Downregulation of miR-145-5p in cancer cells and their derived exosomes may contribute to the development of ovarian cancer by targeting CT

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Abstract. The present study aimed to identify shared microRNAs (miRNAs) in ovarian cancer (OC) cells and their exosomes using microarray data (accession number GSE103708) available from the Gene Expression Omnibus database, including exosomal samples from 13 OC cell lines and 3 normal ovarian surface epithelial cell lines, and their original cell samples. Differentially expressed miRNAs (DE-miRNAs) were identified using the Linear Models for Microarray data method, and mRNA targets of DE-miRNAs were predicted using the miRWalk2 database. The potential functions of target genes were analyzed using Database for Annotation, Visualization and Integrated Discovery and intersected with known OC-associated pathways downloaded from the Comparative Toxicogenomics Database. The associations between crucial miRNAs and target genes, and their clinical associations, were validated using data from The Cancer Genome Atlas. As a result, 16 upregulated and 6 downregulated DE-miRNAs were shared in OC cell lines and their exosomes compared with normal controls. The target genes of 11 common DE-miRNAs were predicted. Among these DE-miRNAs, a low expression of *homo sapiens* (hsa)-miR-145-5p was significantly correlated with a poor prognosis and higher stages. Although 91 target genes were predicted for hsa-miR-145-5p, only 4 genes [connective tissue growth factor (CTGF), myotubularin-related protein 14,

protein phosphatase 3 catalytic subunit alpha and suppressor of cytokine signaling 7] were suggested as risk factors for prognosis. The subsequent Pearson's correlation analysis validated a significant negative correlation between hsa-miR-145-5p and CTGF (r=-0.1126, P=0.02188). According to the results of the functional analysis, CTGF is involved in the Hippo signaling pathway (hsa04390). In conclusion, decreased expression of hsa-miR-145 in OC and OC-derived exosomes may be a crucial biomarker for the diagnosis and treatment of OC.

Introduction

Ovarian cancer (OC) is the most common malignant tumor threatening the health of women in China. In 2015, an estimated 52,100 incident cases were diagnosed, with >22,500 mortalities (1). Although patients with OC are generally treated with a series of standard treatments, including surgery, chemotherapy and radiotherapy, the prognosis remains unsatisfactory for the majority of patients, with an overall 5-year survival rate of ~30% (2). Additionally, advanced OC may only be observed at the point of the initial diagnosis, which may be too late for patients to receive optimal treatment. Therefore, a screen of effective diagnostic biomarkers and the development of novel therapeutic strategies are urgently required.

Based on previous evidence, microRNAs (miRNAs/miRs) serve important roles in the development and progression of OC by specifically binding to the 3'-untranslated region (3'-UTR) of target mRNAs to inhibit translation or induce degradation (3). For example, Qin *et al* (4) observed a significant decrease in the expression of miR-152 in OC specimens and three OC cell lines. The transfection of miR-152 mimic into OC cells inhibited proliferation and migration by targeting the 3'-UTR of the forkhead box protein 1 (FOXP1) and resulted in improved overall survival. Liu *et al* (5) confirmed the overexpression of miR-26a in human OC specimens and revealed correlations between lymph node metastasis, an advanced International Federation of Gynecology and Obstetrics (FIGO) stage and poor survival. miR-216a promoted the metastatic behaviors and epithelial-mesenchymal transition (EMT) of OC cells by inhibiting its direct downstream target phosphatase and tensin homolog and subsequently regulating

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the protein kinase B pathway. Therefore, miRNAs may serve as promising biomarkers for the diagnosis of OC and attractive therapeutic targets.

In addition to their intracellular functions, miRNAs are also packaged into membrane-bound vesicles, including exosomes, and released to the extracellular environment to facilitate tumorigenesis and progression (6). For example, in the study by Ying *et al* (7), OC-derived exosomes released miR-222-3p into macrophages and induced the formation of a tumor-associated macrophage-like phenotype by decreasing the expression of suppressor of cytokine signaling 3 (SOCS3) and activating SOCS3/signal transducer and activator of transcription (STAT)-3 signaling pathway, thereby promoting the growth and metastasis of OC. According to the study by Kanlikilicer *et al* (8), miR-6126 is released from OC cells via exosomes. miR-6126 significantly reduces tumor growth, invasion and migration *in vitro* and *in vivo* by suppressing the expression of integrin-β1. De *et al* (9) observed the anti-neoplastic effects of an *Emblica officinalis* extract on OC that were mediated by the upregulation of cellular and exosomal miR-375 expression. Meng *et al* (10) observed correlations between high levels of exosomal miR-373, miR-200a, miR-200b and miR-200c expression with a higher FIGO stage (III-IV) and a shorter overall survival. Therefore, exosomal miRNAs may also be crucial targets for cancer diagnosis and treatment. Strategies targeting co-expressed miRNAs in cancer cells and their exosomes may represent more effective approaches for inducing cancer remission (11), however, this information has been rarely reported in OC.

The present study aimed to investigate the shared miRNAs in OC cells and their exosomes using microarray data downloaded from Gene Expression Omnibus (GEO) database and validate their clinical significance with The Cancer Genome Atlas (TCGA) datasets. The present study preliminarily identified that downregulated hsa-miR-145 was common in OC and OC-derived exosomes and thus may be a crucial biomarker for the diagnosis and treatment of OC.

Materials and methods

Data collection. The miRNA microarray data (accession number GSE103708) were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) on January 23, 2018; the dataset contained exosomal samples isolated from 13 human epithelial OC cell lines (A2780, ES-2, CAOV3, SKOV3, OV-90, OAW42, MCAS, COV362, RMG-1, RMUG-S, KURAMOCHI, NIH-OVCAR3 and A2780cis), 3 normal ovarian surface epithelial cell lines (HOSE1, HOSE2 and HOSE3) and their original cell samples. Each cell type had one biological repeat, resulting in 32 samples.

Data preprocessing and identification of differentially expressed miRNAs (DE-miRNAs). The GSE Series Matrix and annotations files were retrieved from the Agilent-046064 Unrestricted_Human_miRNA_V19.0_Microarray Array platform GPL18402. The probe names were transferred to gene symbols based on the platform annotation information. When multiple probes were mapped to a given gene, the mean expression value was used. Data were then log2-transformed and quantile normalized using

the Robust Multiarray Average function available in the Bioconductor R package (v3.32.5; http://www.bioconductor.org/packages/release/bioc/html/limma.html). The DE-miRNAs between OC and normal ovarian surface epithelial cells and exosomes and original cells were identified using the Linear Models for Microarray data method in the Bioconductor R package (v3.32.5; http://www.bioconductor.org/packages/release/bioc/html/limma.html) (12). The significance of differences of DE-miRNAs were assessed using the empirical Bayes moderated t-test. Subsequently, the P-value was corrected for multiple comparisons using the Benjamini-Hochberg (BH) procedure (13). miRNAs with a false discovery rate <0.05 and |logFC(fold change)|>1 were considered differentially expressed. The ability of DE-miRNAs to differentiate the samples was tested using the Euclidean distance-based bidirectional hierarchical clustering analysis in the Pheatmap package (v1.0.8; http://cran.r-project.org/web/packages/pheatmap/index.html), following which a heat map was produced. A Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to visualize the shared DE-miRNAs between different exosomes and original cells.

