MicroRNA profiling of serum exosomes in patients with osteosarcoma by high-throughput sequencing

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ABSTRACT

The microRNA expression profile of plasma exosomes in osteosarcoma needs to be further explored. The present study intends to investigate the practicality of plasma exosomal miRNAs as novel biomarkers of osteosarcoma. In the study, exosome-like vesicles were purified from the plasma of patients with osteosarcoma and healthy control. Differential centrifugation was used. The purified vesicles which ranged from 50 to 100 nm in size were identified as exosomes by transmission electron microscopy and western blot. Validating assays in vitro and in vivo were performed via CCK8, reverse transcription-quantitative PCR, flow cytometry, transwell and wound healing assays and xenograft model. High-throughput sequencing identified that 57 miRNAs, 20 of which were upregulated and 37 downregulated, were differentially expressed in patients with osteosarcoma and healthy control ($p<0.01$; fold change ≥3). In comparison to the controls, the expression levels of miR-92a-3p, miR-130a-3p, miR-195–3 p, miR-335–5 p, let-7i-3p were upregulated in the exosomes from patients with osteosarcoma with statistical significance. Studies in vitro and in vivo have proved that osteosarcoma-secreted exosomes from miR-195–3 p upregulated 143B osteosarcoma cells promote cell proliferation and invasion. Overall, the present study identified exosomal miRNAs with dysregulated expression in patients with osteosarcoma, and they may have potential as targets for the treatment of patients with osteosarcoma.

Significance of this study

What is already known about this subject?

► Osteosarcoma is one of the most common malignant bone tumors, and accounts for 34% of malignant bone tumors. In recent years, the studies on regulation of tumor pathogenesis by exosome have obtained significant achievements. miRNA is the one source of osteosarcoma plasma exosome and could also be used as the new diagnostic biomarker for multiple tumors. The microRNA expression profile of plasma exosomes in osteosarcoma needs to be further explored.

What are the new findings?

► The expression levels of miR-92a-3p, miR-130a-3p, miR-195–3 p, miR-335–5 p, let-7i-3p were upregulated in the exosomes from patients with osteosarcoma with statistical significance. Studies in vitro and in vivo have proved that osteosarcoma-secreted exosomes from miR-195–3 p upregulated 143B osteosarcoma cells promote cell proliferation and invasion.

How might these results change the focus of research or clinical practice?

► Exosomal miRNAs with dysregulated expression in patients with osteosarcoma may have potential as targets for the treatment of patients with osteosarcoma.

INTRODUCTION

Osteosarcoma is a primary malignant tumor of bone characterized by the formation of osteoid tissues or immature bone by neoplastic cells. It accounts for 34% of the malignant bone tumors. Osteosarcoma mostly occurs in people aged between 15 and 30 years old, especially in adolescent patients. The number of male patients is higher than that of females. With the application of neoadjuvant chemotherapy, the prognosis and survival rate of patients with osteosarcoma have been greatly improved. However, the 5-year survival rate of patients diagnosed with osteosarcoma is still lower than 60% due to the high rate of metastasis.[1–3]

Exosome is a small extracellular vesicle (EV; 40–100 nm in diameter) released by multiple active cells on the fusion of multi-vesicular endosome and plasmalemma. It consists of multiple components, such as proteins, dsDNA, RNAs, etc.[4] The exosome membrane contains various types of conservative proteins, including membrane skeleton proteins, metabolism-associated proteins (tubulin, actin, myosin, glyceraldehyde triphosphate phosphate dehydrogenase, etc), proteins involved in membrane transport and membrane fusion, such as annexins, multiple G proteins, ALG-2 interacting proteins (Alix), tumor susceptibility gene 101 proteins (TSG101), etc, and proteins belonging to the tetramolecular crosslinking

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Table 1 Clinical characteristics of patients with osteosarcoma

Characteristic	All patients No. (%)
Total	25
Sex	
Male	16 (64)
Female	9 (36)
Age (years)	
<20	7 (28)
≥20	18 (72)
Tumor size	
<8	23 (92)
≥8	2 (8)
Distant metastasis	
Presence	21 (84)
Absence	4 (16)
Differentiation	
Favorable	12 (48)
Moderate	12 (48)
Poor	1 (4)
Clinical stage	
IA–IIA	5 (20)
IIB–IV	20 (80)

family (CD9, CD81, CD82, CD83 and CD63, etc). In addition, specific proteins derived from initiating cells have also been found in exosomes, such as antigen presentation molecules major histocompatibility complex (MHC)-I and MHC-II.[5] In recent years, scientists have obtained significant achievements in understanding the regulation of exosome in tumor pathogenesis.[6–9] It plays important roles in mediating intercellular information exchange and the functional change of receptor cells by transporting proteins and miRNAs related to multiple signaling pathways.[4] Meanwhile, tumor cells may package antitumor drugs into exosomes, thereby inducing drug tolerance.[10] The detection and analysis of exosomes contribute to the early diagnosis of tumors and the development of guided treatment. Therefore, exosomes and their modified products may be considered as an effective drug carrier for tumor treatment.

