Xena project data (11.68 ± 1.97 vs. 10.49 ± 1.05 ; $P < 0.001$; Fig. 1B). Regarding the distinguishability of precursor miR-203a and miR-203a-3p, the AUC of ROC curve was 0.775 ($P < 0.0001$; Fig. 1C) with a sensitivity of 59.24% and a specificity of 89.42%, which implied that precursor miR-203a could be used to distinguish between BC tissue and adjacent breast tissue. The AUC of ROC in the UCSC Xena project was 0.756 ($P < 0.0001$; Fig. 1D) with a sensitivity of 61.51% and a specificity of 88.16 %, which indicated that miR-203a-3p could be used to

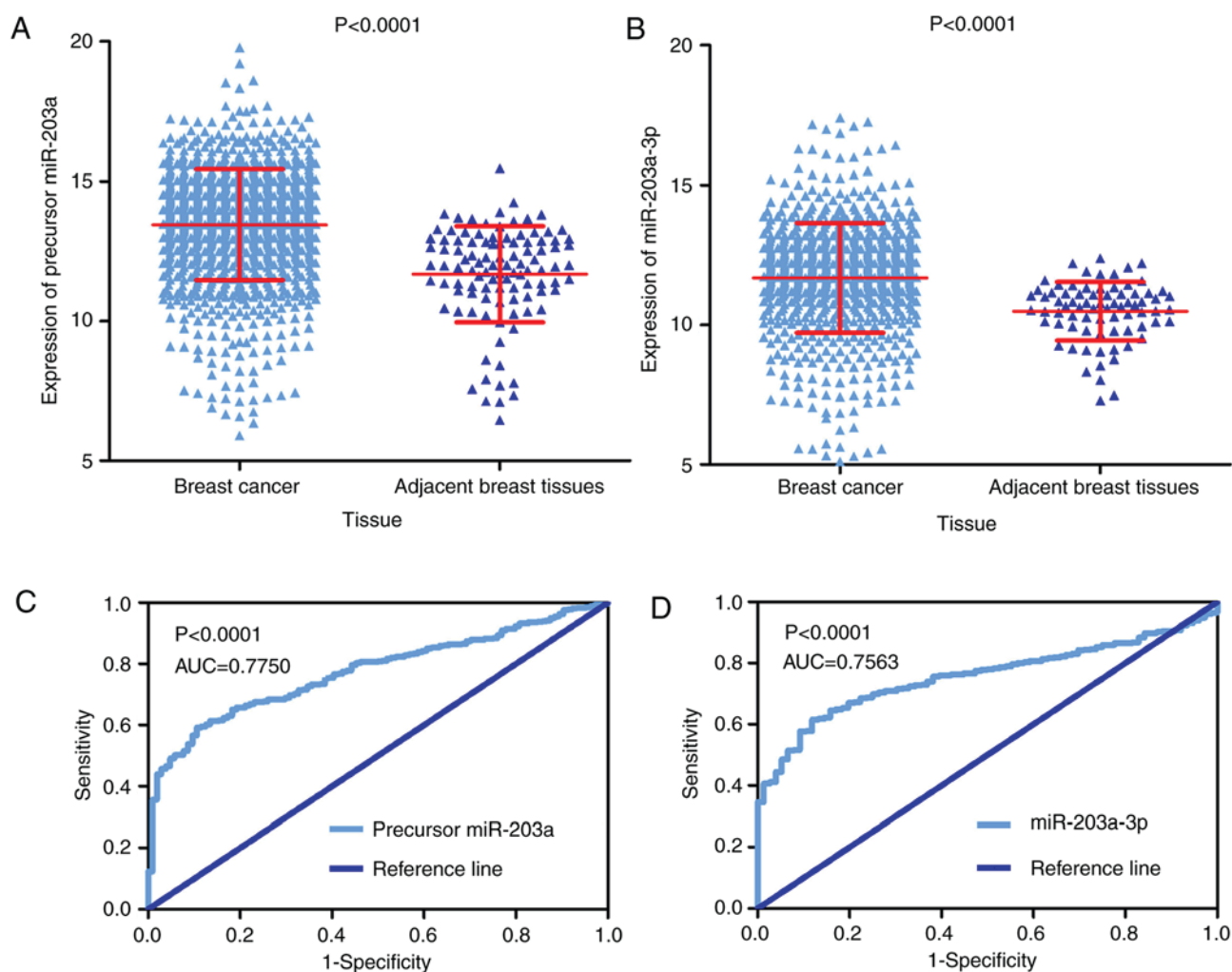


Figure 1. Clinical value of precursor miR-203a and miR-203a-3p in breast cancer tissue compared with adjacent breast tissue. (A) Expression of precursor miR-203a in breast cancer tissue compared with adjacent breast tissue based on TCGA data. (B) The expression of miR-203a-3p in breast cancer tissue compared with adjacent breast tissue based on UCSC Xena project. (C) ROC of precursor miR-203a in breast cancer tissue based on TCGA data. (D) ROC of miR-203a-3p in breast cancer tissue based on UCSC Xena project. miR, microRNA; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz; ROC, receiver operating characteristic; AUC, area under the curve.

distinguish between BC tissue and adjacent breast tissue. It was also identified that the expression of precursor miR-203a was increased in three groups, including the <60 years old group, the negative ER group and the negative PR group, compared with their corresponding groups, the ≥ 60 years old group, the positive ER group and the positive PR group (all $P < 0.05$; Fig. 2A-C, and Table I). The result of the survival analysis indicated that precursor miR-203a possessed no prognostic value in BC (Fig. 3).

Clinical value of miR-203a-3p in BC, using GEO data. Finally, nine GEO microarrays with 611 BC tissue samples and 379 adjacent breast tissue samples were selected for further analysis (Fig. 4). It was identified that the expression of miR-203a-3p was significantly upregulated in BC tissue compared with adjacent breast tissue in 3 GEO microarrays (GSE37407, GSE40525 and GSE58606, all $P < 0.05$; Fig. 5). The ROC curve of these three microarrays also implied that miR-203a-3p could be used to distinguish between BC tissue and adjacent breast tissue (Fig. 6).

Meta-analysis. The result of SMD revealed that the expression of miR-203a-3p was markedly increased in 2,444 BC tissue cases compared with 559 adjacent breast tissue cases. The heterogeneity test indicated that there was significant heterogeneity in the included studies ($I^2 = 91.5\%$; $P = 0.000$; 95% CI, 0.44-0.65; Fig. 7). Therefore, an influence analysis was conducted to seek the source of heterogeneity and it was identified that GSE44281 was significantly different from the other 10 studies (Fig. 8). Following the omission of GSE44281, the level of I^2 was decreased, but still reached 86.9% (Fig. 9). The outcome of a funnel plot asymmetry test indicated that no publication bias was identified in the included studies (Fig. 10). The AUC of sROC reached 0.82 with a sensitivity of 0.70 (0.54-0.82) and a specificity of 0.81 (0.63-0.91), which implied that miR-203a-3p could be used to distinguish between BC tissue and adjacent breast tissue (Figs. 11 and 12).

GO enrichment, KEGG pathway analyses and PPI network. Online prediction tools were used to acquire a total of 4,565 predicted target genes, which had to appear at least four times in searches to qualify. Meanwhile, 2,669 DEGs from