Target gene prediction. The mRNA targets of DE-miRNAs were predicted using the miRWalk database (v2.0; http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) (14). Then, the miRNA-target gene interaction network was constructed and visualized using Cytoscape software (v3.4; www.cytoscape.org/) (15).

Screening prognosis-associated miRNAs and their target genes. The miRNA and mRNA Seq data from OC samples (Level 3) were obtained from the TCGA database (https://tcga-data.nci.nih.gov/). The expression levels of targeted miRNAs and mRNAs in OC were analyzed using the fragment per kilobase per million mapped reads value from the TCGA data.

A univariate Cox regression analysis was performed to screen prognosis-associated miRNAs/mRNAs using the survival package in R (v2.4; https://cran.r-project.org/web/packages/survival/index.html) (16), with the log-rank P<0.05 set as the threshold. Univariate and multivariate Cox regression analyses were used to screen prognosis-associated clinical characteristics, including age, radiation therapy, stage (17), histological grade (18), neoplasm subdivision, lymphatic invasion, recurrence and survival, and then the potential associations between crucial miRNAs and clinical characteristics were also calculated using the survival package. The Kaplan-Meier curve with the log-rank test was plotted using GraphPad Prism software (v5; GraphPad Software, Inc., La Jolla, CA, USA) to determine significant associations between the expression of miRNAs/mRNAs and patient survival outcomes. Pearson's correlation coefficients were calculated to assess the correlations between miRNAs and mRNAs. P<0.05 was considered to indicate a statistically significant difference.

Functional enrichment analysis. The enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) (19) pathways of target genes were predicted by searching the Database for Annotation, Visualization and Integrated Discovery online tool

Table I. Shared differentially expressed miRNAs in exosomes and original cells.

miRNA	Exosome			Original cells		
	logFC	P-value	FDR	logFC	P-value	FDR
hsa-miR-202-3p	-1.14	7.29×10^{-5}	1.48×10^{-2}	-2.14	1.13×10^{-2}	3.92×10^{-2}
hsa-miR-5684	-1.73	5.76×10^{-5}	1.17×10^{-2}	-1.58	1.94×10^{-3}	8.36×10^{-3}
hsa-miR-376a-3p	-1.74	9.67×10^{-6}	1.97×10^{-3}	-1.80	3.36×10^{-3}	1.38×10^{-2}
hsa-miR-141-3p	2.13	8.71×10^{-6}	1.77×10^{-3}	2.01	6.05×10^{-3}	2.29×10^{-2}
hsa-miR-376c-3p	-2.07	4.41×10^{-6}	8.95×10^{-4}	-2.08	6.82×10^{-4}	3.32×10^{-3}
hsa-miR-381-3p	-2.11	6.51×10^{-6}	1.33×10^{-3}	-2.35	4.88×10^{-4}	2.47×10^{-3}
hsa-miR-145-5p	-2.67	1.15×10^{-8}	2.35×10^{-6}	-2.16	1.84×10^{-4}	1.03×10^{-3}
hsa-miR-378i	2.79	2.54×10^{-6}	5.15×10^{-4}	2.13	2.05×10^{-3}	8.70×10^{-3}
hsa-miR-98-5p	1.286	2.42×10^{-4}	4.91×10^{-2}	5.30	4.07×10^{-6}	4.21×10^{-5}
hsa-miR-7-5p	1.48	9.31×10^{-5}	1.90×10^{-2}	4.92	6.08×10^{-5}	3.89×10^{-4}
hsa-miR-374b-5p	1.34	1.57×10^{-4}	3.19×10^{-2}	5.59	5.44×10^{-7}	1.45×10^{-5}
hsa-miR-374a-5p	1.42	1.08×10^{-4}	2.20×10^{-2}	5.74	1.54×10^{-7}	8.57×10^{-6}
hsa-miR-301a-3p	1.56	8.62×10^{-5}	1.76×10^{-2}	5.60	5.44×10^{-7}	1.45×10^{-5}
hsa-miR-17-3p	2.06	1.84×10^{-4}	3.74×10^{-2}	5.42	1.64×10^{-6}	2.15×10^{-5}
hsa-miR-335-5p	3.09	2.31×10^{-4}	4.70×10^{-2}	4.22	1.65×10^{-3}	7.19×10^{-3}
hsa-miR-186-5p	3.34	7.16×10^{-6}	1.46×10^{-3}	3.93	6.75×10^{-3}	2.53×10^{-2}
hsa-miR-148a-3p	2.50	1.50×10^{-5}	3.05×10^{-3}	5.27	5.01×10^{-6}	4.90×10^{-5}
hsa-miR-532-5p	3.35	1.78×10^{-4}	3.63×10^{-2}	4.30	1.21×10^{-3}	5.51×10^{-3}
hsa-miR-660-5p	3.45	1.29×10^{-4}	2.62×10^{-2}	4.43	7.61×10^{-4}	3.64×10^{-3}
hsa-miR-205-5p	4.31	6.74×10^{-6}	1.37×10^{-4}	4.48	7.61×10^{-4}	3.64×10^{-3}
hsa-miR-126-3p	4.15	1.17×10^{-5}	2.37×10^{-3}	4.85	6.98×10^{-5}	4.44×10^{-4}
hsa-miR-200c-3p	4.00	7.13×10^{-7}	1.45×10^{-4}	5.05	2.99×10^{-5}	2.06×10^{-4}

FC, fold change; FDR, false discovery rate; hsa, *homo sapiens*; miRNA, microRNA.

(v6.8; http://david.abcc.ncifcrf.gov) (20). P<0.05 was set as the cut-off criterion. In addition, all known OC-associated pathways were also downloaded from the Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org/) (21). The pathways obtained from these two sources were overlapped to obtain crucial miRNAs involved in OC-associated pathways.

