Expression and potential molecular mechanisms of miR-204-5p in breast cancer, based on bioinformatics and a meta-analysis of 2,306 cases

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Abstract. Breast cancer (BC) is the most common cancer among women worldwide. However, there is insufficient research that focuses on the expression and molecular mechanisms of microRNA (miR)-204-5p in BC. In the current study, data were downloaded from the Cancer Genome Atlas (TCGA), the Gene Expression Omnibus (GEO) and the University of California Santa Cruz (UCSC) Xena databases. They were then used to undertake a meta-analysis that leveraged the standard mean difference (SMD) and summarized receiver operating characteristic (sROC) to evaluate the expression of the precursor miR-204 and mature miR-204-5p in BC. Additionally, an intersection of predicted genes, differentially expressed genes (DEGs) from the TCGA database and the GEO database were plotted to acquire desirable putative genes. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and protein-protein interaction (PPI) network analyses were performed to assess the potential pathways and hub genes of miR-204-5p in BC. A decreased trend in precursor miR-204 expression was detected in 1,077 BC tissue samples in comparison to 104 para-carcinoma tissue samples in the TCGA database. Further, the expression of mature miR-204-5p was markedly downregulated in 756 BC tissue samples in comparison to 76 para-carcinoma tissue samples in the UCSC Xena database. The outcome of the SMD from meta-analysis also indicated that the expression of miR-204-5p was markedly reduced in 2,306 BC tissue samples in comparison to 367 para-carcinoma tissue samples. Additionally, the ROC and sROC values indicated that miR-204-5p had a great discriminatory capacity for BC. In GO analysis, 'cell development', 'cell surface activity', and 'receptor agonist activity' were the most enriched terms; in KEGG analysis, 'endocytosis' was significantly enriched. Rac GTPase activating protein 1 (RACGAP1) was considered the hub gene in the PPI network. In conclusion, miR-204-5p may serve a suppressor role in the oncogenesis and advancement of BC, and miR-204-5p may have crucial functions in BC by targeting RACGAP1.

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Key words: breast cancer, microRNA-204-5p, The Cancer Genome Atlas, Gene Expression Omnibus, target genes

Introduction

Breast cancer (BC) is the most common form of cancer among women worldwide. In the United States, ~1 in 8 women will be diagnosed with BC during their lifetime (1,2). Despite advancements in antineoplastic strategies including surgical treatment, adjuvant chemotherapy and radiotherapy, prognosis remains poor (3-6). Furthermore, the use of various prognostic markers including the cyclase associated actin cytoskeleton regulatory protein 2, lactate dehydrogenase A, AMP-activated protein kinase, Midline-2 and Claudin 12, have been reported to improve BC patient outcomes (7-10). However, in the United States, 66,120 new BC cases and 40,920 BC mortalities are likely to occur in 2018, representing growth rates of 30 and 14% relative to the previous year, respectively (11). The onset and progression of BC is a multifactorial process, associated with genetic, endocrine and external environmental factors (12). Hereditary phenomena appear in 5-10% of BC patients, and germline gene mutations, particularly in breast cancer type 1 susceptibility protein (BRCA)1 and BRCA2, closely correlate with hereditary BC (13). Further investigation is essential in understanding the complex molecular mechanisms underlying BC, while concurrently identifying more potential target genes.

miRNAs are small, endogenous non-coding RNAs, 18-22 nucleotides in length (14,15). By suppressing protein translation or enhancing the downregulation of mRNA transcripts, miRNAs have a regulatory role in the expression of target proteins (16,17). Additionally, miRNAs have been reported to have effects on the chemosensitivity, proliferation, migration, apoptosis, metastasis and invasion of BC through targeting gene regulation (18-23). The clinical features of

miR-204-5p have been widely discussed in the context of various cancers, such as hepatocellular carcinoma, laryngeal squamous cell carcinoma, melanoma, oral squamous cell carcinoma, colorectal cancer, papillary thyroid carcinoma and endometrial carcinoma (24-30). Nevertheless, the expression status and molecular mechanisms underlying miR-204-5p in BC remains unclear. Therefore, in the present study, the expression of the precursor miR-204 and mature miR-204-5p in BC was investigated, using data downloaded from the Cancer Genome Atlas (TCGA), University of California Santa Cruz (UCSC) Xena and Gene Expression Omnibus (GEO) databases. In addition to the data obtained from the TCGA and UCSC Xena databases, a meta-analysis involving GEO microarrays was undertaken to evaluate the expression of miR-204 and miR-204-5p in BC. Furthermore, the putative genes selected from the intersection of the predicted genes, differentially expressed genes (DEGs) from the TCGA database, and DEGs from the GEO database were used to determine Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichments; in order to examine the molecular mechanisms underlying miR-204-5p in BC, and to construct a protein-protein interaction (PPI) network to draw interaction maps of the identified DEGs.

Materials and methods

Breast cancer samples in the TCGA and UCSC Xena databases. Precursor miR-204 expression data, as well as data on several corresponding clinical parameters, including age, gender, vital status, pathologic stage, tumor status, node status, metastasis status, estrogen receptor (ER) status, the human epidermal growth factor receptor (HER2) status and the progesterone receptor (PR) status were obtained from the TCGA database (http://cancergenome.nih.gov/) (31). Additionally, data on the expression of mature miR-204-5p were acquired from the UCSC Xena database (http://xena.ucsc.edu/).

BC microarrays in the GEO database. BC microarrays were drawn from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) (32), using the following search terms: (neoplasm* OR cancer OR adenocarcinoma OR malignant* OR carcinoma OR tumor OR tumor) and (breast OR mammary). Next, BC microarrays were selected for further meta-analysis, based on the following inclusion criteria: i) Patients were diagnosed with BC tissue samples and para-carcinoma tissue samples; ii) the microRNA profile was available; and iii) corresponding clinical parameters were provided.

Predicted target genes and differentially expressed genes of miR-204-5p in breast cancer. The target genes of miR-204-5p were acquired from miRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) (33), which contains 12 tools: MicroT4, TargetScan, miRanda, miRNAMap, PICTAR2, RNA22, miRBridge, miRDB, PITA, miRMap, RNAhybrid, and miRWalk. To ensure accuracy, the potential predicted target genes of miR-204-5p were extracted, if they emerged more than five times among those twelve tools. Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/index.html) (34) was used to download DEGs from the TCGA database, using the following criteria: log2 |fold change (FC)|>1 and P<0.05. In addition, the Gene-Cloud of Biotechnology Information (35) was used to analyze BC microarrays obtained from the GEO database, using the following search logic: (Affymetrix) and (neoplasm* OR cancer OR adenocarcinoma OR malignant* OR carcinoma OR tumor) and (breast OR mammary). DEGs were selected from the GEO database, again under the following criteria: log2 |FC|> 1 and P<0.05.

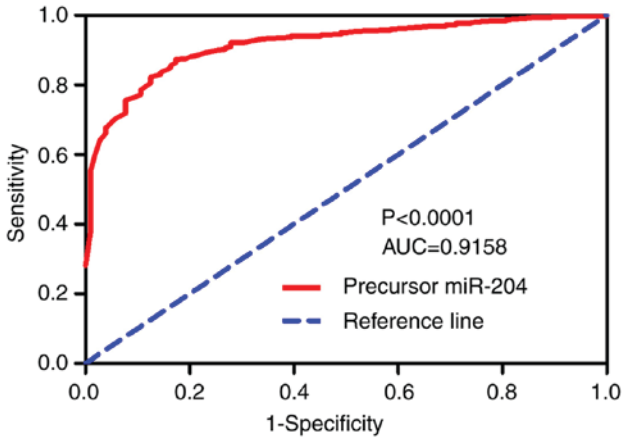

Figure 1. ROC curve of the precursor miR-204 in breast cancer based on The Cancer Genome Atlas database. The AUC of the ROC curve of miR-204 was 0.9158, indicating that it possessed a great discriminatory capability for breast cancer. ROC, receiver operating characteristic; AUC, area under the curve; miR, microRNA.

Enrichment analyses and the protein-protein interaction network. An intersection of potential target genes, DEGs from the TCGA database and DEGs from the GEO database were plotted to obtain putative genes. Subsequently, GO (http://www.geneontology.org/) (36,37) and KEGG (https://www.genome.jp/kegg/) (38,39) analyses were undertaken to identify the potential biological processes and possible pathways of the selected putative genes in BC. A functional network graph of GO was drawn by the ORA Sample Run WEB-based Gene Set Analysis Toolkit (http://www.webgestalt.org/option.php#). Moreover, the PPI network was generated by Cytoscape 3.5.0, to build interaction maps of the putative genes (40,41). Additionally, a Spearman's correlation analysis was created to identify the correlation between miR-204-5p and the hub gene.

Statistical analysis. SPSS 22.0 (IBM Corp., Armonk, NY, USA) statistical software package was used to perform an independent-sample t-test to estimate the expression of the precursor miR-204 and mature miR-204-5p in BC tissue samples and para-carcinoma tissue samples, and to determine the expression of the precursor miR-204 in groups differentiated in terms of the aforementioned clinical parameters. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was evaluated to assess the discriminatory capability of the precursor miR-204 for BC, in which an AUC >0.7 was considered to denote a great discriminatory capability. In addition, the Kaplan-Meier curve was undertaken to evaluate the prognostic value of the

Table I. Clinical parameters and the expression level of precursor miR-204.