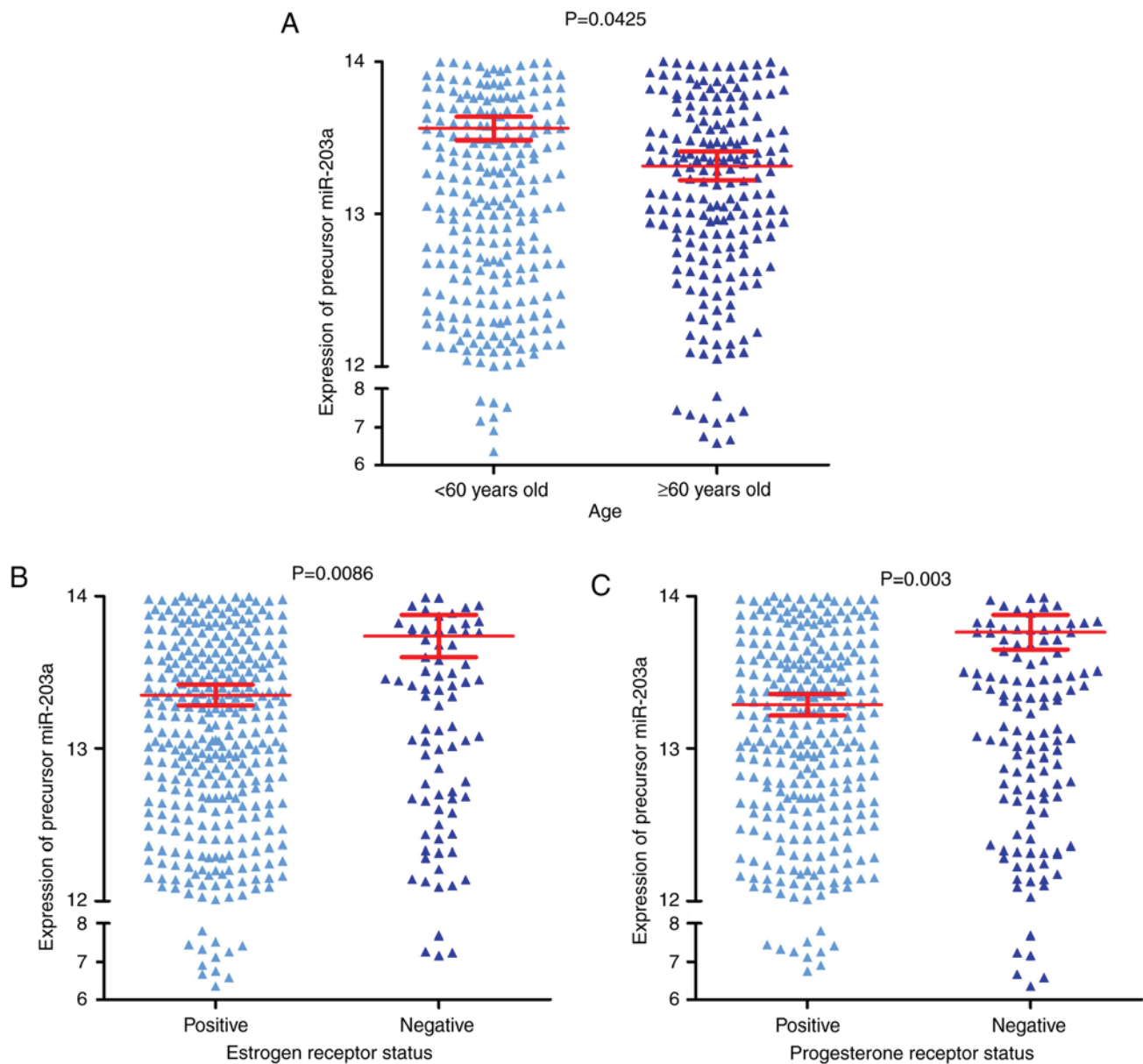


Figure 2. Expression of precursor miR-203a in groups divided by clinical parameters. (A) ≥ 60 years vs. < 60 years. (B) Positive estrogen receptor status vs. negative estrogen receptor status. (C) Positive progesterone receptor status vs. negative progesterone receptor status. miR, microRNA.

GEO and 2,138 DEGs from TCGA were acquired. Of these DEGs, 89 genes intersected with the predicted target genes (Fig. 13). The result of GO enrichment analysis indicated that the overlapped genes were associated with 'plasma membrane integrity', 'cell surface receptor linked signal transduction' and '3',5'-cyclic nucleotide phosphodiesterase activity' (Fig. 14; Table II). In addition, a pathway termed 'purine metabolism' was identified to be closely associated with miR-203a-3p expression in BC via its target genes, including phosphodiesterase 1C (PDE1C), adenylate cyclase 5 (ADCY5), phosphodiesterase 1A (PDE1A), phosphodiesterase 5A (PDE5A) and phosphodiesterase 8B (PDE8B; Table III). Notably, the expression of three genes (PDE1A, PDE1C and PDE8B) was significantly reduced in BC tissue compared with adjacent breast tissue. The other genes demonstrated a reduced trend in BC tissue compared to adjacent breast tissue, but no statistical significance was observed (Fig. 15).

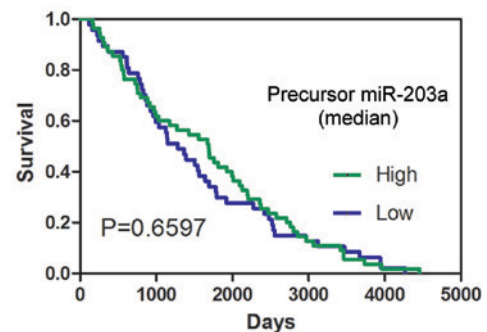


Figure 3. The survival curve of precursor miR-203a in breast cancer. The survival curve implied that miR-203a featured no prognosis value in breast cancer. miR, microRNA.

Spearman's correlation analysis identified that ADCY5 was negatively correlated with miR-203a-3p. A minor negative

Table I. Expression of precursor miR-203a in groups divided from clinical parameters.

Clinical parameters	n	miR-203a expression		
		Mean \pm standard deviation	T or F	P-value
Tissue			9.83 ^a	<0.001 ^c
Normal	104	11.69 \pm 1.72		
Breast cancer	1,077	13.45 \pm 1.97		
Age (years)			-2.01 ^a	0.045 ^c
\leq 60	592	13.56 \pm 1.88		
>60	485	13.32 \pm 2.08		
Sex			-1.10 ^a	0.295
Female	1,065	13.46 \pm 1.98		
Male	12	12.91 \pm 1.71		
Vital status			-0.84 ^a	0.401
Alive	975	13.43 \pm 1.98		
Dead	102	13.60 \pm 1.89		
Pathologic stage			F=1.253 ^b	
Stage I	181	13.44 \pm 1.65		0.289
Stage II	609	13.46 \pm 2.11		
Stage III	244	13.50 \pm 1.89		
Stage IV	20	12.61 \pm 1.89		
T			F=0.707 ^b	
T1	279	13.54 \pm 1.72		0.548
T2	620	13.45 \pm 2.07		
T3	135	12.27 \pm 2.00		
T4	40	13.26 \pm 2.16		
N			-1.14 ^a	0.254
No	508	13.37 \pm 2.00		
Yes	549	13.51 \pm 1.96		
M			1.81 ^a	0.085
No	893	13.44 \pm 1.94		
Yes	21	12.69 \pm 1.87		
Estrogen receptor status			-2.63 ^a	0.009 ^c
Positive	795	13.35 \pm 1.91		
Negative	232	13.74 \pm 2.13		
Progesterone receptor status			-3.52 ^a	<0.001 ^c
Positive	689	13.29 \pm 1.88		
Negative	335	13.76 \pm 2.09		
HER2 status			0.60 ^a	0.549
Positive	164	13.55 \pm 0.15		
Negative	564	13.45 \pm 0.08		

^aT-test was applied. ^bOne-way analysis of variance was applied. ^cP<0.05 was considered to indicate a statistically significant difference. T, N and M based on TNM staging. miR, microRNA; HER2, human epidermal growth factor receptor 2.

correlation was identified between the other four genes and miR-203a-3p, but no statistical significance was observed (Fig. 16). The ROC demonstrated that all these genes could be used to distinguish between BC tissue and adjacent breast tissue (Fig. 17). Through the PPI network, four hub genes were identified: Epidermal growth factor receptor, ADCY5, metalloproteinase inhibitor 3 and insulin-like growth

factor 1 (IGF1; Fig. 18). Depending on data from TCGA, it was identified that only IGF1 was predominantly decreased in BC tissue compared with adjacent breast tissue (Figs. 15A, 19 and 20A). As the expression of the hub genes should be decreased in BC tissue compared with adjacent breast tissue, IGF1 was identified as the hub gene of miR-203a-3p in BC. Furthermore, IGF1 exhibited a distinction between BC tissue

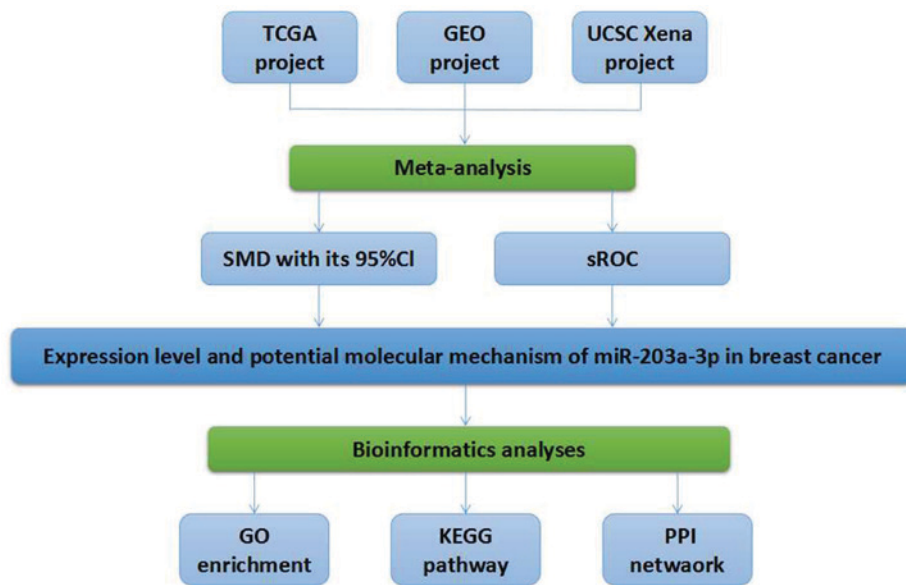


Figure 4. Flow chart of the present study. TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; UCSC, University of California Santa Cruz; SMD, standard mean difference; CI, confidence interval; sROC, summarized receiver operating characteristic; miR, microRNA; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction.