Results

Identification of DE-miRNAs between cancer and normal cells. In the exosomal samples, 77 miRNAs were differentially expressed between 13 OC cancer and 3 normal cells, including 37 upregulated and 40 downregulated miRNAs. For the original cell samples, 259 DE-miRNAs were identified between 13 OC cancer and 3 normal cells, including 87 upregulated and 172 downregulated miRNAs. According to the Venn diagram, 22 DE-miRNAs with similar expression trends (16 upregulated and 6 downregulated) were shared between exosomal and original cell samples (Fig. 1; Table I), suggesting that these 22 DE-miRNAs may be particularly crucial for OC development in an exosomal or non-exosomal manner. Therefore, these DE-miRNAs were examined in subsequent analyses.

Comparison of target genes of DE-miRNAs between OC cells and normal controls. Subsequent to searching the miRWalk 2.0 database, only the target genes of 11 shared

DE-miRNAs [upregulated: *homo sapiens* (has)-miR-126-3p, hsa-miR-141-3p, hsa-miR-17-3p, hsa-miR-186-5p, hsa-miR-301a-3p, hsa-miR-335-5p, hsa-miR-532-5p, hsa-miR-7-5p, hsa-miR-374b-5p, and hsa-miR-98-5p; downregulated: hsa-miR-145-5p] were obtained. These common miRNAs and their target genes were then used to construct a miRNA-mRNA regulatory network, as demonstrated in Fig. 2. This network included 4,198 nodes (11 miRNAs and 4187 target genes) and 462 edges (interaction associations).

Screening prognosis-associated miRNAs and their target genes. The miRNA/mRNA expression and clinical data associated with OC were also extracted from the TCGA database to validate the clinical importance of the miRNAs identified in the present study. Subsequently, the miRNA and mRNA expression data were matched with the clinical data for 414 patients. The expression of 11 shared DE-miRNAs was extracted from the TCGA data and then combined with the clinical data to screen the prognosis-associated miRNAs. Notably, hsa-miR-98-5p (upregulated; P=0.01046), hsa-miR-145-5p (downregulated; P=0.0178) and hsa-miR-17-3p (upregulated; P=0.0294) were significantly correlated with the survival outcomes of patients with OC. The Kaplan-Meier analysis additionally revealed a poor prognosis for patients with low expression levels of hsa-miR-145-5p, as expected (Fig. 3). Similarly, the subsequent univariate analyses revealed an
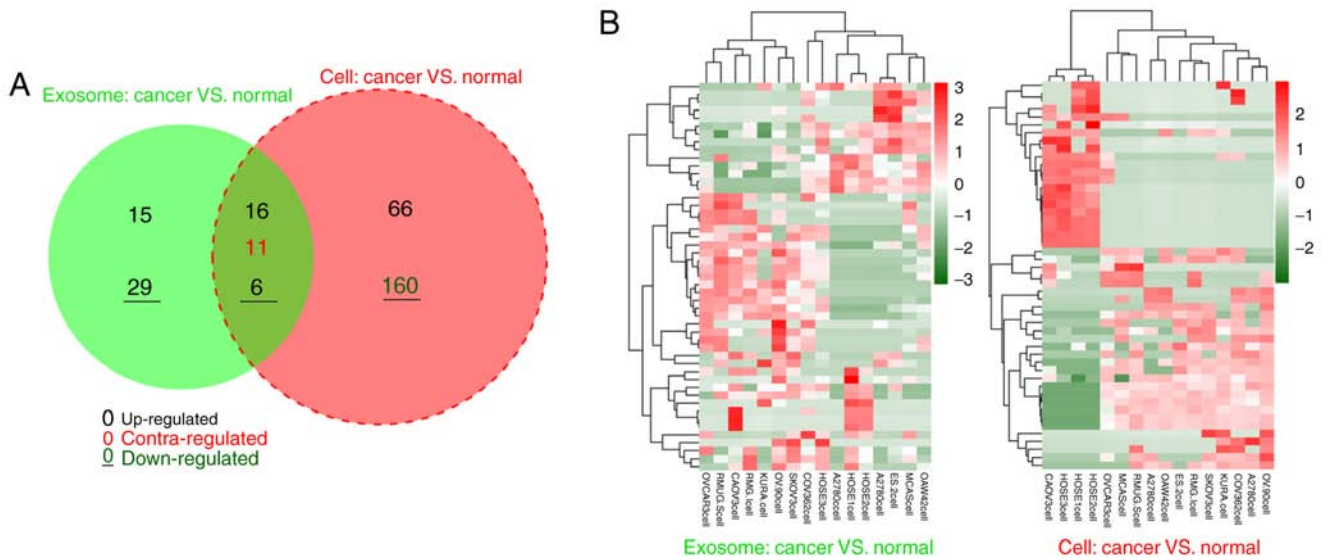
Figure 1. Shared differentially expressed miRNAs in 13 ovarian cancer cells and their exosomes compared with normal cells. (A) Venn diagram. (B) Heat map. Contra-regulated in the Venn diagram indicates there was a different expression trend in ovarian cancer cells and their exosomes. Red in the heat map indicates the high expression of miRNAs; the green in the heat map indicates the lower expression of miRNAs. miRNA, microRNA.
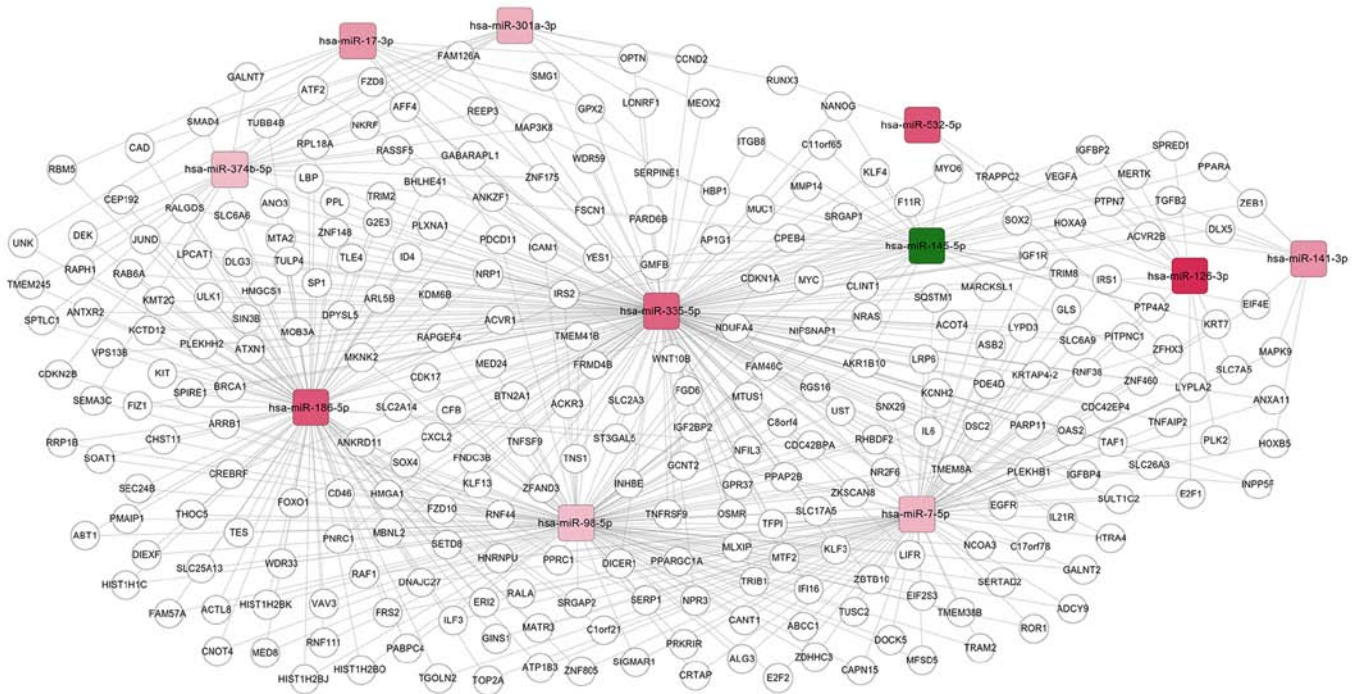