Clinical feature	n	Expression level of precursor microRNA-204		
		Mean ± standard deviation	t	P-value
Tissue				
BC	1,077	2.284±1.983	-17.535	<0.001[a]
Para-carcinoma	104	5.775±1.391		
Age (years)				
<60	571	2.492±2.016	1.741	<0.001
≥60	506	2.049±1.921		
Sex				
Female	1,065	2.297±1.987	3.148	0.043[a]
Male	12	1.129±1.267		
Vital status				
Alive	975	2.325±2.005	2.100	0.036[a]
Dead	102	1.892±1.718		
Pathological stage				
Stages I-II	790	2.256±1.962	-1.417	0.157
Stages III-IV	264	2.456±2.061		
Tumor				
T1-2	899	2.236±1.965	-1.772	0.077
T3-4	175	2.527±2.063		
Pathological stage				
Stage I	181	2.643±1.959	F=4.179	0.006[a]
Stage II	609	2.140±1.950		
Stage III	244	2.499±2.048		
Stage IV	20	1.935±2.205		
Tumor				
T1	279	2.643±1.906	F=2.250	0.081
T2	620	2.054±1.966		
T3	135	2.781±2.090		
T4	40	1.668±1.733		
Node				
No	508	2.207±1.937	-1.395	0.163
Yes	549	2.377±2.019		
Metastasis				
No	893	2.151±1.916	0.726	0.468
Yes	21	1.843±1.843		
ER status				
Positive	795	2.316±1.980	0.592	0.554
Negative	232	2.228±2.001		
PR status				
Positive	689	2.392±2.004	2.276	0.023[a]
HER2 status				
Negative	335	2.092±1.929	4.831	<0.001[a]
Positive	159	1.565±0.137		
Negative	555	2.407±0.084		

Clinical features were only present in BC samples, but clinical features were not available for every BC sample. [a]P<0.05. BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, receptor tyrosine-protein kinase erbB2. One-way analysis of variance was applied to determine significance for pathological and tumor stages.

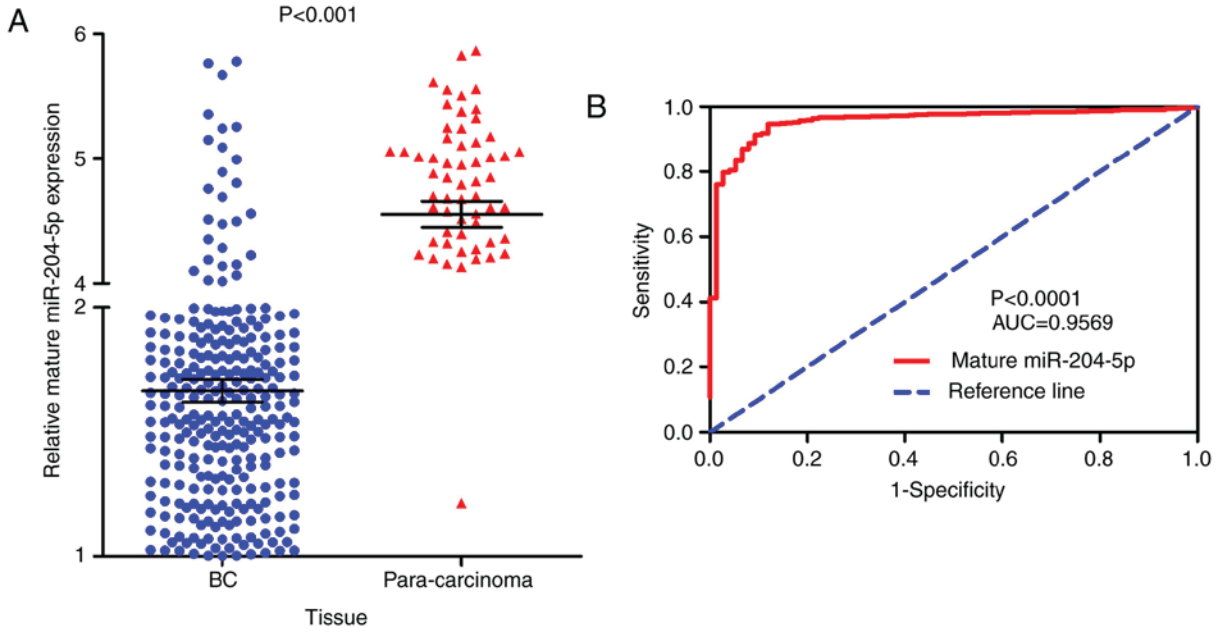

Figure 2. Expression and receiver operating characteristic curve of mature miR-204-5p in breast cancer based on the University of California Santa Cruz Xena database. (A) The expression of mature miR-204-5p was significantly decreased in breast cancer tissue samples, compared with para-carcinoma tissue samples. (B) The AUC was 0.9569, indicating that mature miR-204-5p had a great discriminatory capability for breast cancer. AUC, area under the curve; miR, microRNA.

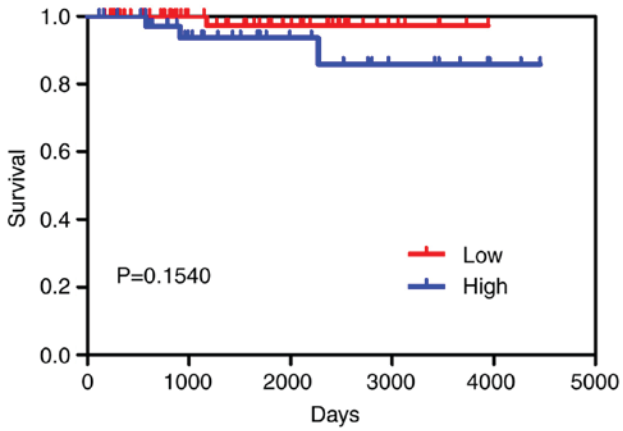

Figure 3. Survival curve of the precursor miR-204 in breast cancer. No significant correlation was identified between the precursor miR-204 and survival outcome in breast cancer.

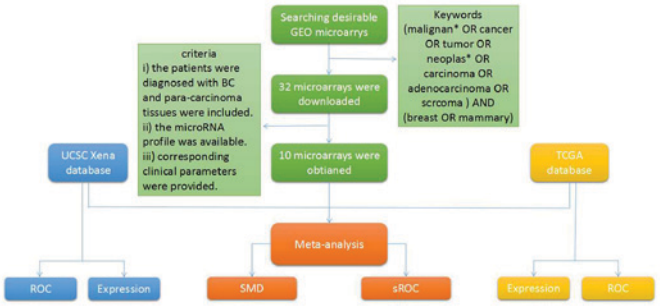

Figure 4. Flow chart for obtaining GEO microarrays and estimating the expression and discriminatory capacity of precursor miR-204 and mature miR-204-5p in breast cancer. GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas; BC, breast cancer; ROC, receiver operating characteristic; s, summarized.

precursor miR-204 in BC, and a log-rank test was performed to compare the high and low miR-204 expression groups. STATA 12.0 (StataCorp LLC, College Station, TX, USA) was used to undertake the meta-analysis. The standard mean difference (SMD) was adopted to determine the expression of miR-204-5p in BC and para-carcinoma tissue samples. Concurrently, the heterogeneity of the BC microarrays, and the data obtained from the TCGA and UCSC Xena databases, were estimated via a heterogeneity test, with I^2<50% signifying no heterogeneity. A random-effects model may be conducted if the heterogeneity existed. The sensitivity analysis was performed to seek the source of the heterogeneity. Additionally, publication bias was calculated using Deek's funnel plot dissymmetry tests, with P<0.05 indicating an obvious publication bias. Subsequently, summary (s)ROCs were determined, to calculate the discriminatory capability of miR-204-5p in BC.

Results

Expression level of the precursor miR-204 and mature miR-204-5p in breast cancer. A downregulation of miR-204 was detected in 1,077 BC tissue samples in comparison to 104 para-carcinoma tissue samples based on the TCGA database (2.284±1.983 vs. 5.775±1.391, P<0.001, Table I). The AUC of the miR-204 ROC curve was 0.9158, with a sensitivity of 87.28% and specificity of 82.69%, thus indicating that precursor miR-204 possesses a great discriminatory capability for BC (P<0.0001; Fig. 1). In addition, the expression of mature miR-204-5p was significantly decreased in 756 BC tissue samples compared with 76 para-carcinoma tissue samples based on the UCSC Xena database (1.66422±1.251283 vs. 4.55208±0.905784; P<0.001; Fig. 2A). Additionally, mature

miR-204-5p also featured a great discriminatory capability for BC (P<0.0001; Fig. 2B). Additionally, downregulation of the miR-204 was determined to be significant in several groups, including individuals aged ≥60 years, those who were dead, negative PR status, positive HER2 status and pathological stage IV (all P<0.05, Table I). No significant correlation was identified between the precursor miR-204 and survival outcome in BC, as determined by the Kaplan-Meier curve (P=0.154; Fig. 3).

Meta-analysis. A total of 10 GEO microarrays containing 473 BC tissue samples and 187 para-carcinoma tissue samples were acquired (Fig. 4; Table II) (42-51). It was revealed that in two microarrays (GSE40525 and GSE44124), the expression of miR-204-5p was markedly reduced in BC tissue samples, in comparison to para-carcinoma tissue samples (both P<0.05; Fig. 5). Additionally, the ROC curve result implied that in four microarrays (GSE32922, GSE35412, GSE40525 and GSE58606), miR-204-5p possessed a great discriminatory capability for BC (all P<0.05; Fig. 6). Regarding the meta-analysis, a significant heterogeneity outcome was achieved by the heterogeneity test. Thus, a random-effects model was undertaken to calculate the SMD and 95% confidence interval, the SMD outcome demonstrated that the expression of miR-204-5p was reduced in BC tissue samples in comparison to para-carcinoma tissue samples (I^2=95.7%; P<0.001; Fig. 7). The influence analysis demonstrated no significant difference (Fig. 8). Additionally, no significant publication bias was detected via Deek's funnel plot asymmetry test (P=0.14; Fig. 9). In addition, the diagnostic likelihood ratio (DLR) positive, DLR negative, diagnostic score, and odds ratio values were 3.78 (1.98-7.24), 0.27 (0.15-0.50), 2.64 (1.53-3.75) and 13.99 (4.61-42.51), respectively (Figs. 10 and 11). In addition, the prior probability and post-probability positive and negative reached 20, 49 and 6%, respectively (Fig. 12). Finally, the AUC of the sROC was 0.86 (0.82-0.89), with a sensitivity of 79% (64-88%) and a specificity of 79% (64-89%), thus indicating a great discriminatory capability of miR-204-5p for BC (Figs. 13 and 14).