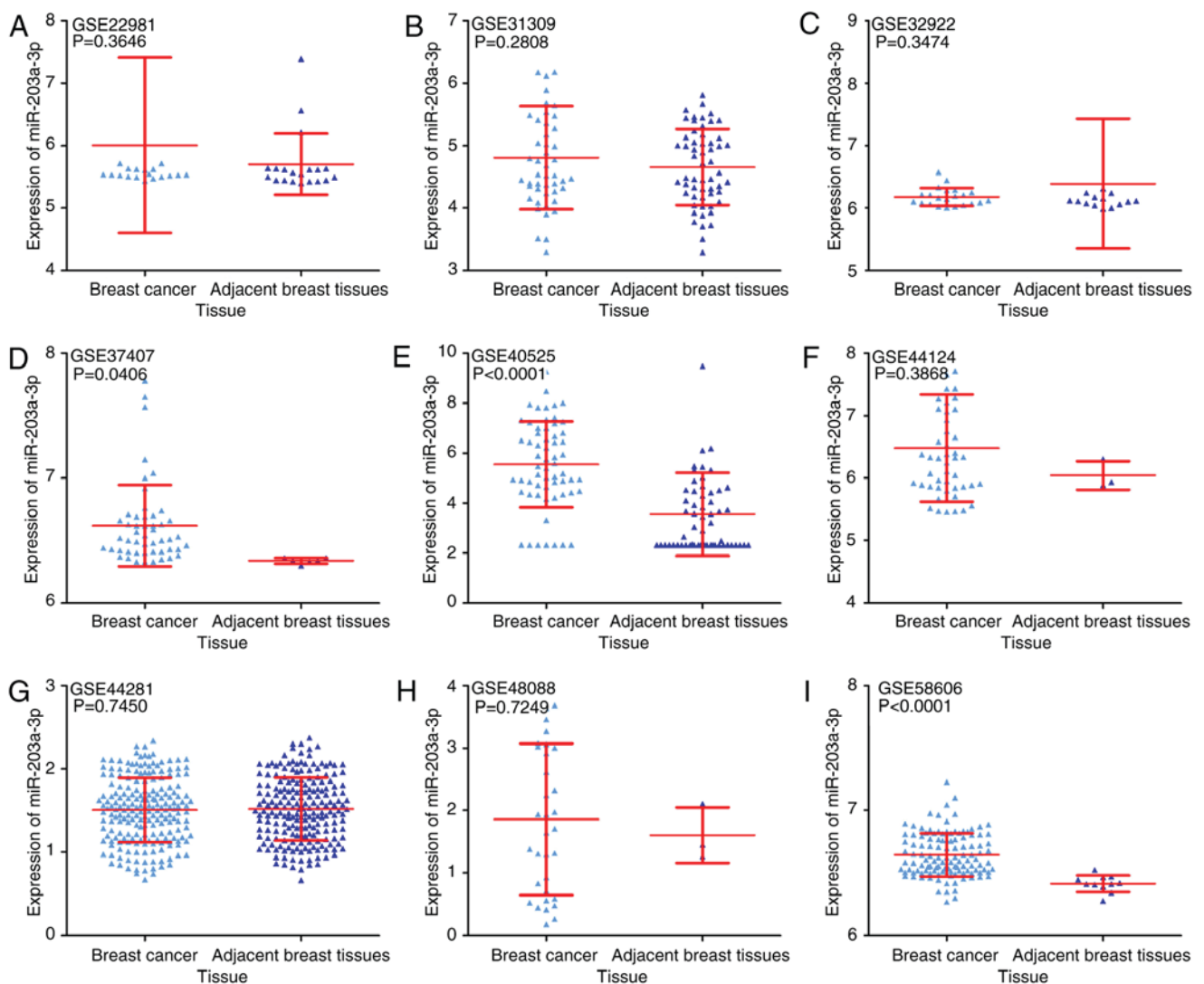


Figure 5. Expression of miR-203a-3p in breast cancer tissue and adjacent breast tissue in Gene Expression Omnibus datasets. (A) GSE22981, (B) GSE31309, (C) GSE32922, (D) GSE37407, (E) GSE40525, (F) GSE44124, (G) GSE44281, (H) GSE48088 and (I) GSE58606. miR, microRNA.

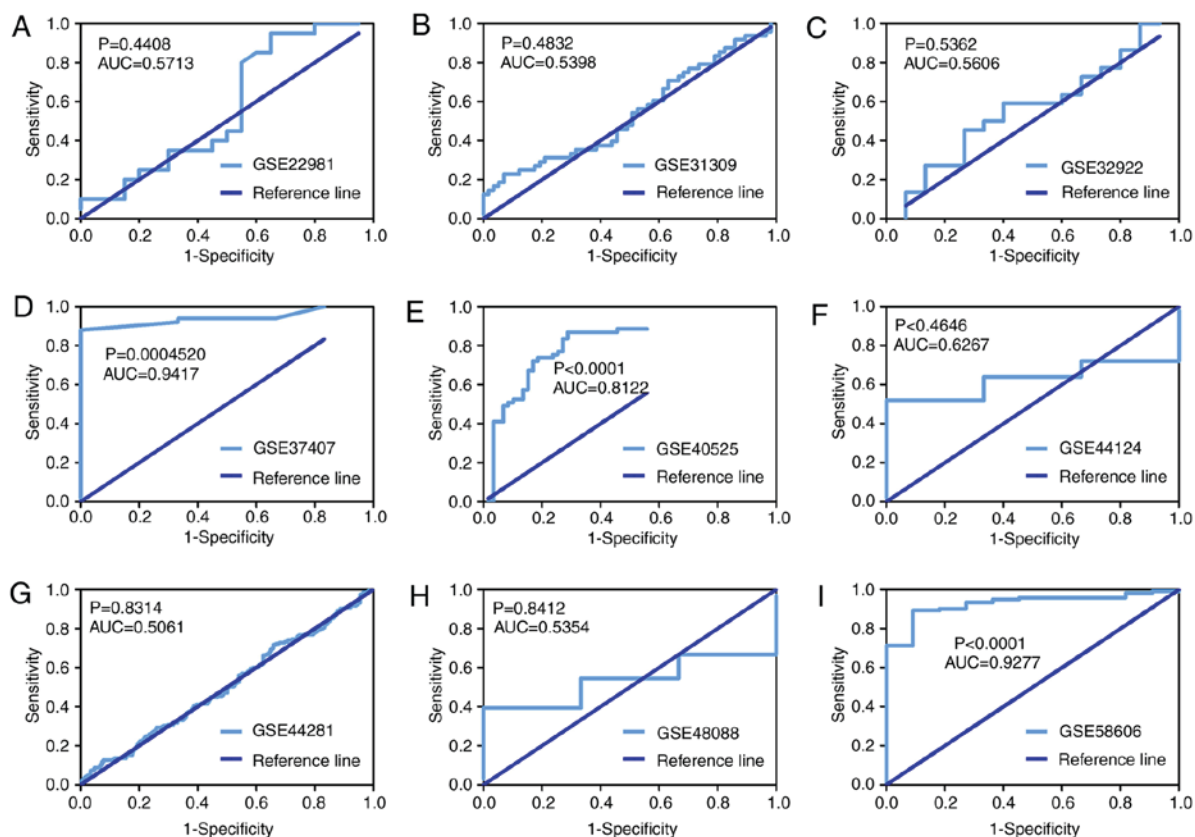


Figure 6. Receiver operating characteristics of microRNA-203a-3p in breast cancer tissue and adjacent breast tissue in Gene Expression Omnibus datasets. (A) GSE22981, (B) GSE31309, (C) GSE32922, (D) GSE37407, (E) GSE40525, (F) GSE44124, (G) GSE44281, (H) GSE48088 and (I) GSE58606. AUC, area under the curve.