miRNAs have been used as novel diagnostic biomarkers in multiple cancers and can be packaged in plasma exosomes secreted by osteosarcoma cells.[11] However, few articles on the functional research of miRNAs are published to date. In the present study, we performed microRNA profiling using the high-throughput sequencing method to analyze the plasma exosome from patients with osteosarcoma and healthy subjects. We also investigated the impact of plasma exosome on osteosarcoma cells to further elucidate the pathogenesis of osteosarcoma.

MATERIALS AND METHODS

Sample collection

Each participant gave a written informed consent. In all, 25 patients with osteosarcoma and 10 healthy subjects were recruited at the Sixth Hospital of Ningbo and the third Affiliated Hospital of Soochow University from January 2011 to December 2018. All patients underwent pathological biopsy to confirm the diagnosis of osteosarcoma and received no chemotherapy, radiotherapy, primary surgical treatment or other curative methods before the study. Patients with any predisposing systemic disorders were excluded. Fasting blood samples were collected using 10 mL ethylene diamine tetraacetic acid (EDTA) tubes from all participants, followed by the centrifugation at 2000 g for 10 min at 4 °C. The supernatants were stored in 750 µl aliquots at −80 °C for further use.

Cell culture

Osteosarcoma cells (U2OS, 143B) and normal osteoblasts were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 5% penicillin/streptomycin in a 5% CO_2 atmosphere at 37°C.

Exosome preparation and size determination

Plasma samples were centrifuged at 1600 g for 20 min at 4 °C and then at 10 000 g for 30 min. The supernatants were collected and processed using a 0.22 µm disposable filter. Then the samples were centrifuged at 1 00 000 g for 2 hours at 4 °C. After washed with 10 mL PBS, the exosomes were resuspended in 0.1 mL PBS and stored at −80 °C for further use. Their diameters were measured by Zetasizer Nano according to the manufacturer's instructions. The prepared exosome samples were observed using an EM-2010 transmission electron microscope (TEM) at 250,000× magnification.

Western blotting

The total proteins were extracted using RIPA lysis buffer containing protease inhibitor cocktail. The protein concentration was measured by bicinchoninic acid (BCA) protein assay kit and 20 µg of the protein was separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to nitrocellulose membranes. After blocking with 5% skimmed milk diluted in PBS-Tween (0.5% Tween-20) for 2 hours at room temperature, the membranes were incubated with the following primary antibodies at 4°C overnight: mouse anti-CD63 (1/1000), rabbit anti-TSG101 (1/1000) or mouse anti-HSP90 (1/1000). After immersing in the anti-mouse secondary antibody (1/1,000) at room temperature for 1 hour, the bands were visualized by X-ray films exposed with Pierce enhanced chemiluminescent visualization reagent.

Isolation of exosomal RNAs and small RNA sequencing

The exosomal RNAs in the plasma samples were isolated using RNeasy Mini Spin kit according to the manufacturer's protocols. The concentration and purity of the RNA extractions were detected by the Agilent 2100 bioanalyzer system. Small RNA sequencing was conducted using the BGISEQ-500 sequencer. Significantly interfered results were excluded. Distinctive adapter sequences or RNA small sequences that showed a perfect match in the human genome database (ftp://ncbi.nlm.nih.gov/genomes/Homo_sapiens) were considered valid for subsequent analysis. Target mRNAs were identified by TargetScan (version 7.1, http://www.targetscan.org/vert_71/) based on the seed sequences. The expression level of miRNAs was analyzed using the DEGseq method.[12]