Putative genes of miR-204-5p in breast cancer. In total, 1,417 and 6,158 DEGs were acquired from the TCGA and GEO databases, respectively. Additionally, 2,913 predicted target genes were obtained. The intersection was plotted and 164 putative genes were obtained for use in further bioinformatics analyses (Fig. 15).

Bioinformatics analyses. With regards to the GO analysis (Fig. 16), the putative genes of miR-204-5p were identify to have mainly participated in 'cell development' in biological process (BP) terms (Figs. 16A and 17), and were enriched in 'cell surface' in cellular component (CC) terms (Figs. 16B and 18). In addition, for molecular function (MF), the putative genes were predominantly enriched in receptor agonist activity (Figs. 16C and 19). Regarding the KEGG pathway analysis, the most enriched pathway of the putative genes was 'endocytosis' (Fig. 20). A total of eight pathway-associated genes were obtained: ADP-ribosylation factor 3 (ARF3), C-C chemokine receptor type 5 (CCR5), C-X-C chemokine receptor type 4 (CXCR4), receptor tyrosine-protein kinase erbB-3 (ERBB3), proteinase-activated receptor 1 (F2R), Ras-related

Table II. Introduction of the GEO microarrays.

					BC			Para-carcinoma				
Author, year	Dataset	Country	Year	Platform	n	Mean	SD	n	Mean	SD	Area under the curve	(Refs.)
Fassan *et al*, 2009	GSE17155	Italy	2009	GPL8871	33	4.91	0.77	5	4.77	0.94	0.9706	(42)
Zhao *et al*, 2010	GSE22981	USA	2011	GPL8179	20	9.26	1.23	20	9.05	1.43	0.5879	(43)
Schrauder *et al*, 2012	GSE31309	Germany	2012	GPL14132	48	4.63	0.90	57	4.50	0.70	0.5318	(44)
Tanic *et al*, 2013	GSE32922	Spain	2013	GPL7723	22	6.03	0.44	15	6.00	0.09	0.7182	(45)
Romero-Cordoba *et al*, 2012	GSE35412	Mexico	2012	GPL9731	34	9.53	2.33	6	4.44	0.76	0.9706	(46)
Gravgaard *et al*, 2012	GSE37407	Sweden	2012	GPL13703	50	9.79	0.28	8	9.86	0.10	0.5113	(47)
Biagioni *et al*, 2012	GSE40525	Israel	2012	GPL8227	61	2.69	0.75	59	3.74	1.57	0.7463	(48)
Feliciano *et al*, 2013	GSE44124	Spain	2014	GPL14767	50	5.60	0.14	3	5.88	0.29	0.7800	(49)
Peña-Chilet *et al*, 2014	GSE48088	Spain	2014	GPL14613	33	0.48	0.25	3	0.76	0.24	0.8232	(50)
Matamala *et al*, 2015	GSE58606	Spain	2015	GPL18838	122	7.05	0.21	11	7.16	0.14	0.7042	(51)

BC, breast cancer; SD, standard deviation.

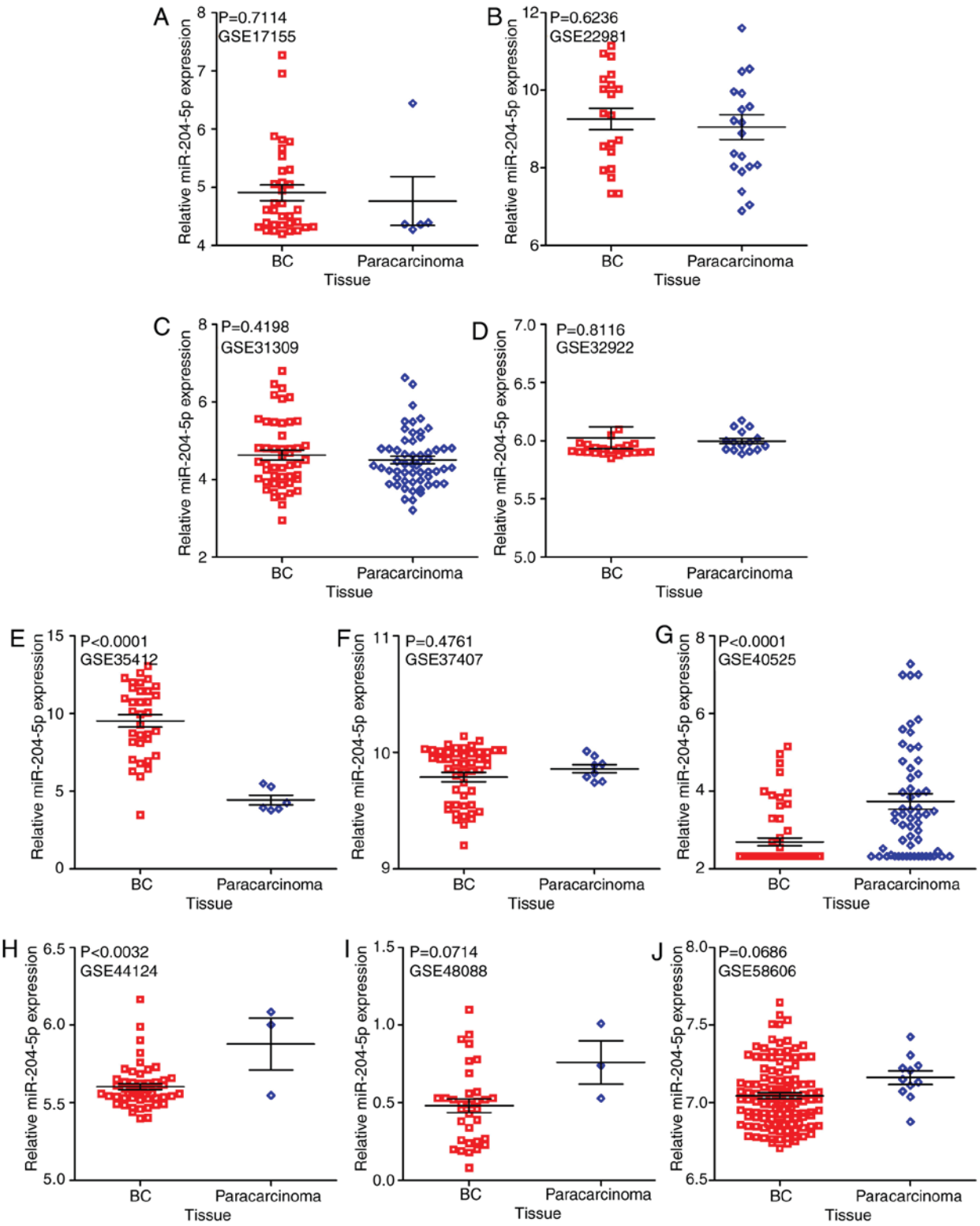

Figure 5. Expression of miR-204-5p in breast cancer tissues in comparison to para-carcinoma tissues based on the Gene Expression Omnibus database. (A) GSE17155. (B) GSE22981. (C) GSE31309. (D) GSE32922. (E) GSE35412. (F) GSE37407. (G) GSE40525. (H) GSE44124. (I) GSE48088. (J) GSE58606. BC, breast cancer; miR, microRNA.

protein Rab-10 (RAB10), Rab11 family-interacting protein 1 (RAB11FIP1) and proto-oncogene tyrosine-protein kinase receptor Ret (RET).

Furthermore, it was identified that RAB10 was significantly upregulated in BC tissue samples compared to para-carcinoma tissue samples (Fig. 21). Additionally, ROC curve analysis suggested that RAB10 possessed a great discriminatory capability for BC (Fig. 22). In terms of the PPI network, in the current study, estrogen receptor 1 (ESR1), ribonucleotide reductase regulatory subunit M2 (RRM2) and Rac GTPase activating protein 1 (RACGAP1) exhibited the highest degrees (Fig. 23). However, both ESR1 and RRM2 did