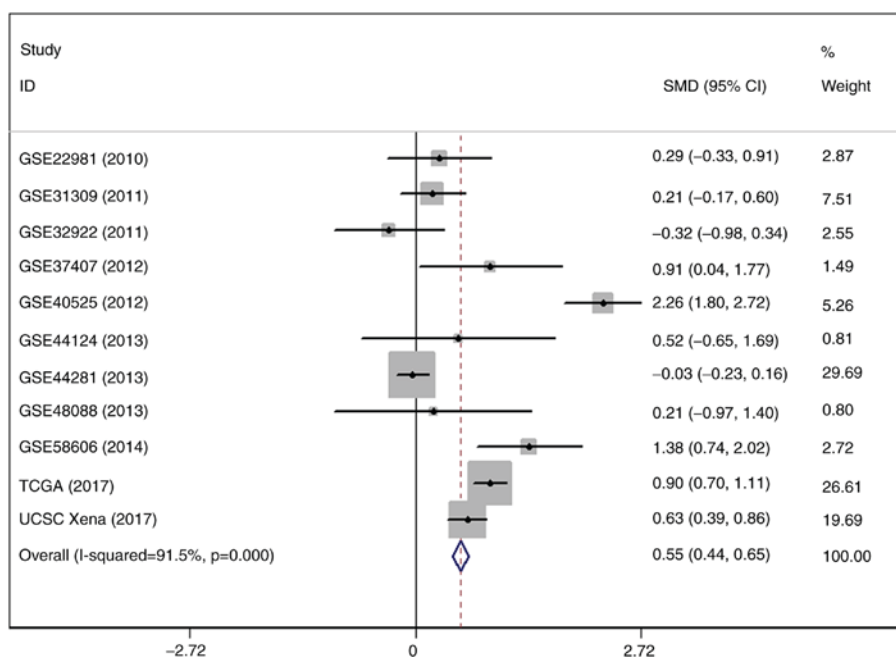


Figure 7. Forest plot to evaluate the expression of miR-203a-3p in breast cancer tissue and adjacent breast tissue prior to deleting GSE44281 (2013). The expression of miR-203a-3p was markedly increased in breast cancer tissue compared to adjacent breast tissue. miR, microRNA; SMD, standard mean difference; CI, confidence interval; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.

and adjacent breast tissue with an AUC of ROC that reached 0.9348 (Fig. 20B). Additionally, a slight negative correlation was identified between IGF1 and miR-203a-3p according to

the Spearman's correlation analysis; however, the correlation was not statistically significant ($r=-0.1611$; $P=0.4038$; Fig. 20C).

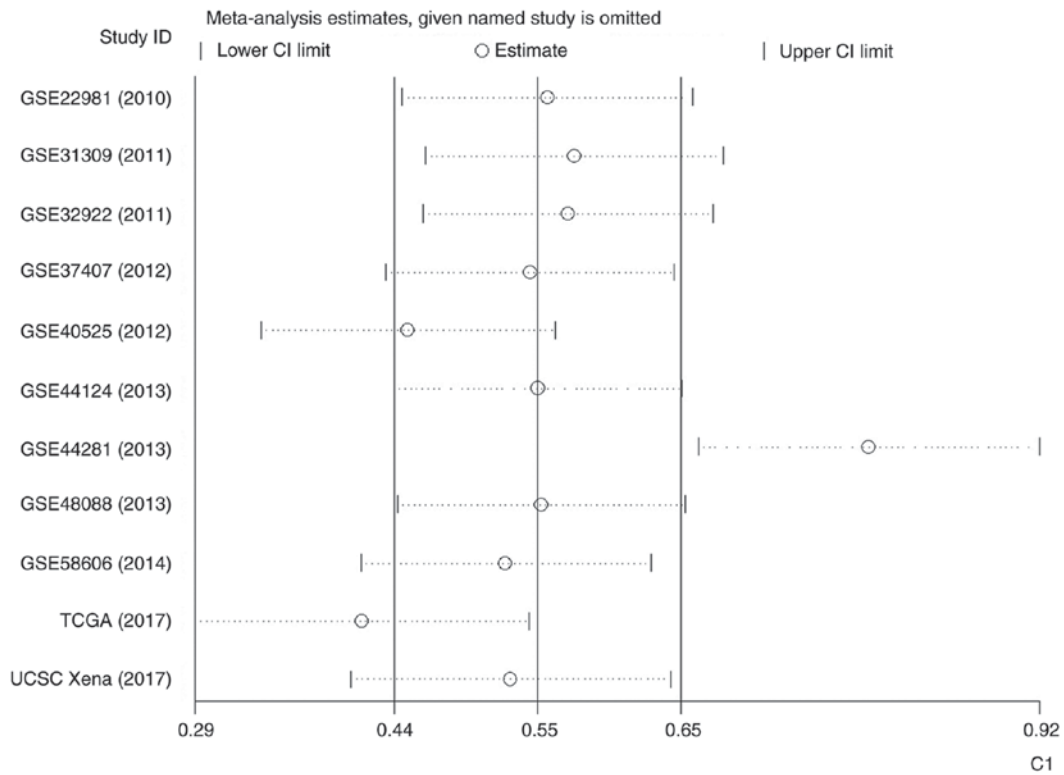


Figure 8. Influence analysis of the included studies. GSE44281 (2013) was substantially different from the other 10 studies. CI, confidence interval; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.

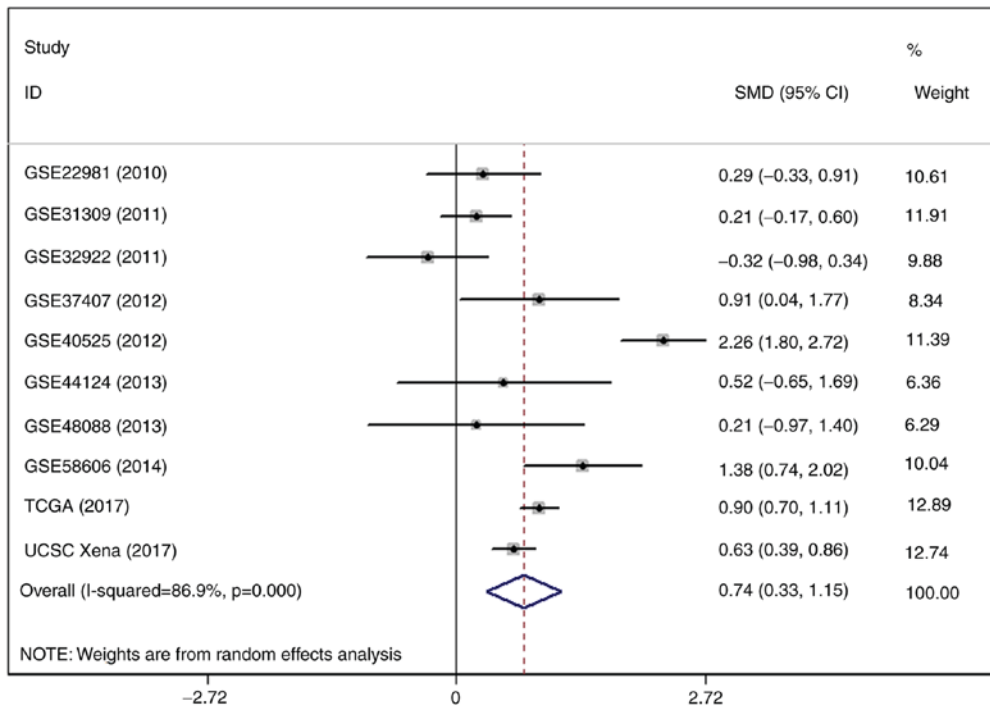


Figure 9. Forest plot to evaluate the expression of miR-203a-3p in breast cancer tissue and adjacent breast tissue following deleting GSE44281 (2013). The expression of miR-203a-3p was markedly increased in breast cancer tissue compared with adjacent breast tissue. miR, microRNA; SMD, standard mean difference; CI, confidence interval; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.

Discussion

Previous studies have identified that miR-203a-3p is significantly associated with various cancers; a trend of miR-203a-3p elevation

has been detected in hepatocellular (33) and colorectal (34) carcinoma. By contrast, downregulated miR-203a-3p expression was detected in gastric cancer (35), prostate cancer (36), non-small-cell lung carcinoma (37) and esophageal cancer (38).

Table II. GO enrichment of the 89 overlapped genes.