Figure 2. Regulatory network comprising 11 common differentially expressed miRNAs in ovarian cancer cells and their exosomes, and their target genes. Red squares represent upregulated miRNAs, and green squares represent downregulated miRNAs. The changes in red shades indicated different numbers of target genes (darker shades = higher numbers). hsa, *homo sapiens*; miRNA, microRNA.

association between hsa-miR-145-5p expression and the tumor stage (Table II), which was also an independent prognosis factor with survival (Table III; Fig. 4).

The 91 target genes of hsa-miR-145-5p were included in the screen of prognosis-associated genes. As a result, 11 genes, including ADP ribosylation factor-like GTPase 6 interacting protein 5 (P=0.017), connective tissue growth factor (CTGF; P=0.047), matrix metallopeptidase 12 (P=0.0068), myotubularin-related protein 14 (MTMR14; P=0.03), p21 (RAC1)-activated kinase 4 (P=0.026), protein

phosphatase 3 catalytic subunit alpha (PPP3CA; P=0.018), suppressor of cytokine signaling 7 (SOCS7; P=0.0084), STAT1 (P=0.015), tropomyosin 3 (P=0.0015), tetraspanin 6 (P=0.036), vascular endothelial growth factor A (P=0.0049) and AT-rich interaction domain 4B (P=0.015), were obtained, among which 4 genes (CTGF, exp(coef)=1.1; MTMR14, exp(coef)=1.42; PPP3CA, exp(coef)=1.31; SOCS7, exp(coef)=1.43) were suggested to be risk factors for prognosis. Therefore, a Kaplan-Meier survival curve was drawn for these 4 genes to reveal their associations with survival.

Table II. Associations between miRNAs and clinical characteristics using data from The Cancer Genome Atlas data.

Clinical characteristics	hsa-miR-17-3p P-value	hsa-miR-145-5p P-value	hsa-miR-98-5p P-value
Age (59.44±11.42)	0.10	0.30	0.26
Radiation therapy (yes/no)	0.90	0.61	6.62×10^{-3}
Neoplasm subdivision (bilateral/left/right)	0.95	0.09	0.30
Stage (II/III/IV)	0.01	0.01	0.39
Lymphatic invasion (yes/no)	0.53	0.65	0.12
Histologic grade (G1-G2/G3-G4)	0.26	0.85	0.88
Recurrence (yes/no)	0.14	0.74	0.09

hsa, *homo sapiens*; miRNA, microRNA. Data are presented as the mean ± standard deviation. The survival package was used for the statistical analysis.
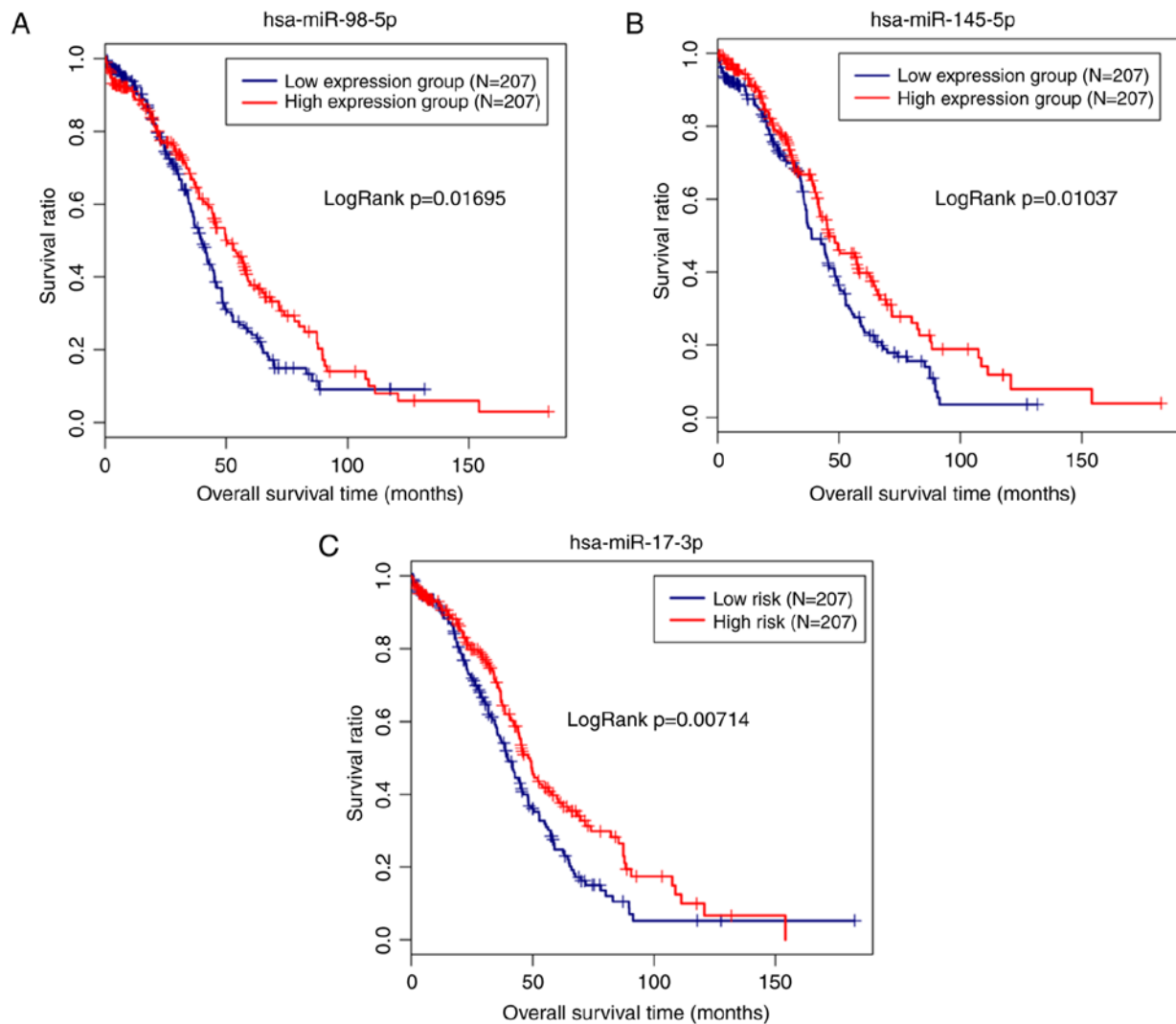