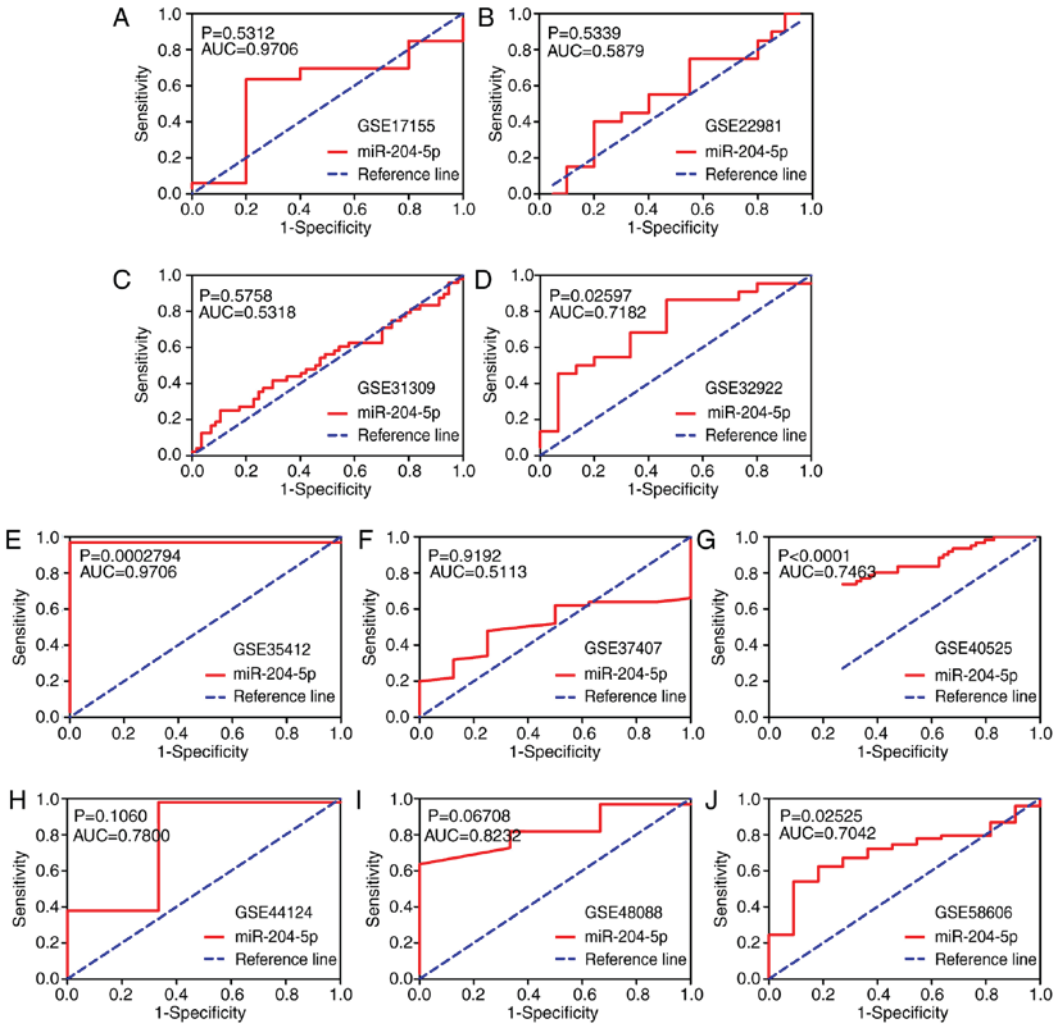

Figure 6. Receiver operating characteristic curve of miR-204-5p in breast cancer tissues in comparison to para-carcinoma tissues based on the Gene Expression Omnibus database. (A) GSE17155. (B) GSE22981. (C) GSE31309. (D) GSE32922. (E) GSE35412. (F) GSE37407. (G) GSE40525. (H) GSE44124. (I) GSE48088. (J) GSE58606. miR, microRNA; AUC, area under the curve.

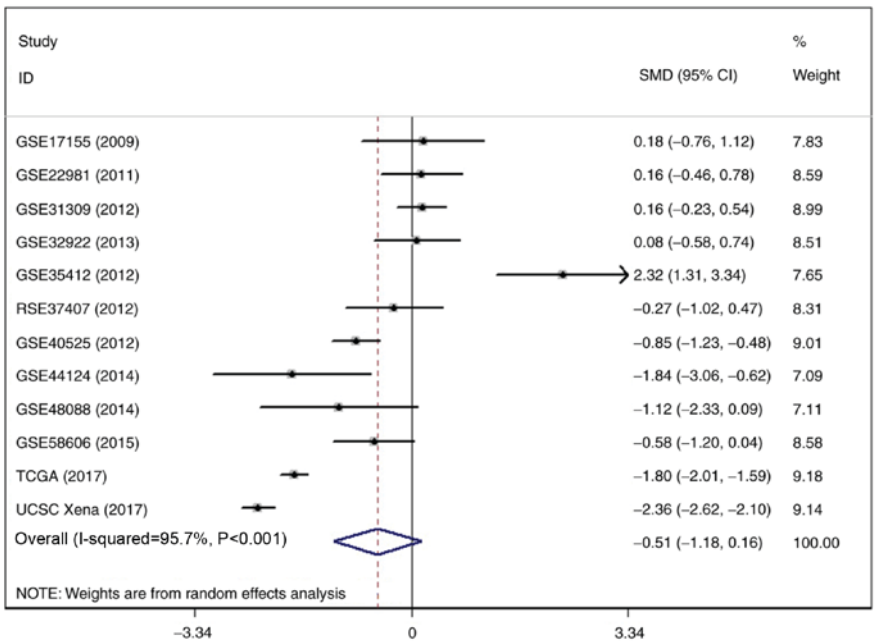

Figure 7. Heterogeneity test of the included studies. A suppressed trend was discovered in the breast cancer tissues, in comparison to para-carcinoma tissues. SMD, standard mean difference; CI, confidence interval.

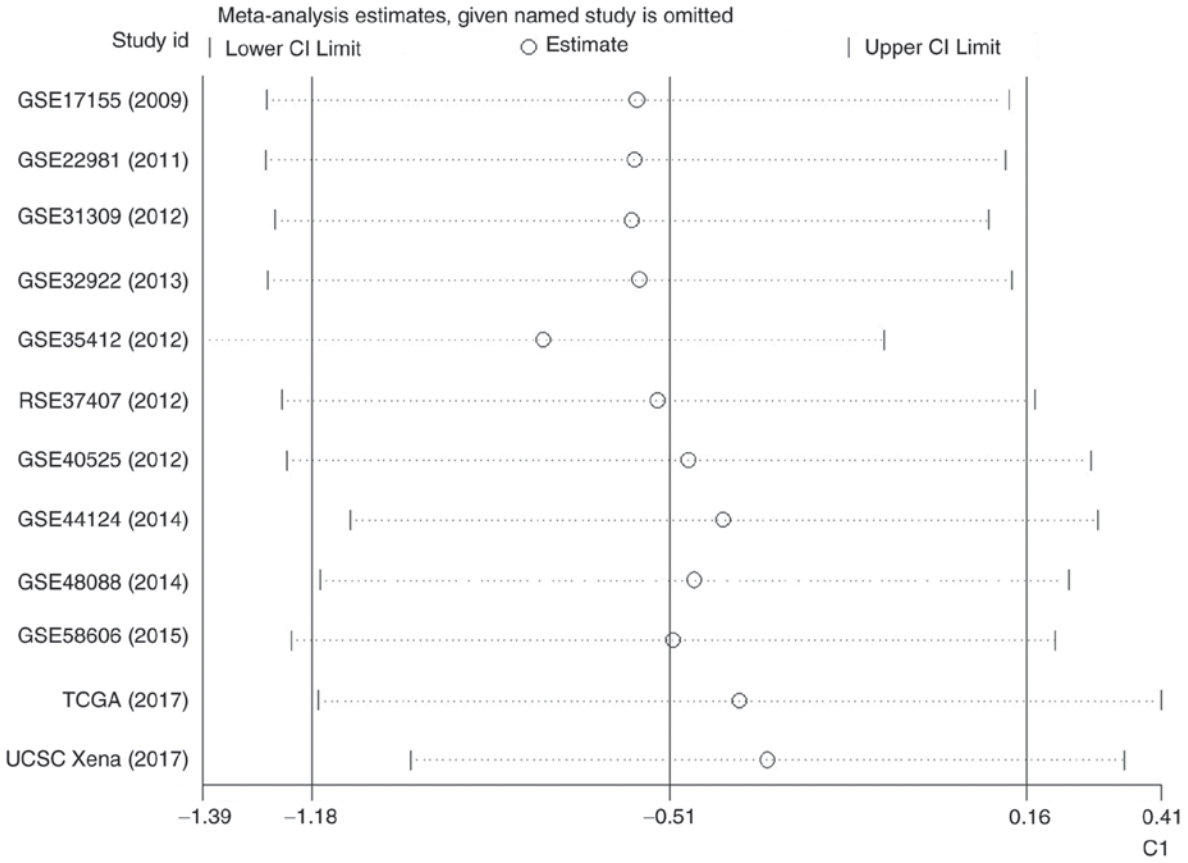

Figure 8. Influence analysis of the included studies. The influence analysis demonstrated no significant difference.

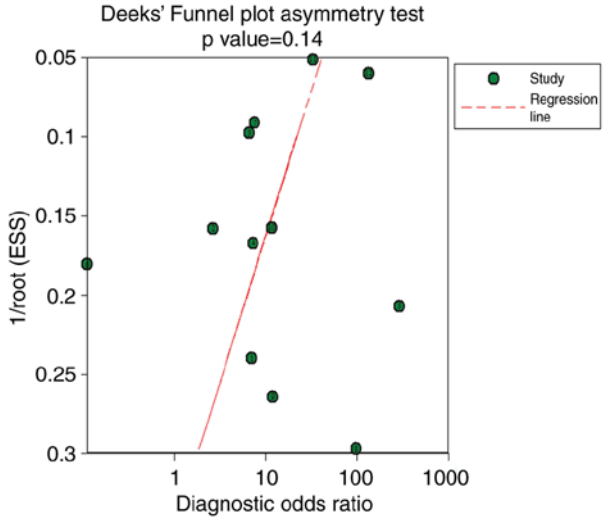

Figure 9. Publication bias in the included studies. The results of Deek's funnel plot asymmetry test implied there was no publication bias.

not have significant negative correlations with miR-204-5p, and RRM2 mRNA expression levels were not significantly lower in para-carcinoma tissue (data not shown). Therefore, RACGAP1 was selected as the hub gene. RACGAP1 mRNA expression levels were significantly increased in BC tissue samples in comparison to para-carcinoma tissue samples (Fig. 24A). Furthermore, the ROC curve indicated that RACGAP1 had great discriminatory capability for BC (Fig. 24B). Of note, a markedly negative correlation trend was identified between RACGAP1 and miR-204-5p (P=0.0166; r=-0.3592; Fig. 24C).