GO ID	Term	Count	Ontology	P-value
GO:0007166	Cell surface receptor linked signal transduction	22	BP	3.877x10 ⁻⁴
GO:0007167	Enzyme linked receptor protein signaling pathway	8	BP	2.061x10 ⁻³
GO:0019932	Second-messenger-mediated signaling	6	BP	7.787x10 ⁻³
GO:0030335	Positive regulation of cell migration	4	BP	1.140x10 ⁻²
GO:0030334	Regulation of cell migration	5	BP	1.194x10 ⁻²
GO:0009725	Response to hormone stimulus	7	BP	1.247x10 ⁻²
GO:0007242	Intracellular signaling cascade	14	BP	1.281x10 ⁻²
GO:0050806	Positive regulation of synaptic transmission	3	BP	1.367x10 ⁻²
GO:0040017	Positive regulation of locomotion	4	BP	1.477x10 ⁻²
GO:0051272	Positive regulation of cell motion	4	BP	1.477x10 ⁻²
GO:0005887	Integral to plasma membrane	16	CC	6.200x10 ⁻⁴
GO:0031226	Intrinsic to plasma membrane	16	CC	7.800x10 ⁻⁴
GO:0044459	Plasma membrane part	22	CC	1.800x10 ⁻³
GO:0016021	Integral to membrane	37	CC	1.200x10 ⁻²
GO:0044433	Cytoplasmic vesicle part	5	CC	1.400x10 ⁻²
GO:0005886	Plasma membrane	28	CC	2.100x10 ⁻²
GO:0031224	Intrinsic to membrane	37	CC	2.100x10 ⁻²
GO:0031091	Platelet α granule	3	CC	3.200x10 ⁻²
GO:0030659	Cytoplasmic vesicle membrane	4	CC	3.200x10 ⁻²
GO:0012506	Vesicle membrane	4	CC	4.000x10 ⁻²
GO:0004114	3',5'-cyclic-nucleotide phosphodiesterase activity	4	MF	3.300x10 ⁻⁴
GO:0004112	Cyclic-nucleotide phosphodiesterase activity	4	MF	3.740x10 ⁻⁴
GO:0008081	Phosphoric diester hydrolase activity	5	MF	1.330x10 ⁻³
GO:0004117	Calmodulin-dependent cyclic-nucleotide phosphodiesterase activity	2	MF	1.700x10 ⁻²
GO:0003690	Double-stranded DNA binding	4	MF	1.788x10 ⁻²
GO:0003779	Actin binding	6	MF	3.819x10 ⁻²
GO:0008144	Drug binding	3	MF	4.182x10 ⁻²
GO:0043566	Structure-specific DNA binding	4	MF	4.981x10 ⁻²
GO:0004714	Transmembrane receptor protein tyrosine kinase activity	3	MF	5.588x10 ⁻²
GO:0060090	Molecular adaptor activity	3	MF	5.588x10 ⁻²

GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function.

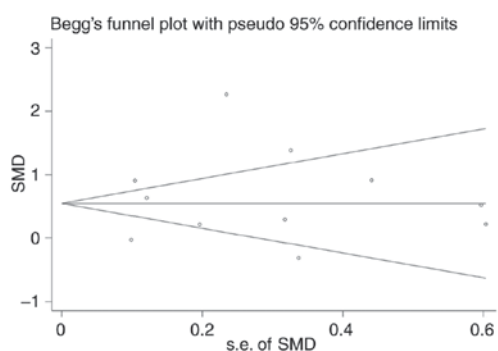


Figure 10. The funnel plot indicated that no publication bias was identified. s.e., standard error; SMD, standard mean difference.

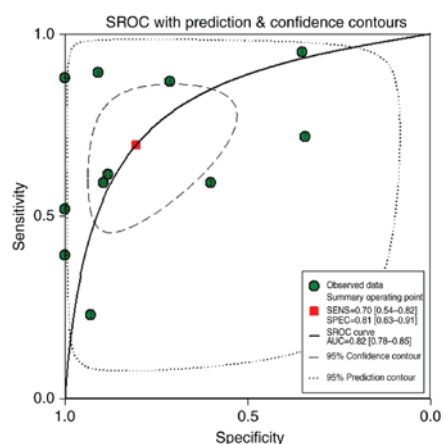


Figure 11. sROC of microRNA-203a-3p in breast cancer based on data from The Cancer Genome Atlas, Gene Expression Omnibus and University of California Santa Cruz Xena projects. The AUC was 0.82, indicating an apparent distinguishability between breast cancer tissue and adjacent breast tissue. sROC, summarized receiver operating characteristic; SENS, sensitivity; SPEC, specificity; AUC, area under the curve.

However, only one study has identified the expression and potential functions of miR-203a-3p in BC; Gomes *et al* (21) reported that the expression of miR-203a-3p was markedly upregulated in 109 BC samples compared with matched normal

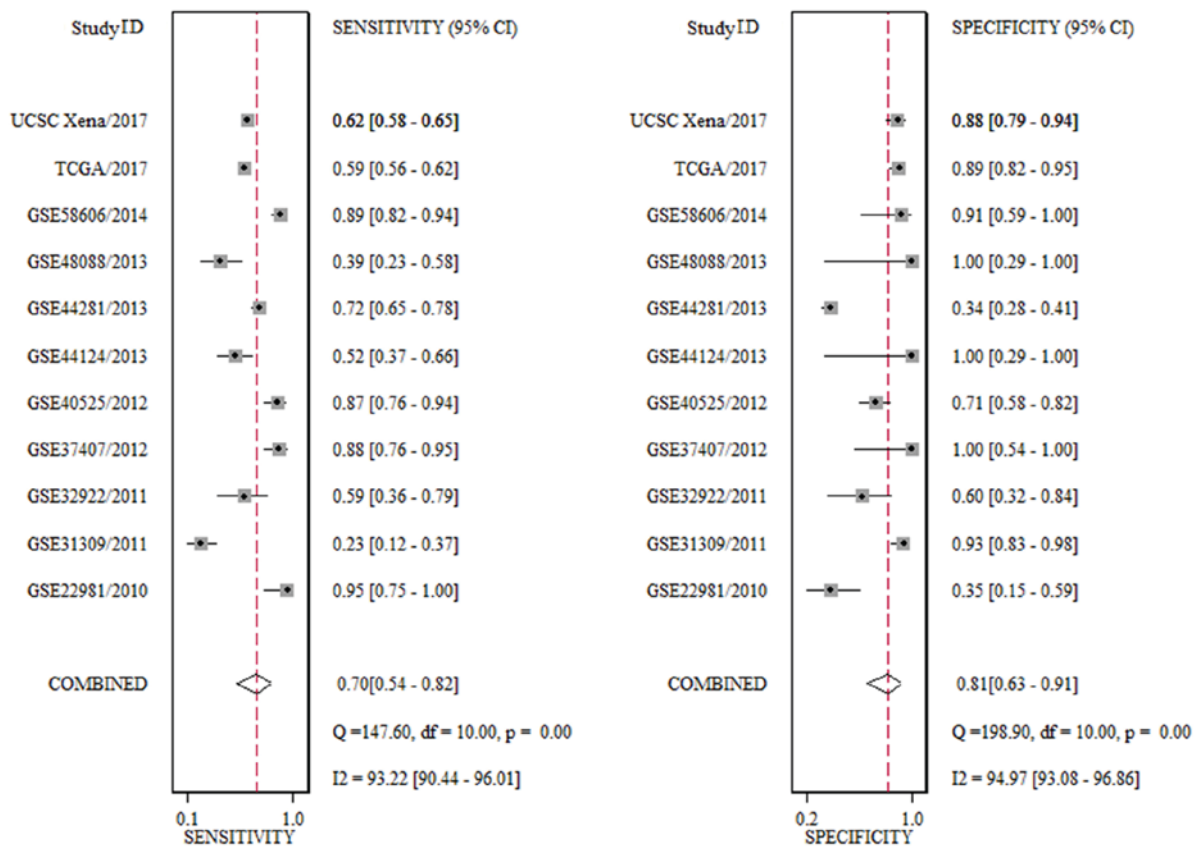


Figure 12. The sensitivity and specificity of sROC. The sensitivity and specificity of sROC were 0.70 (0.54-0.82) and 0.81 (0.63-0.91), respectively. sROC, summarized receiver operating characteristic; CI, confidence interval; UCSC, University of California Santa Cruz; TCGA, The Cancer Genome Atlas.

breast samples and also identified that upregulated expression of miR-203a-3p was established in five clinic pathological characteristics groups: Tumor size ≤ 18.5 mm, HER2-negative, PR-positive, ER-positive and high Ki-67 index groups.