Figure 3. Kaplan-Meier analysis of the associations between miRNAs and overall survival of patients with ovarian cancer. (A) miR-98-5p. (B) miR-145-5p. (C) miR-17-3p. hsa, *homo sapiens*; miRNA, microRNA.

As anticipated, a poor prognosis was observed for patients expressing high levels of these 4 genes, and the difference was significant (Fig. 5). However, only a significant negative correlation was observed between hsa-miR-145-5p and CTGF (r=-0.1126, P=0.02188) using Pearson's correlation analysis (Fig. 6).

Table III. Prognosis-associated clinical characteristics using data from The Cancer Genome Atlas data.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Radiation therapy (yes/no)	0.84	0.53-1.34	0.47	-	-	-
Neoplasm subdivision (bilateral/left/right)	0.99	0.80-1.21	0.89	-	-	-
Lymphatic invasion (yes/no)	1.14	0.70-1.86	0.59	-	-	-
Recurrence (yes/no)	1.73	1.45-2.20	0.21	-	-	-
Age (59.44±11.42)	1.02	1.01-1.03	3.78×10^{-3}	1.02	1.01-1.03	2.49×10^{-3}
Stage (II/III/IV)	1.38	1.04-1.82	0.03	1.36	1.02-1.83	0.04
Histologic grade (G1-G2/G3-G4)	1.39	0.96-2.00	0.04	1.35	0.93-1.95	0.04

HR, hazard ratio; CI, confidence interval. Data are presented as the mean ± standard deviation. The survival package was used for the statistical analysis.
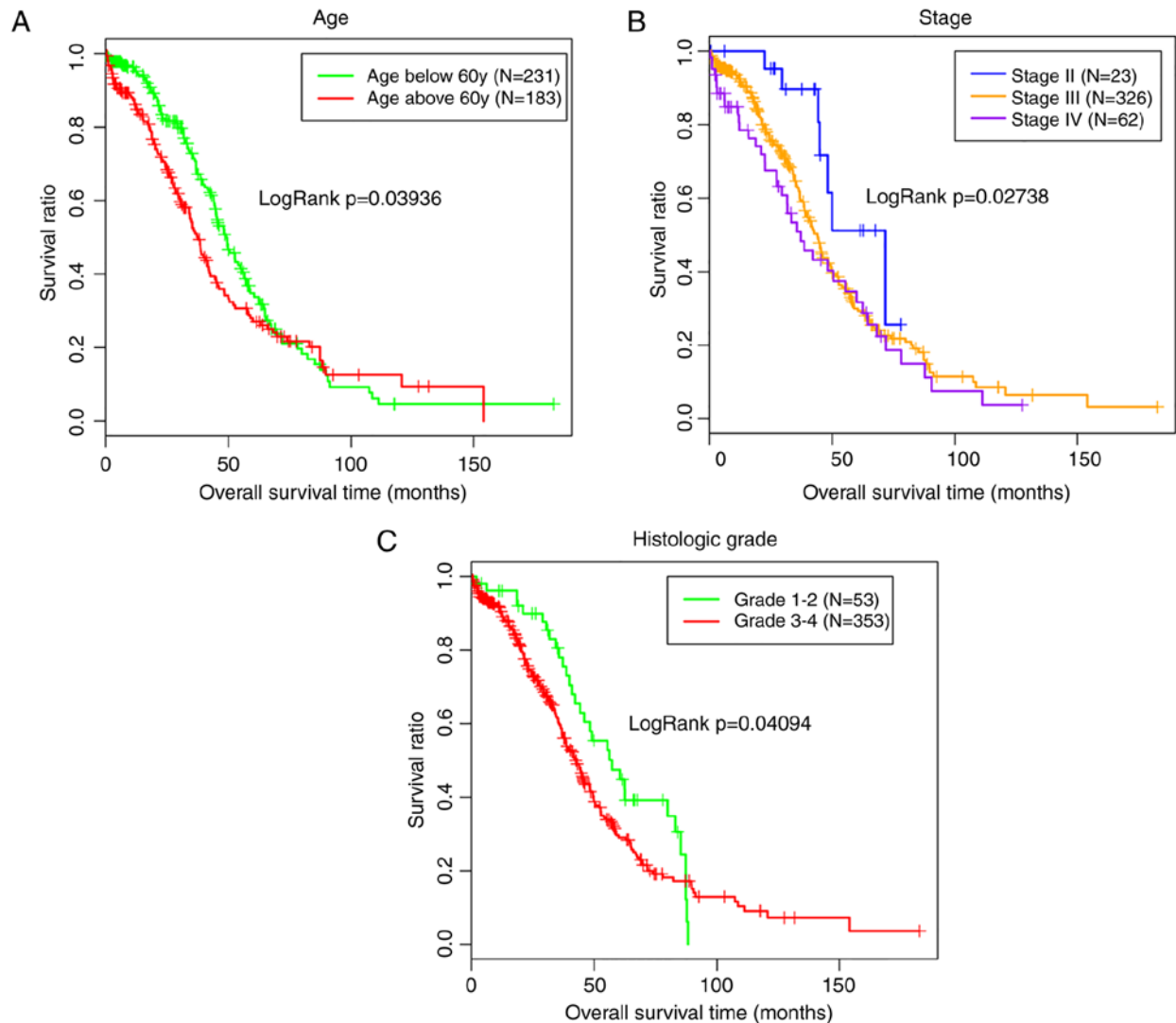

Figure 4. Kaplan-Meier analysis of the associations between the clinical characteristics and survival outcomes of patients with ovarian cancer. (A) Age. (B) Stage. (C) Histological grade.