Discussion

To date, many studies have reported the decreased expression of miR-204-5p in various cancers, including hepatocellular carcinoma (24), non-small cell lung cancer (52), gastric cancer (53), oral squamous cell carcinoma (27), prostate cancer (54) and esophageal cancer (55). Further, recent studies have focused on the expression of miR-204-5p in BC. For example, Wang *et al* (56) discovered that the expression level of miR-204-5p was obviously reduced in 24 BC tissue samples in comparison to corresponding normal tissue samples. In addition, they reported a decrease in miR-204-5p expression in two BC cell lines (MDA-MB-231 and MCF-7), compared with MCF-10A, a breast epithelial cell line (56). Shen *et al* (57) reported that the expression of miR-204-5p was markedly suppressed in BC cells. They also demonstrated that the expression of miR-204-5p was markedly reduced in MCF-7 cells in comparison to HBL-100 cells, which are normal breast epithelial cells. In addition, they revealed that the upregulation of miR-204-5p inhibits the invasion, proliferation and migration, and enhances the apoptosis of BC cells.

Nonetheless, in-depth research featuring abundant samples is still required. In the current study, the expression of miR-204-5p in BC was evaluated in data obtained from

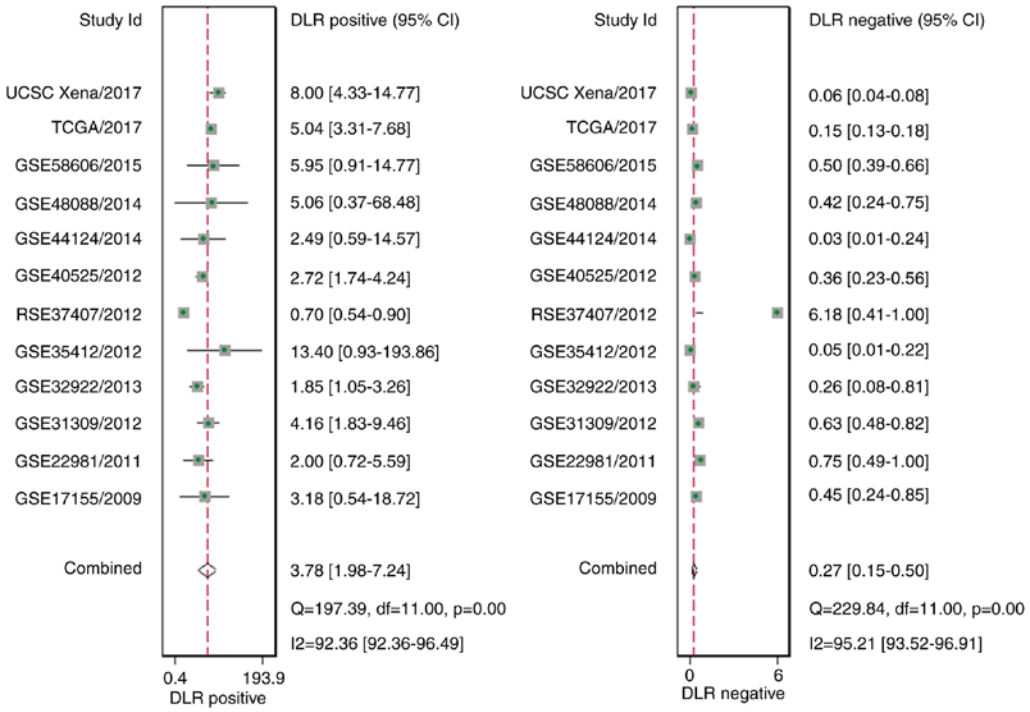

Figure 10. DLR positive and DLR negative of the included studies. The DLR positive and DLR negative were 3.78 (1.98-7.24) and 0.27 (0.15-0.50), respectively. DLR, diagnostic likelihood ratio; TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz.

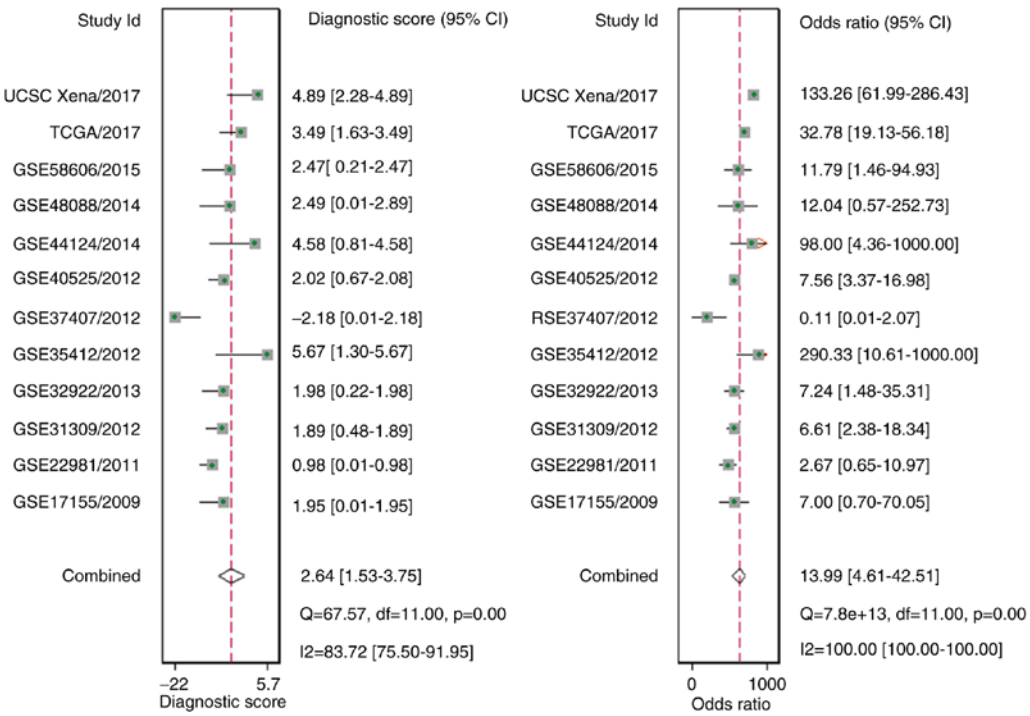

Figure 11. Diagnostic score, and odds ratio of the included studies. The diagnostic score, and odds ratio values were 2.64 (1.53-3.75) and 13.99 (4.61-42.51), respectively. TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz; CI, confidence interval.

the TCGA, GEO, and UCSC Xena databases. In the TCGA database, a decreased trend in precursor miR-204 expression was identified in 1,077 BC tissue samples, in comparison with 104 para-carcinoma tissue samples. In addition, in the UCSC Xena database, the expression of mature miR-204-5p was notably reduced in 756 BC tissue samples, compared with 76 para-carcinoma tissue samples. Furthermore, a number of the GEO microarrays indicated that the expression of miR-204-5p was downregulated in BC tissue samples, and the SMD in the meta-analysis also showed that the expression of miR-204-5p was notably lower in 2,306 BC tissue samples, compared with the 291 para-carcinoma tissue samples. The AUCs of the ROC

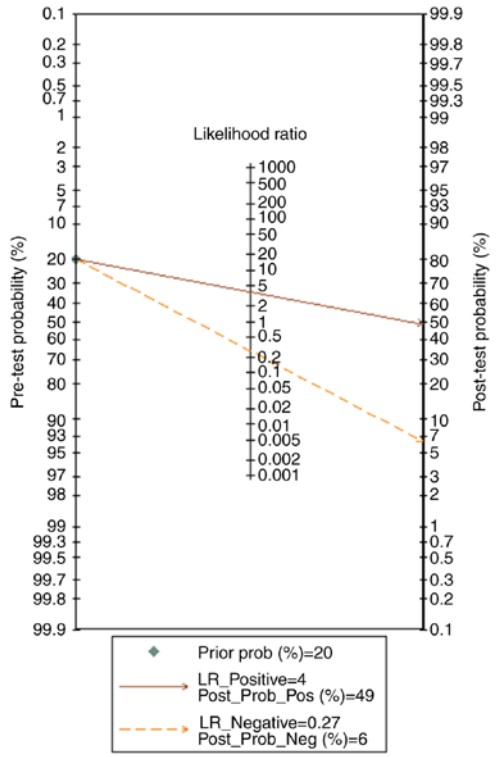

Figure 12. Prior probability and post-probability positive and negative of the included studies. The prior probability, post probability positive and negative reached 20, 49 and 6%, respectively. LR, likelihood ratio.