Since the sample size of the study by Gomes *et al* (21) was not large or varied enough, the current study combined data from three projects with a larger sample size to ensure the accuracy of the results. It was identified that the expression of precursor miR-203a was significantly elevated in 1,077 BC tissue samples compared with 104 adjacent breast tissue samples in TCGA project data. In the UCSC Xena project, the expression of miR-203a-3p was significantly increased in 756 BC tissue cases compared with 76 adjacent breast tissue cases. In addition, an elevated trend was detected in BC tissues compared with adjacent breast tissue in three GEO microarrays. The outcome of the comprehensive meta-analysis indicated that the expression of miR-203a-3p trended toward overexpression in 2,444 BC tissue cases compared with 559 adjacent breast tissue cases. Additionally, ROC and sROC suggested that miR-203a-3p could be used to distinguish between BC tissue and adjacent breast tissue. It was detected that upregulated miR-203a-3p was associated with age (<60 -year-old patients), PR-negative BC tissue and ER-negative BC tissue. Regarding the prognosis value of miR-203a-3p in BC, no prognostic value was observed. Taken together, it was hypothesized that miR-203a-3p may enhance the development and oncogenesis of BC.

GO enrichment and KEGG pathway analyses were conducted to identify the potential molecular mechanism of the role of miR-203a-3p in BC. The predicted miR-203a-3p

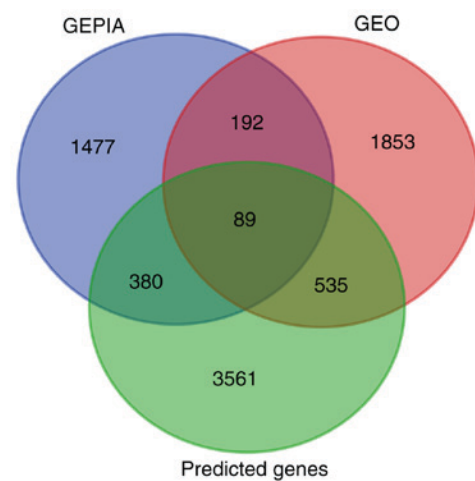


Figure 13. Venn diagram showing the intersection of differentially expressed genes from GEO, GEPIA and predicted target genes. In all, 89 overlapped genes were identified. GEPIA, gene expression profiling interactive analysis; GEO, Gene Expression Omnibus.

target genes were significantly enriched in three biological processes: ‘Plasma membrane’, ‘cell surface receptor linked signal transduction’ and ‘3’,5’-cyclic nucleotide phosphodiesterase activity’. Therefore, it was hypothesized that miR-203a-3p may influence BC via the above processes. In addition, a pathway termed ‘purine metabolism’ was closely associated with miR-203a-3p target genes. The expression and ROCs of the pathway-related genes were assessed; the

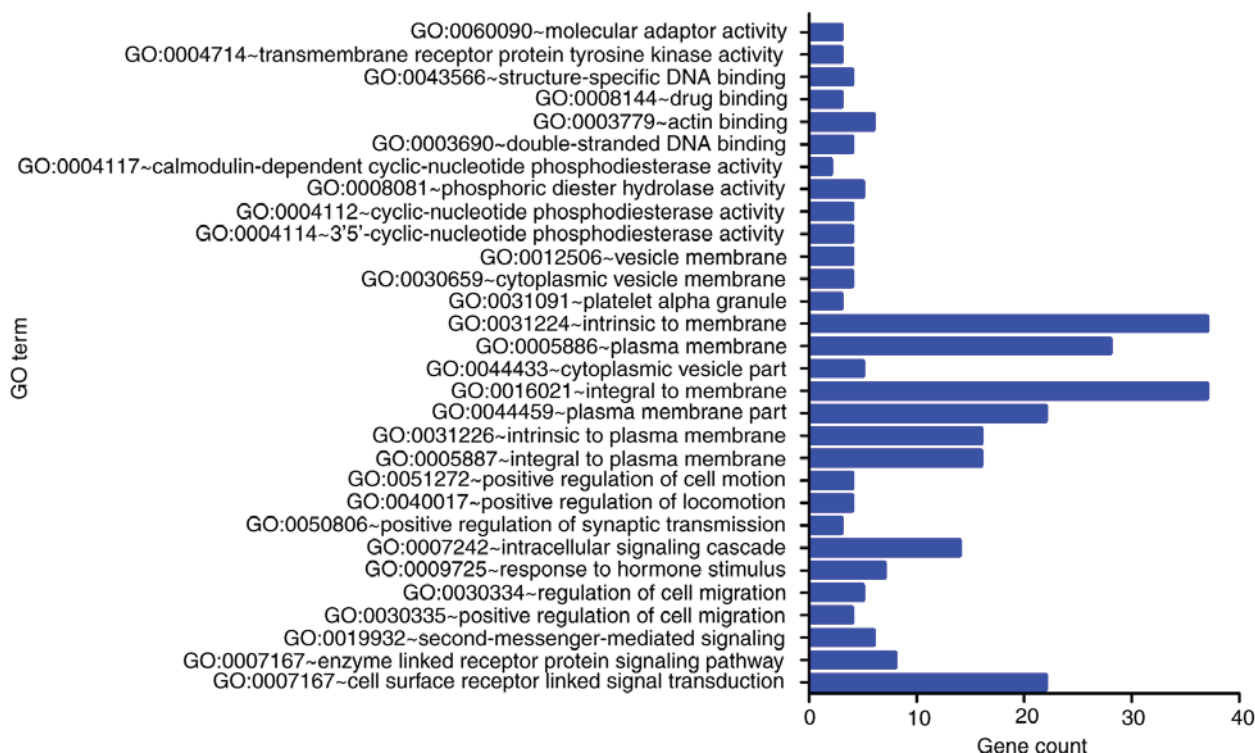


Figure 14. GO enrichment of the 89 overlapped genes. The 89 overlapped genes were correlated with plasma membrane integrity, cell surface receptor linked signal transduction and 3',5'-cyclic nucleotide phosphodiesterase activity. GO, gene ontology.

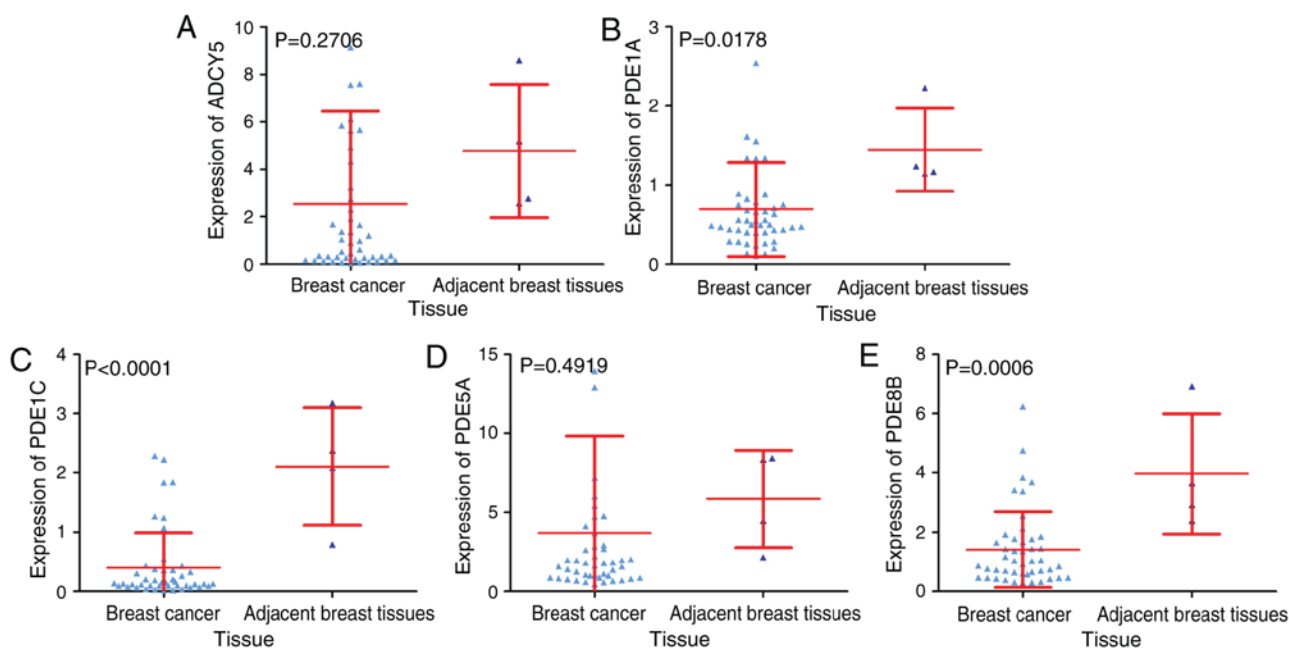


Figure 15. Expression of genes in the purine metabolism pathway. (A) ADCY5, (B) PDE1A, (C) PDE1C, (D) PDE5A and (E) PDE8B. ADCY5, adenylate cyclase 5; PDE1A, phosphodiesterase 1A; PDE1C, phosphodiesterase 1C; PDE5A, phosphodiesterase 5A; PDE8B, phosphodiesterase 8B.

expression of three genes (PDE1C, PDE1A and PDE8B) was significantly decreased in BC tissue compared with adjacent breast tissue and the expression of other genes (PDE5A and ADCY5) was marginally reduced in BC tissue compared with adjacent breast tissue, but the change was not statistically significant. ROCs from these five genes indicated that each was able to distinguish BC from adjacent normal tissue. In

addition, it was detected that ADCY5 expression was negatively correlated with miR-203a-3p expression. Taken together, the findings indicate that miR-203a-3p may be involved in purine metabolism in BC by targeting ADCY5, PDE1C, PDE1A, PDE5A and PDE8B.