According to the functional analysis, the target genes of hsa-miR-145-5p participated in OC development by affecting 26 KEGG pathways, 20 of which overlapped with 56 known OC-associated pathways downloaded from the CTD (Table IV). CTGF was identified to be involved in the Hippo signaling pathway (hsa04390).
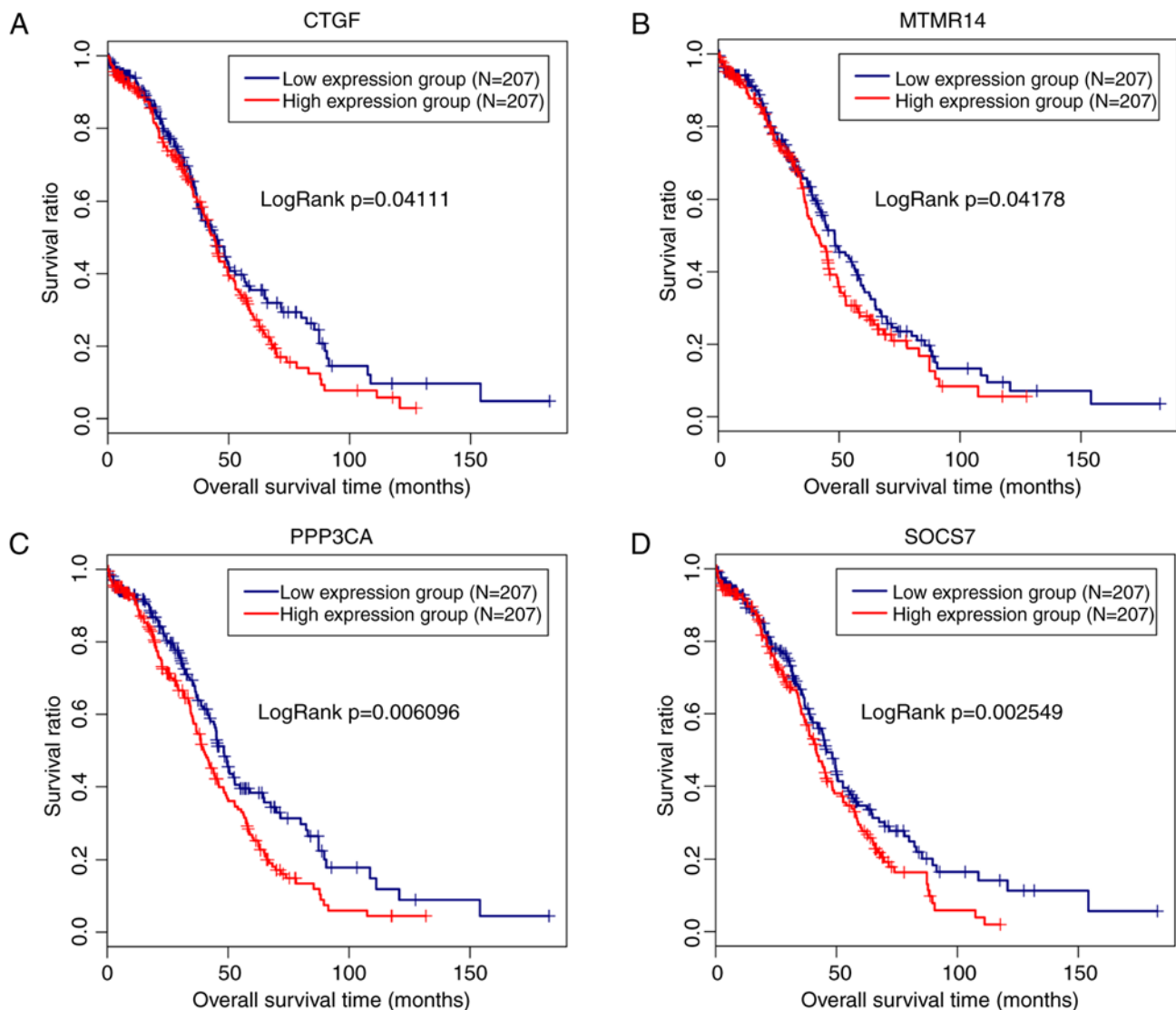
Figure 5. Kaplan-Meier analysis of the associations between the target genes of microR-145-5p and survival outcomes of patients with ovarian cancer. (A) CTGF. (B) MTMR14. (C) PPP3CA. (D) SOCS7. CTGF, connective tissue growth factor; MTMR14, myotubularin-related protein 14; PPP3CA, protein phosphatase 3 catalytic subunit alpha; SOCS7, suppressor of cytokine signaling 7.

Discussion

In the present study, the miRNA expression profiles of OC cells and their exosomes compared with normal ovarian surface epithelial cell lines were examined using a microarray. A total of 22 miRNAs were co-expressed in exosomes and the OC cells from which they were derived. Among these miRNAs, the downregulation of hsa-miR-145-5p and its negatively regulated target gene CTGF were additionally demonstrated to be associated with the prognosis of patients with OC by affecting the Hippo signaling pathway, and therefore they may be potentially important diagnostic biomarkers and therapeutic targets for OC.

Based on extensive evidence, miR-145-5p functions as a tumor suppressor gene in various cancers, including OC. For example, Zhang et al (22) observed the downregulation of miR-145-5p in gastric cancer tissues compared with the adjacent normal tissues. Low expression of miR-145-5p was significantly associated with lymph node metastasis, metastasis stage and