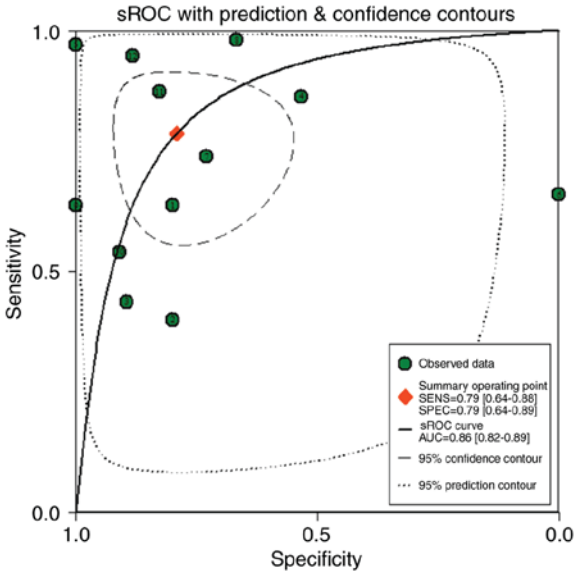

Figure 13. sROC values of the included studies. The AUC of the sROC curve was 0.86 (0.82-0.89), indicating a great discriminatory capability of miR-204-5p in breast cancer. sROC, summarized receiver operating characteristic.

and sROC curves implied that t miR-204 and miR-204-5p exhibited great discriminatory capacity in BC. Next, the prognostic value of miR-204-5p in BC was determined. Prior analysis of BC samples suggested that the decreased expression of miR-204-5p correlates with poor overall survival and disease-free survival in BC (58). Ye *et al* (59) demonstrated that miR-204-5p had no prognostic value in BC through analyzing

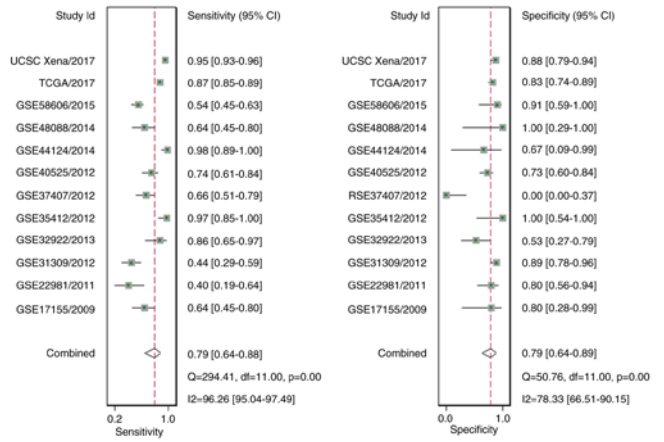

Figure 14. Sensitivity and specificity values of the included studies. The sensitivity and specificity values of the included studies were 79% (64-88%) and 79% (64-89%), respectively. TCGA, The Cancer Genome Atlas; UCSC, University of California Santa Cruz; CI, confidence interval.

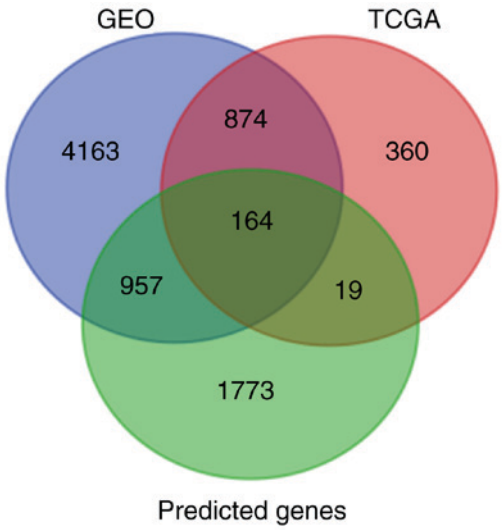

Figure 15. Intersection of the DEG predicted DEGs, DEGs from the TCGA database and the GEO database. In total, 164 putative genes were acquired. GEO, Gene Expression Omnibus; TCGA, The Cancer Genome Atlas.

563 BC tissue samples obtained from the TCGA database. In the current study, no obvious correlation was found between the precursor miR-204 and survival outcome in BC based on analyzing 1,077 BC tissue samples from TCGA database. Additionally, it was identified that miR-204 downregulation was significant in several groups, including age, vital status, PR status, HER2 status and pathological stage. Taken together with the results of these two aforementioned studies, it was hypothesized that miR-204-5p may acts as a tumor suppressor in the oncogenesis and progression of BC.

GO and KEGG analysis was performed to investigate the potential biological processes and pathways of miR-204-5p in BC. 'Cell development', 'cell surface activity' and 'receptor agonist activity' were considered the most enriched processes in GO analyses. Thus, it was suggested that miR-204-5p may participate these processes in BC, by targeting its corresponding target genes. However, further study is needed to verify the molecular mechanisms underlying miR-204-5p and these

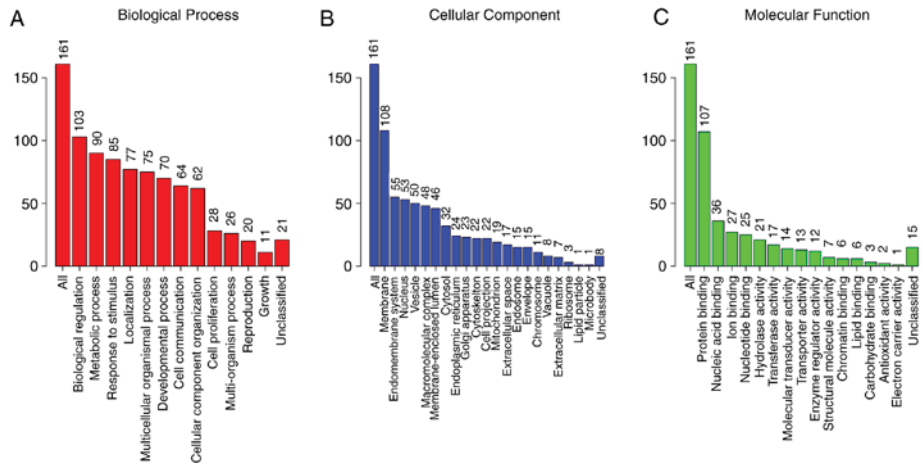

Figure 16. Gene Ontology analysis of the 164 putative genes, in terms of (A) biological process, (B) cellular component and (C) molecular function.

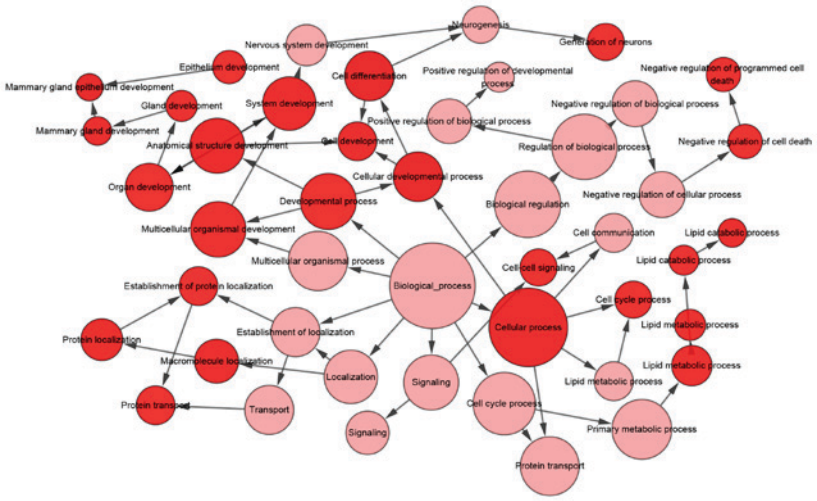

Figure 17. Gene network analysis of the 164 putative genes in terms of BP. The putative genes of microRNA-204-5p were found to have predominantly participated in cell development. The color intensity is proportional to enrichment significance and the circle size indicates the number of enriched genes. BP, biological process.

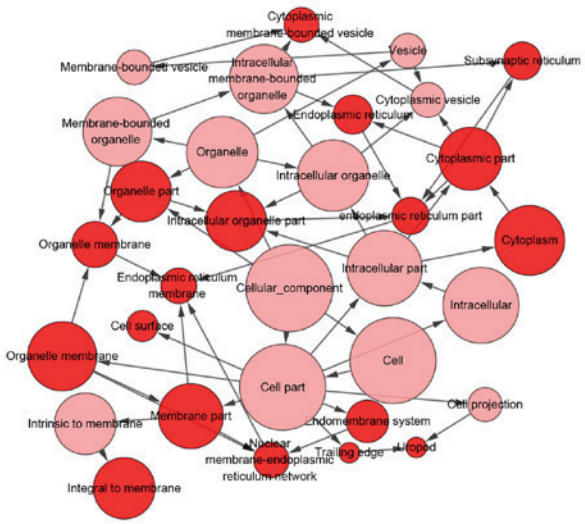

Figure 18. Gene network analysis of the 164 putative genes in terms of CC. The putative genes of microRNA-204-5p were found to have predominantly participated in cell surface for CC. The color intensity is proportional to enrichment significance and the circle size indicates the number of enriched genes. CC, cellular component.

three processes in BC. Concurrently, in the KEGG analyses, 'endocytosis' was found to be the most enriched, which was associated with ARF3, CCR5, CXCR4, ERBB3, F2R, RAB10, RAB11FIP1 and RET. The expression and ROC curves of the eight pathway-related genes were estimated, and it was determined that RAB10 expression was significantly increased in BC tissue samples, compared with para-carcinoma tissue samples. In addition, RAB10 featured a great discriminatory capacity for BC within non-cancerous breast tissue samples. Hence, it was proposed that miR-204-5p may possess a vital effect on BC via genes associated with endocytosis, including RAB10. However, the role of endocytosis in BC is unclear and further investigation is urgently required.