Finally, the hub gene IGF1 was selected for further investigation. IGF1 is regarded as a vital gene in regulating

Table III. KEGG pathway of the 89 overlapped genes.

ID	Term	Count	P-value
hsa00230:	Purine metabolism	5	9.189x10 ⁻³
hsa04020:	Calcium signaling pathway	5	1.483x10 ⁻²
hsa05214:	Glioma	3	4.645x10 ⁻²
hsa05218:	Melanoma	3	5.755x10 ⁻²
hsa05212:	Pancreatic cancer	3	5.899x10 ⁻²
hsa04914:	Progesterone-mediated oocyte maturation	3	8.051x10 ⁻²
hsa05215:	Prostate cancer	3	8.540x10 ⁻²
hsa04666:	Fc γ R-mediated phagocytosis	3	9.545x10 ⁻²
hsa00230:	Purine metabolism	5	9.189x10 ⁻³

KEGG, Kyoto Encyclopedia of Genes and Genomes.

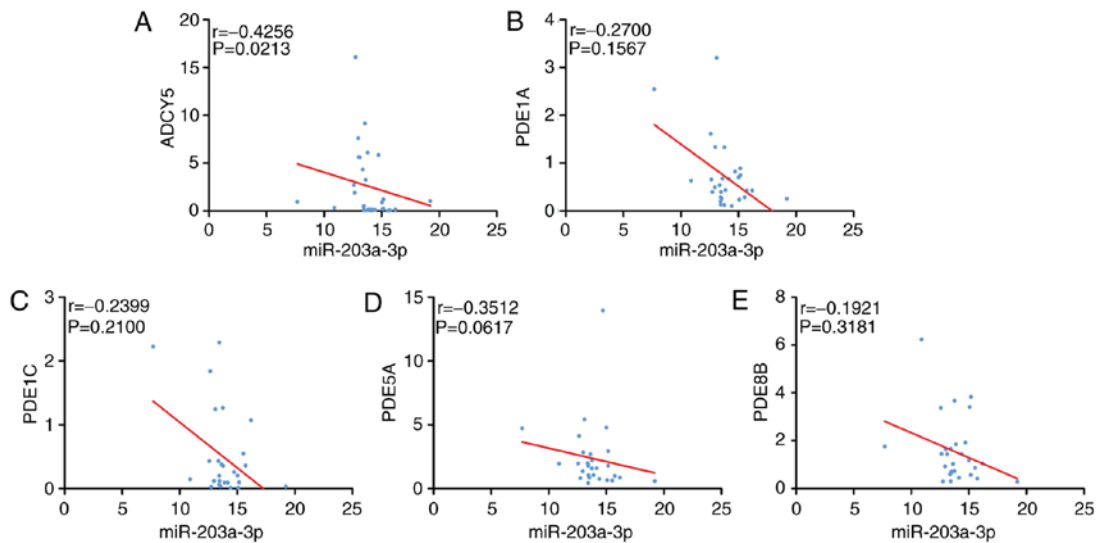


Figure 16. The correlation between pathway-associated genes and miR-203a-3p based on The Cancer Genome Atlas project data. (A) ADCY5. (B) PDE1A. (C) PDE1C. (D) PDE5A. (E) PDE8B. ADCY5, adenylate cyclase 5; PDE1A, phosphodiesterase 1A; PDE1C, phosphodiesterase 1C; PDE5A, phosphodiesterase 5A; PDE8B, phosphodiesterase 8B; miR, microRNA.

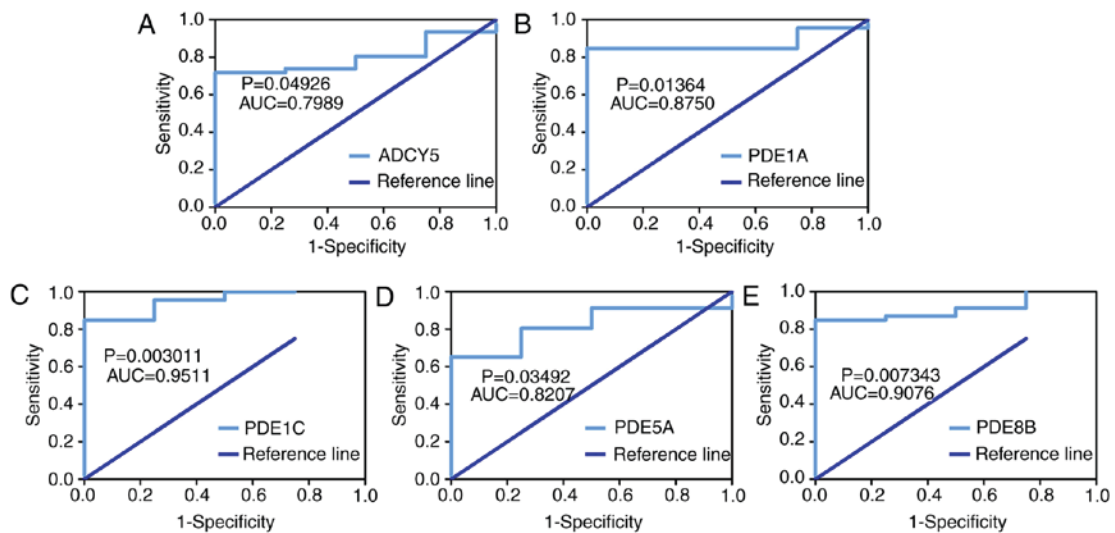


Figure 17. Receiver operating characteristic of genes in 'purine metabolism' pathway. (A) ADCY5, (B) PDE1A, (C) PDE1C, (D) PDE5A and (E) PDE8B. AUC, area under the curve; ADCY5, adenylate cyclase 5; PDE1A, phosphodiesterase 1A; PDE1C, phosphodiesterase 1C; PDE5A, phosphodiesterase 5A; PDE8B, phosphodiesterase 8B.

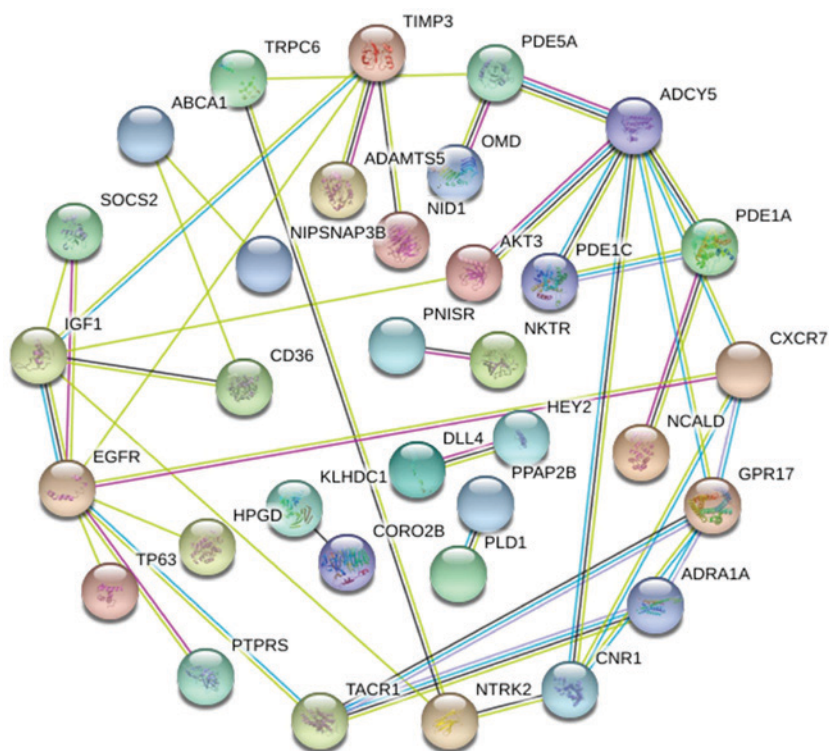


Figure 18. The protein-protein interaction network of miR-203a-3p in breast cancer. IGF1 was identified as the hub gene of miR-203a-3p in breast cancer. Each node represents a different gene. The line color indicates the type of evidence for interaction. The magenta line and blue lines signify known interaction; the green line signifies predicted interaction; the black line and purple line signify other interactions. miR, microRNA; IGF1, insulin-like growth factor 1.