distant metastasis, ultimately leading to poorer overall survival. In the study by Ozen et al (23), overexpression of miR-145-5p inhibited proliferation and decreased the migration of prostate cancer cells. Similarly, miR-145-5p is also an important target for non-small cell lung cancer (NSCLC) as miR-145-5p overexpression suppressed the EMT in NSCLC cells, which is an important biological process associated with cancer migration and metastasis (24). Upon transfection of miR-145-5p, the angiogenesis ability of SW480 colon carcinoma cells was significantly inhibited (25). Concomitantly, the overexpression of miR-145 significantly suppressed the proliferation, migration and invasion of OC cells and inhibited tumor growth and metastasis in vivo (26,27). Furthermore, tumor protein 53 (TP53) upregulated the expression of miR-145 to enhance its tumor suppressor roles (28-30). However, TP53 mutations occur in ~95% of high-grade serous OC (31). Therefore, miR-145 may be specifically downregulated in advanced OC. Consistent with these studies, miR-145 was expressed at lower levels in all 13 OC cell lines and its downregulation was significantly
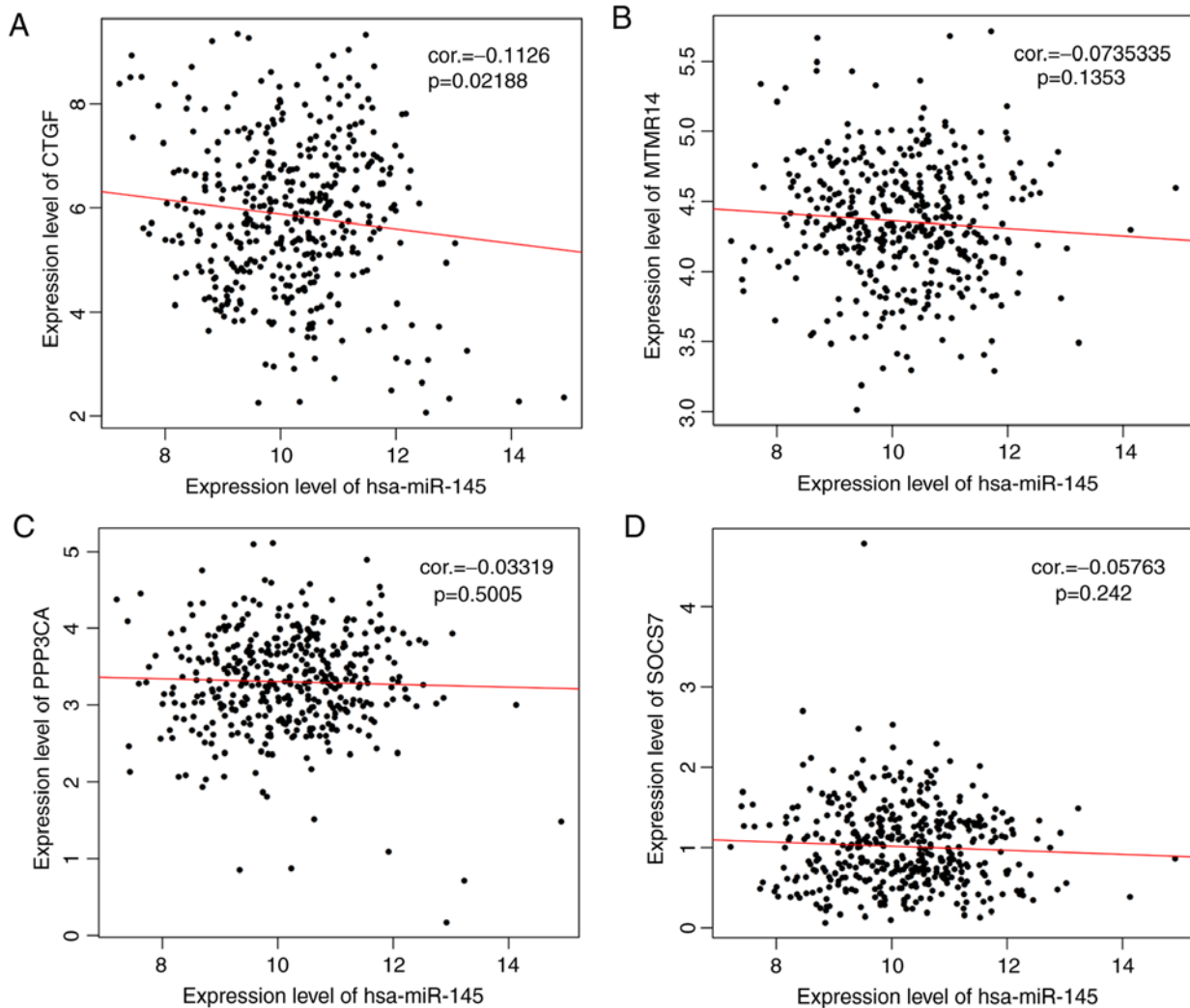
Figure 6. Pearson's correlation analysis of the negative correlations between the target genes and miR-145-5p in patients with ovarian cancer. (A) CTGF. (B) MTMR14. (C) PPP3CA. (D) SOCS7. CTGF, connective tissue growth factor; MTMR14, myotubularin-related protein 14; PPP3CA, protein phosphatase 3 catalytic subunit alpha; SOCS7, suppressor of cytokine signaling 7; hsa, *homo sapiens*; miRNA, microRNA; cor, correlation coefficient.

associated with a shorter survival and higher tumor stage in the present study.

Although miR-145 serves crucial roles in the development of cancer, the majority of studies have focused on the original cells, and the expression of miR-145 in exosomes and its effect on OC remains largely unknown. However, one previous study revealed that the exposure of pancreatic ductal adenocarcinoma to miR-145-5p-enriched tumor-associated stroma exosomes cells decreased cell viability in a dose-dependent manner (32). To the best of our knowledge, the present study is the first to demonstrate the significant downregulation of miR-145-5p expression in OC exosomes, indicating that the enhanced secretion of miR-145 via exosomes may be an underlying approach to treating OC (33,34). In addition, exosomes secreted from primary tumors are transferred and released to the peripheral circulation (35-37). Therefore, the expression of exosomal miRNAs detected in cancer cells is generally similar to their levels in blood. This hypothesis has been verified in the study by Hannafon *et al* (38), in which miR-1246 and miR-21 were first detected at significantly higher levels in exosomes from breast cancer cells and then

validated in patient-derived orthotopic xenograft models, and mouse and human plasma exosomal samples. According to their analysis of the receiver operating characteristic curves, the diagnostic accuracy (area under the curve) of the combination of plasma exosomal miR-1246 and miR-21 levels was 72.66% for breast cancer. Consistent with these data, we hypothesize that the exosomal miR-145-5p identified in OC cells may also be a potential biomarker for the detection of malignant OC.

miRNAs are considered to participate in disease development by regulating target genes through interactions with complementary sequences in the 3'UTR. Several studies have explored the mechanisms of miR-145-5p in cancer. For example, Matsushita *et al* (39) predicted putative binding sites for miR-145-5p in 1,735 genes following a search of the microRNA.org database, and additionally confirmed that ubiquitin-like with PHD and ring finger domains 1 may be an indirect target of miR-145-5p to regulate bladder cancer cell aggressiveness in subsequent dual luciferase reporter assays. Wu *et al* (27) revealed a negative regulatory effect of miR-145 on levels of the ribosomal protein S6 kinase, polypeptide 1

Table IV. Functional enrichment for the target genes of microR-145.