In researching the target genes of miR-204-5p in BC, Flores-Peréz *et al* (60) found that transforming growth factor β receptor 2 (TGFβR2) and angiopoietin 1 (ANGPT1) are crucial in BC tumor angiogenesis; BC cell migration and proliferation decreases when TGFβR2 is suppressed, and the suppression of TGFβR2 and ANGPT1 inhibits angiogenesis. Furthermore, Zeng *et al* (61) identified a negative correlation trend between miR-204-5p and SIX homeobox 1 (Six1) expression in

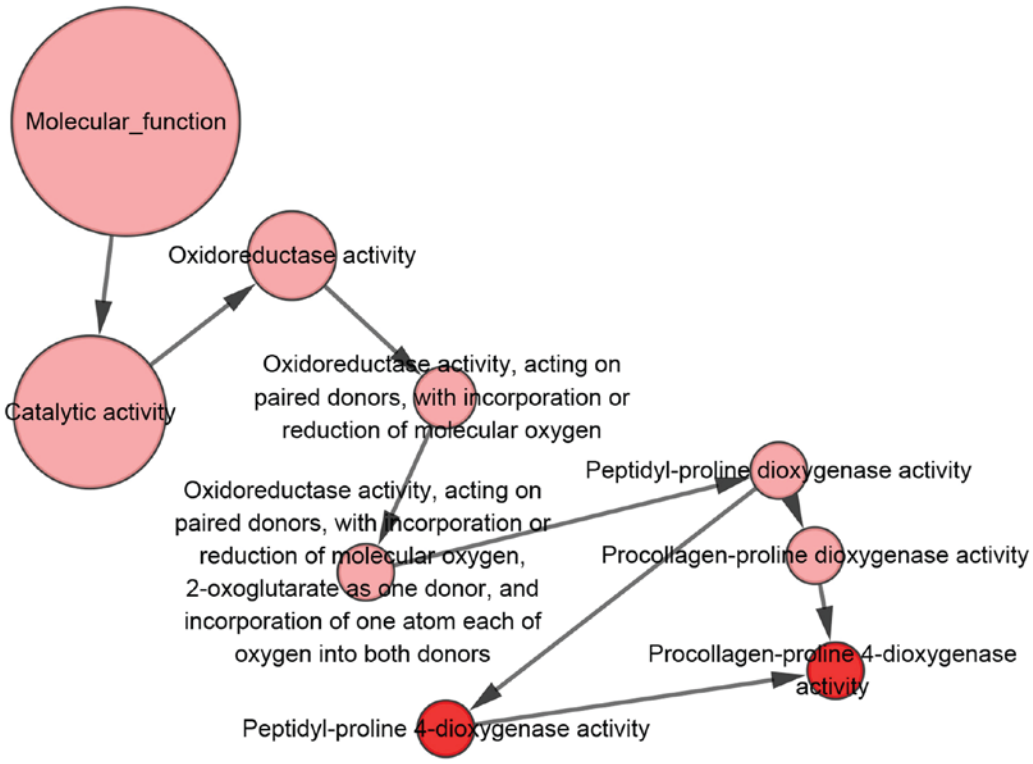

Figure 19. Gene network analysis of the 164 putative genes in terms of MF. The putative genes of microRNA-204-5p were found to have predominantly participated in receptor-agonist activity for MF. The color intensity is proportional to enrichment significance and the circle size indicates the number of enriched genes. MF, molecular function.

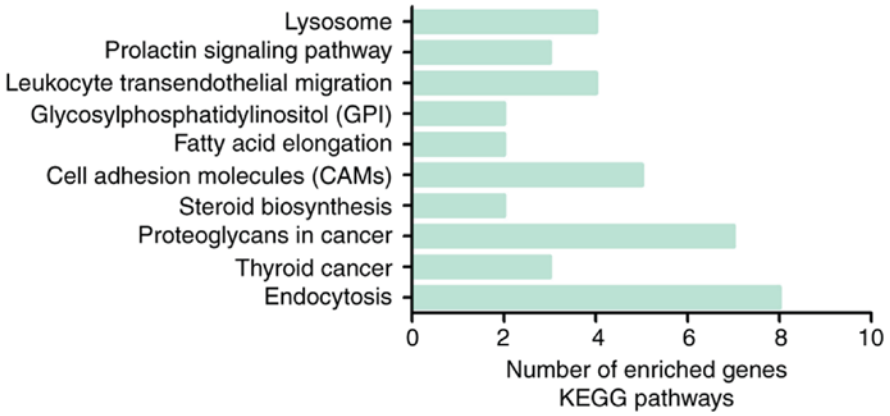

Figure 20. KEGG pathways of the 164 putative genes. The most significantly enriched pathway of the putative genes was 'endocytosis'. KEGG, Kyoto Encyclopedia of Genes and Genomes.

BC tissue samples, and when miR-204-5p mimics or Six1 siRNA was transfected, the expression of chromodomain helicase DNA binding protein 1 was markedly increased, thus enhancing epithelial-mesenchymal transition and affecting the invasion and migration of BC cells (61). Various target genes of miR-204-5p have been confirmed in previous studies, including traditional serrated adenoma, MX dynamin like GTPase 1, thioredoxin interacting protein, Src-associated in mitosis 68 kDa protein and forkhead box A1 (57,62-64). However, more target genes need to be determined. Accordingly, a PPI network was generated in the present study. The hub gene RACGAP1 was selected as an example for further investigation. RACGAP1 is involved in cell cytokinesis, transformation, migration, metastasis and growth (65,66). In BC specifically, it has been reported that RACGAP1 is critical in enhancing basal-like breast cancer proliferation and oncogenicity (67,68). Furthermore, in untransformed cells, RACGAP1 stimulates malignant phenotypes, and elevated RACGAP1 expression is correlated with poor BC outcomes (67,68). In the current study, RACGAP1 expression was evaluated in BC tissue sample data obtained from the TCGA database. RACGAP1 was upregulated BC tissue samples, compared with para-carcinoma tissue samples. In addition, a strong negative correlation trend was identified between RACGAP1 and miR-204-5p. Thus, it was proposed that miR-204-5p may serve a crucial role in BC by targeting RACGAP1. However, these conclusions were made

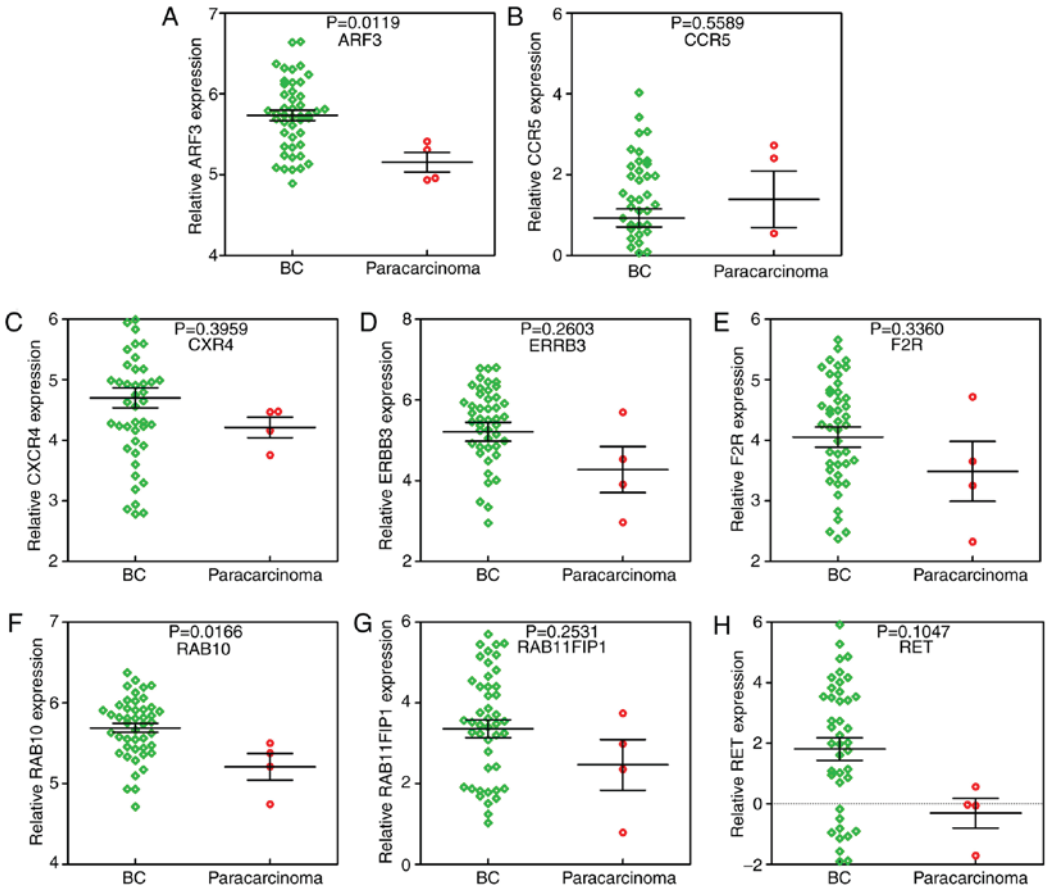

Figure 21. Expression level of pathway-associated genes, based on data from The Cancer Genome Atlas database. (A) ARF3. (B) CCR5. (C) CXCR4. (D) ERBB3. (E) F2R. (F) RAB10. (G) RAB11FIP1. (H) RET. BC, breast cancer; ARF3, ADP-ribosylation factor 3; CCR5, C-C chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4; ERBB3, receptor tyrosine-protein kinase erbB-3; F2R, proteinase-activated receptor 1; RAB10, Ras-related protein Rab-10; Rab11 family-interacting protein 1; RET, proto-oncogene tyrosine-protein kinase receptor Ret.