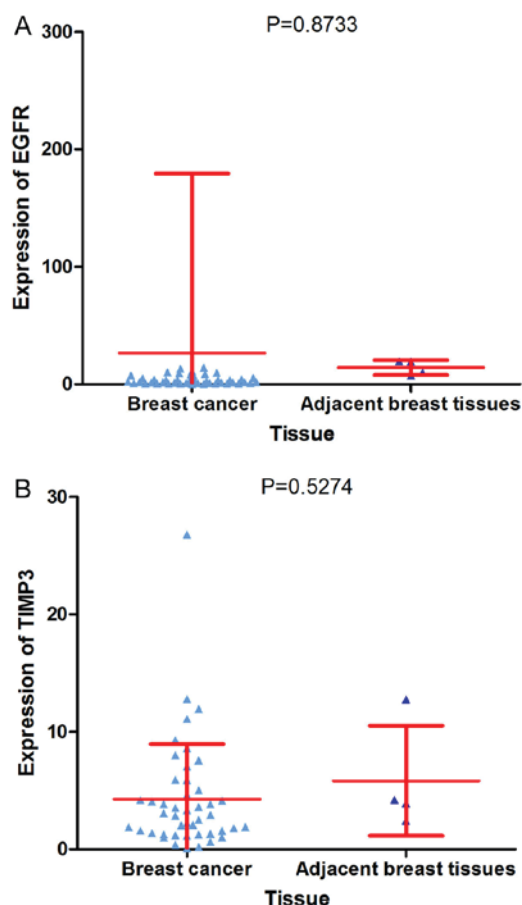


Figure 19. The expression of EGFR and TIMP3 based on The Cancer Genome Atlas project. (A) EGFR and (B) TIMP3. EGFR, epidermal growth factor receptor; TIMP3, metalloproteinase inhibitor 3.

cell differentiation, apoptosis and proliferation in BC. IGF1 polymorphisms may enhance the risk for BC (39). De Santi *et al* (40) demonstrated that IGF1 is comprised a pro-form and mature form. The IGF1 pro-form enhances cell proliferation in BC via IGF1 receptor. The current study evaluated the expression and diagnostic ability of IGF1 in BC tissue and adjacent breast tissue. It was identified that the expression of IGF1 was reduced in BC tissue compared with adjacent breast tissue and IGF1 could be used to distinguish BC tissue; however, the negative correlation between IGF1 and miR-203a-3p expression was not statistically significant. The findings of the present study suggested that miR-203a-3p may be involved in certain pivotal processes in BC by targeting IGF1.

Although certain findings were acquired from the comprehensive meta-analysis and bioinformatics analyses, there are limitations of the current study. The heterogeneity test indicated that there was significant heterogeneity in the included studies; although an attempt was made to solve this. Unfortunately, the level of I^2 was still $>50\%$ following the omission of the source of heterogeneity. It was hypothesized that the following factors may have resulted in the significant heterogeneity: i) The GEO microarrays were acquired from different countries with four microarrays obtained from Spain (GSE32922, GSE44124, GSE48088 and GSE58606), two microarrays obtained from USA (GSE22981, GSE44281) and GSE31309, GSE37407 and GSE40525 were acquired from Germany, Sweden and Israel, respectively; ii) the approaches for determining the expression of miR-203a-3p were different across the different studies. Various platforms were conducted to analyze GEO microarrays. Furthermore,

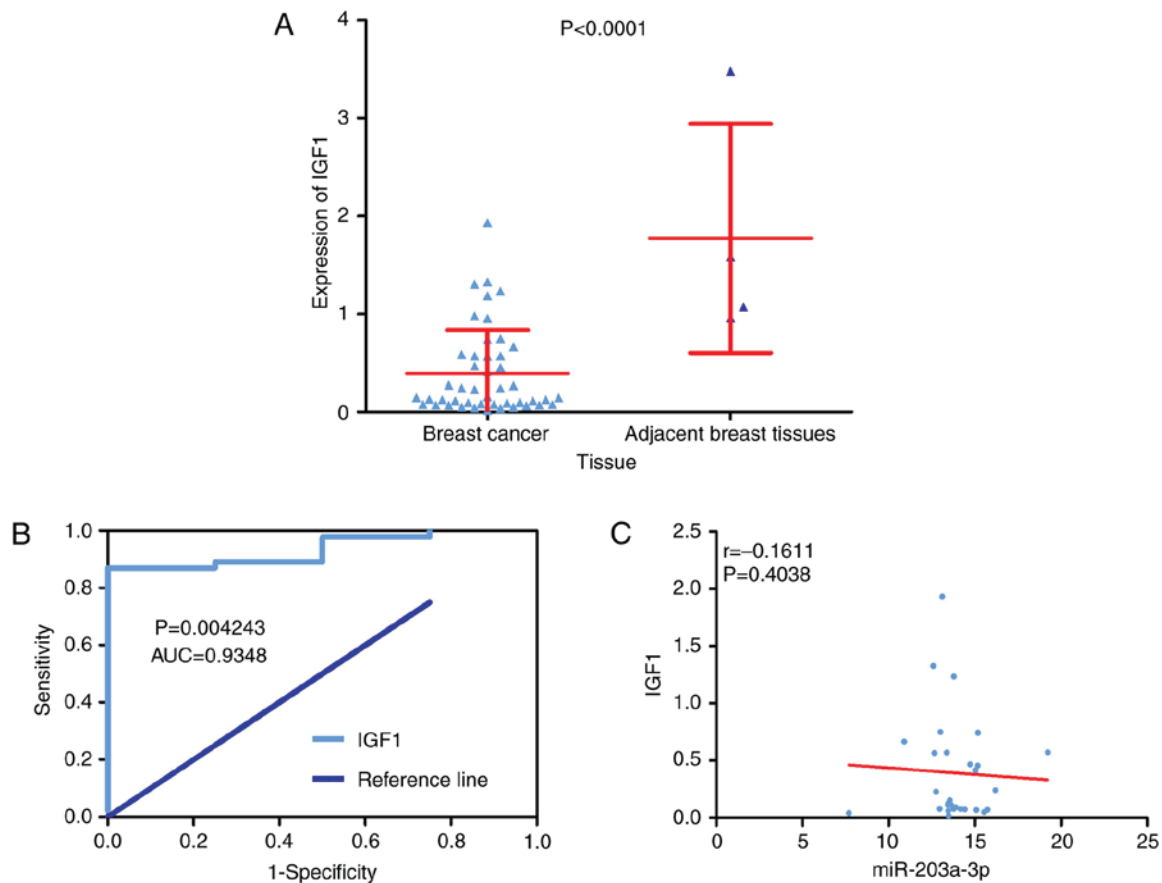


Figure 20. Clinical relevance of IGF1 in breast cancer. (A) The expression of IGF1 was markedly reduced in breast cancer tissue compared with adjacent breast tissue. (B) Receiver operating characteristics of IGF1 in breast cancer tissue. (C) Correlation between IGF1 and miR-203a-3p. miR, microRNA; AUC, area under the curve; IGF1, insulin-like growth factor 1.

in vitro or *in vivo* experiments to support the hypothesis of the present study were not performed, which is a major limitation. Thus, *in vitro* and *in vivo* studies should be performed in the near future.

In general, the present study established that the expression of miR-203a-3p was markedly elevated in BC tissue compared with adjacent breast tissue. Thus, it is hypothesized that miR-203a-3p may enhance the development and oncogenesis of BC. In addition, the target gene IGF1 was identified as a hub gene of miR-203a-3p in BC while the expression of IGF1 was significantly reduced in BC tissue compared with adjacent breast tissue.

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Availability of data and materials

The data and materials of the present study are available from the corresponding authors on reasonable request.

Authors' contributions

CF, JZha, RH and JM collected and analyzed the data. KC and JZho conceived the study and wrote the manuscript. All authors read the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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