Term	P-value	Genes
hsa05219:Bladder cancer	3.91×10^{-7}	NRAS, CDKN1A, VEGFA, MDM2, CDK4, MYC, MMP1
hsa04550:Signaling pathways regulating pluripotency of stem cells	5.82×10^{-5}	NRAS, IGF1R, NANOG, POU5F1, SOX2, MYC, FZD7, KLF4
hsa05200:Pathways in cancer	8.73×10^{-5}	NRAS, IGF1R, CDKN1A, HDAC2, VEGFA, MDM2, STAT1, CDK4, MYC, FZD7, MMP1, TPM3
hsa04151:PI3K-Akt signaling pathway	1.45×10^{-4}	NRAS, IGF1R, CDKN1A, EIF4E, ITGB8, IFNB1, VEGFA, MDM2, CDK4, IRS1, MYC
hsa05220:Chronic myeloid leukemia	1.57×10^{-4}	NRAS, CDKN1A, HDAC2, MDM2, CDK4, MYC
hsa05205:Proteoglycans in cancer	5.32×10^{-4}	NRAS, IGF1R, CDKN1A, NANOG, VEGFA, MDM2, MYC, FZD7
hsa05161:Hepatitis B	5.72×10^{-4}	NRAS, CDKN1A, IFNB1, TIRAP, STAT1, CDK4, MYC
hsa05214:Glioma	1.19×10^{-3}	NRAS, IGF1R, CDKN1A, MDM2, CDK4
hsa05202:Transcriptional misregulation in cancer	1.24×10^{-3}	IGF1R, ERG, CDKN1A, FLI1, HDAC2, MDM2, MYC
hsa04115:p53 signaling pathway	1.33×10^{-3}	PPM1D, CDKN1A, SERPINE1, MDM2, CDK4
hsa05218:Melanoma	1.65×10^{-3}	NRAS, IGF1R, CDKN1A, MDM2, CDK4
hsa05206:MicroRNAs in cancer	4.11×10^{-3}	NRAS, CDKN1A, IRS2, PAK4, VEGFA, MDM2, IRS1, MYC
hsa04919:Thyroid hormone signaling pathway	9.03×10^{-3}	NRAS, HDAC2, MDM2, STAT1, MYC
hsa04110:Cell cycle	1.20×10^{-2}	CDKN1A, HDAC2, MDM2, CDK4, MYC
hsa04360:Axon guidance	1.31×10^{-2}	NRAS, PAK4, ROBO2, PPP3CA, SRGAP1
hsa05216:Thyroid cancer	1.84×10^{-2}	NRAS, MYC, TPM3
Shsa04390:Hippo signaling pathway	2.32×10^{-2}	CTGF, SOX2, SERPINE1, MYC, FZD7
hsa05215:Prostate cancer	2.54×10^{-2}	NRAS, IGF1R, CDKN1A, MDM2
hsa05166:HTLV-I infection	3.66×10^{-2}	NRAS, CDKN1A, PPP3CA, CDK4, MYC, FZD7
hsa05169:Epstein-Barr virus infection	4.78×10^{-2}	CDKN1A, HDAC2, MDM2, MYC, MAP2K6

hsa, *homo sapiens*; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; p53, tumor protein 53; HTLV-I, human T-lymphotropic virus type 1.

(P70S6K1) and mucin 1, cell surface associated (MUC1) proteins in OC cells. Overexpression of p70S6K1 and MUC1 restored the colony formation and invasion abilities that were inhibited by miR-145. As indicated in the study by Chen *et al* (40), transfection of an miR-145 agomiR (activator) into OC cells downregulated the expression of its direct target tripartite motif containing 2 and subsequently induced apoptosis. However, the mechanisms of miR-145 in OC are not well understood. In the present study, 91 target genes of miR-145 were screened using the miRwalk2.0 database, and the subsequent clinical correlation analysis using TCGA data suggested that the Hippo signaling pathway-associated gene CTGF may be an important target of miR-145, due to its significant association with a poor prognosis and negative correlation with miR-145 expression. The data from the present study appear to be consistent with previous studies, as high CTGF expression promotes the migration and peritoneal adhesion of OC cells, resulting in a poor prognosis; these changes were abrogated by a human monoclonal antibody against CTGF FG-3019 (41). CTGF is a key downstream intermediate in the Hippo-YAP1/TEAD cascade that controls cell growth and initiates OC formation (42-44). CTGF was indicated to be a direct target of miR-145 using a dual luciferase reporter gene assay, and miR-145 negatively regulated CTGF to affect the proliferation, migration and invasion of esophageal squamous cell carcinoma (45) and malignant glioma cells transfected with miR-145 mimics and CTGF small interfering RNA (46). Nevertheless, to the best of our knowledge, studies of this nature in OC have not been described, requiring additional investigation. Notably, we hypothesized that the upregulation of CTGF in OC cells due to the downregulation of miR-145 may be one potential contributor to the decreased sorting of miR-145 into exosomes and subsequent transfer to acceptor cells, including cancer associated fibroblasts, endothelial cells and tumor-associated macrophages, to inhibit the pro-tumor environment (47). Furthermore, CTGF may also be a crucial target of exosomal miR-145 in the tumor environment. Low expression of miR-145 in exosomes was insufficient to block the transcription of CTGF, leading to its overexpression, acceleration of tumor cell invasion and the carcinogenesis of adjacent normal ovarian epithelial cells (48,49).

The present study had certain limitations: Firstly, 13 OC cell lines were used to screen crucial miRNAs in the present study. Due to the presence of underlying differences in phenotype, the expression of miRNAs varied among these cell lines, which lead to unexpected and paradoxical results for specific miRNAs, including miR-98 and miR-17. According to the prognosis analysis, miR-98 and miR-17 should be expressed at lower levels in OC, but in fact they

were upregulated in OC and exosomes. Secondly, the TCGA data did not include a normal control, and potential deviations in the identified correlations between the identified miRNAs and clinical characteristics may have occurred. Thirdly, although the analysis preliminarily revealed a negative correlation between miR-145 and its target gene CTGF in clinical samples, *in vitro* and *in vivo* experiments using OC models are required. Fourthly, the loss of exosomal miR-145 as a potentially important factor contributing to OC pathogenesis was identified, but the mechanism underlying its formation and whether modification of exosomes by transfection with miR-145 decrease oncogenesis by affecting the tumor environment remain unknown (33,34,50). Fifthly, although previous studies have suggested that the levels of exosomal miRNAs in cancer cells are consistent with their levels in the peripheral circulation (35-38), the expression of exosomal miR-145 in plasma samples from patients with OC requires additional verification to confirm its underlying value as a biomarker.

According to the data from the present study, the decreased expression of hsa-miR-145 in OC and OC-derived exosomes may be required for the development of OC via the targeted modulation of a downstream intermediate in the Hippo signaling pathway, CTGF. Therefore, upregulation of hsa-miR-145 in exosomes and cells to inhibit CTGF expression may be a potential therapeutic approach for OC.

Availability of data and materials

The microarray data GSE103708 were downloaded from the GEO database in NCBI (http://www.ncbi.nlm.nih.gov/geo/).

Authors' contributions

WH, QH and XX conceived the design of the original study. WH and YF conducted the statistical analysis and drafted the manuscript. ZS, YY and YZ were involved with the interpretation of the data. QH and XX participated in critical revisions of the manuscript. All authors read and approved the final 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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