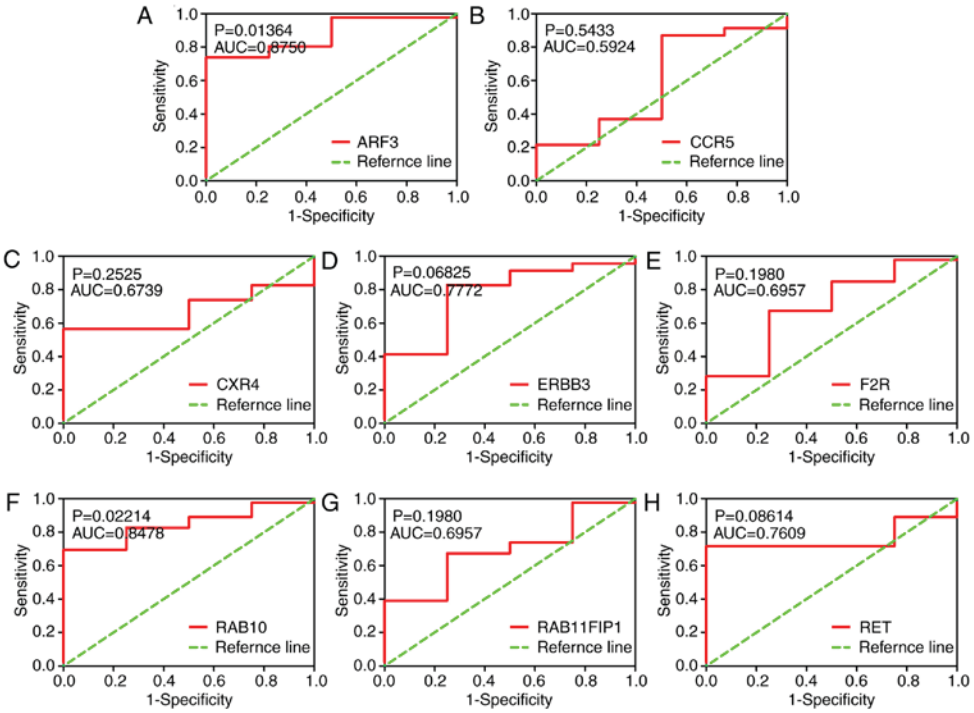

Figure 22. Receiver operating characteristic curves of pathway-associated genes, based on data from The Cancer Genome Atlas database. (A) ARF3. (B) CCR5. (C) CXCR4. (D) ERBB3. (E) F2R. (F) RAB10. (G) RAB11FIP1. (H) RET. AUC, area under the curve; ARF3, ADP-ribosylation factor 3; CCR5, C-C chemokine receptor type 5; CXCR4, C-X-C chemokine receptor type 4; ERBB3, receptor tyrosine-protein kinase erbB-3; F2R, proteinase-activated receptor 1; RAB10, Ras-related protein Rab-10; Rab11 family-interacting protein 1; RET, proto-oncogene tyrosine-protein kinase receptor Ret.

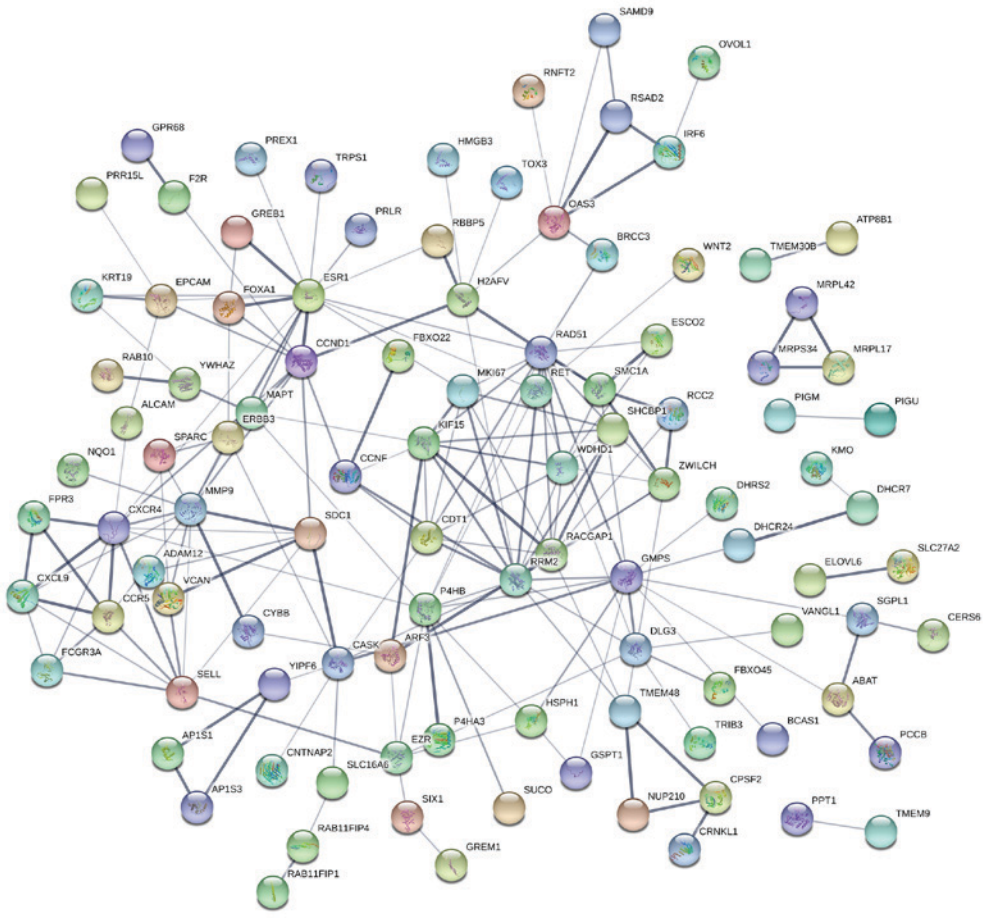

Figure 23. Protein-protein interaction network of the 164 putative genes. In the current study, RACGAP1 was selected as the hub gene. RACGAP1, Rac GTPase-activating protein 1.

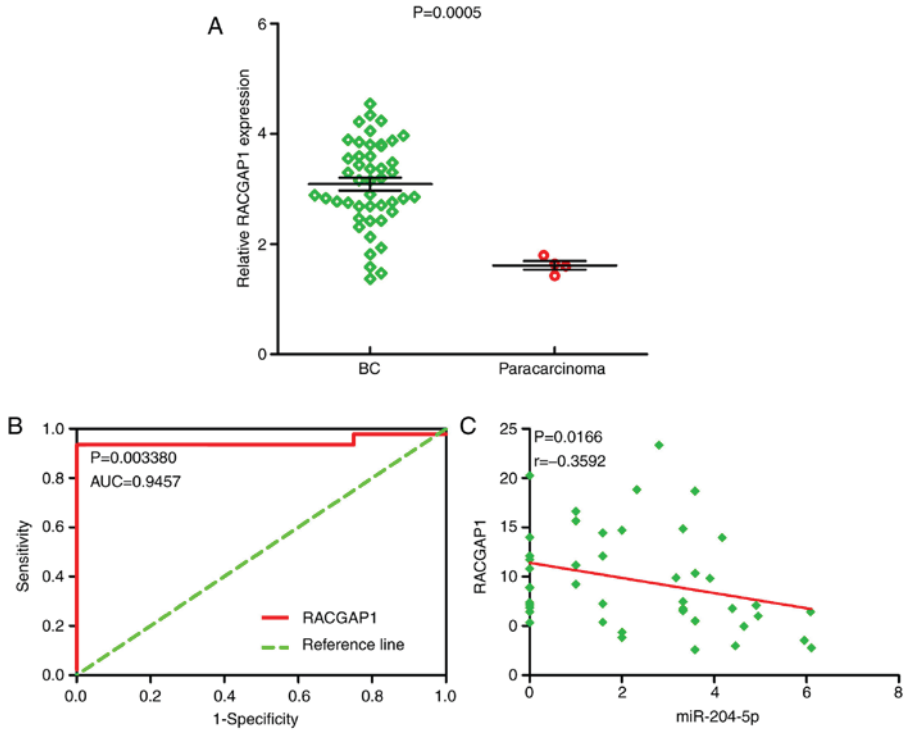

Figure 24. Expression level and receiver operating characteristic curve of RACGAP1 in breast cancer. (A) The expression level of RACGAP1 was markedly increased in breast cancer tissues in comparison to para-carcinoma tissues, based on data from The Cancer Genome Atlas database. (B) RACGAP1 had a great discriminatory capacity in breast cancer. (C) The correlation between miR-204-5p and RACGAP1. BC, breast cancer; RACGAP1, Rac GTPase-activating protein 1; AUC, area under the curve; miR, microRNA.

based on online tools, so further *in vivo* and *in vitro* investigations should be performed to verify the molecular mechanisms of RACGAP1 and miR-204-5p in BC.

There are several limitations to the present study. First, a high degree of I^2 existed in the heterogeneity test. Thus, a random effects model was conducted to reduce the degree of I^2-however, it still exceeded 50%. This may be a result of using various measures and platforms used to analyze the data. The nine GEO microarrays were acquired from six countries; GSE32922, GSE44124, GSE48088 and GSE58606 were obtained from Spain, and GSE17155, GSE22981, GSE31309, GSE35412, GSE37407 and GSE40525 were obtained from Italy, the USA, Germany, Mexico, Sweden and Israel, respectively. Second, a dual luciferase reporter assay was not performed to verify the correlation between miR-204-5p and the hub gene. Thus, in-depth investigations with *in vivo* and *in vitro* experiments should be performed in the future.

In conclusion, the results of the present study identified that miR-204-5p expression was downregulated in BC tissue samples in comparison to para-carcinoma tissue samples; this suggested that miR-204-5p might function as a suppressor in the oncogenesis and advancement of BC. Furthermore, it was revealed that RACGAP1 may be a crucial target gene of miR-204-5p, and the expression of RACGAP1 was markedly increased in BC tissue samples in comparison to para-carcinoma tissue samples. Notably, a significant negative correlation was identified between RACGAP1 and miR-204-5p in BC. Therefore, it was concluded that miR-204-5p may serve a crucial role in BC by targeting RACGAP1.

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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

AGL and ZFW collected and analyzed the TCGA data. HWJ and JJZ collected and analyzed the GEO data. RQH, GC and JM collected and analyzed the UCSC data. KTC and JCZ conducted meta-analysis 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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