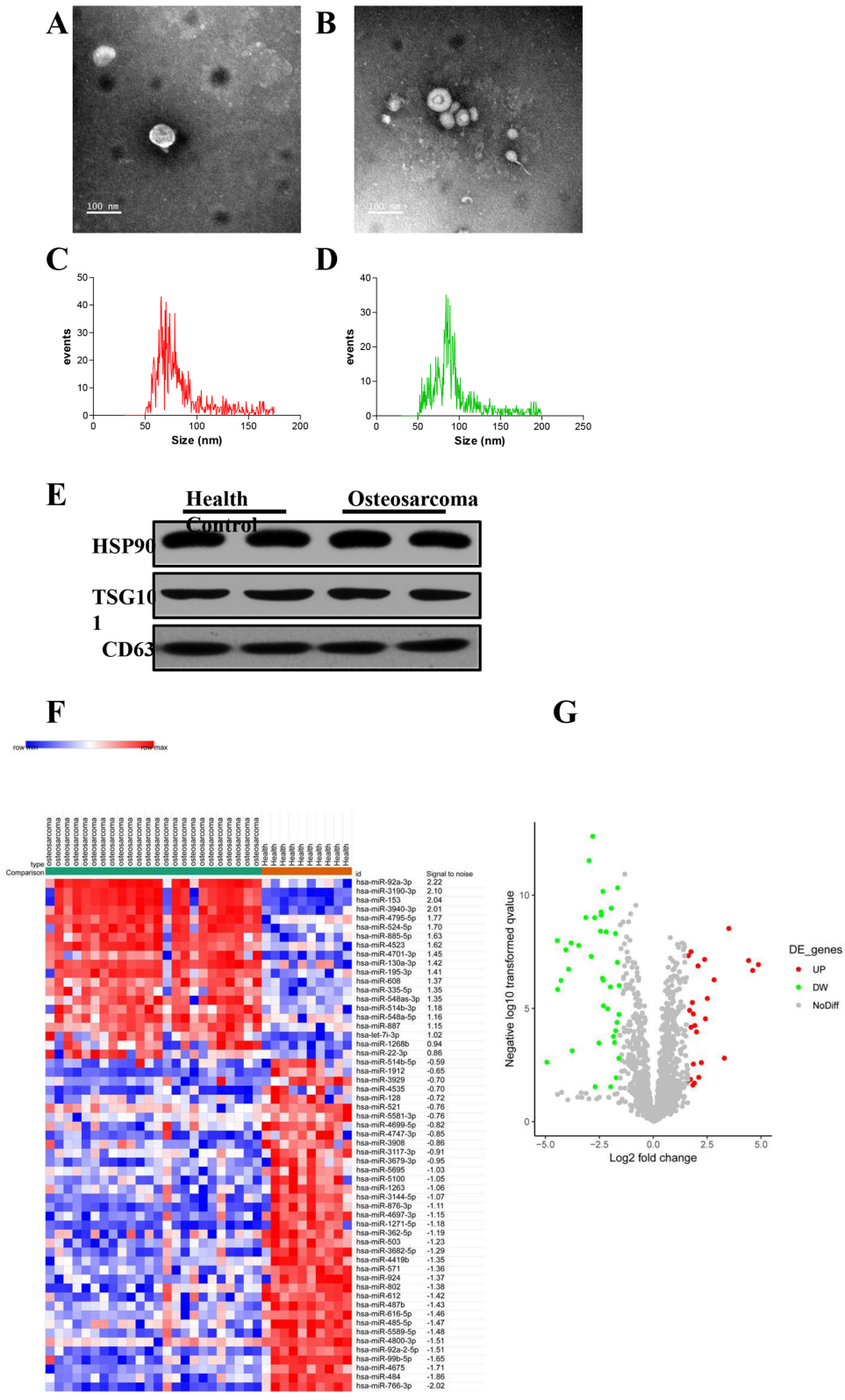

Figure 1 Identification of vesicles similar to exosomes. Diameter range of those exosome-like vesicles from (A) normal controls and (B) patients with osteosarcoma. TEM results of purified exosome-like vesicles from (C) normal controls and (D) patients with osteosarcoma. Scale bar: 100 nm. (E) Western blot of Hsp90, TSG101 and CD63 proteins in the vesicles. Hsp90, heat shock protein 90 KDa; TSG101, tumor susceptibility gene 101; CD63, granulophysin. (F) top differentially expressed miRNAs between were showed as a heat map (>3 fold; p<0.01). (G) Volcano plot. Plotted along the x-axis there were the means of log2 fold-change, and along the y-axis the negative logarithm of p values were plotted. Red spots showed enhanced miRNAs and green spots showed inhibited miRNAs with a >3.0 fold change. TEM, transmission electron microscope; TSG, tumor susceptibility gene

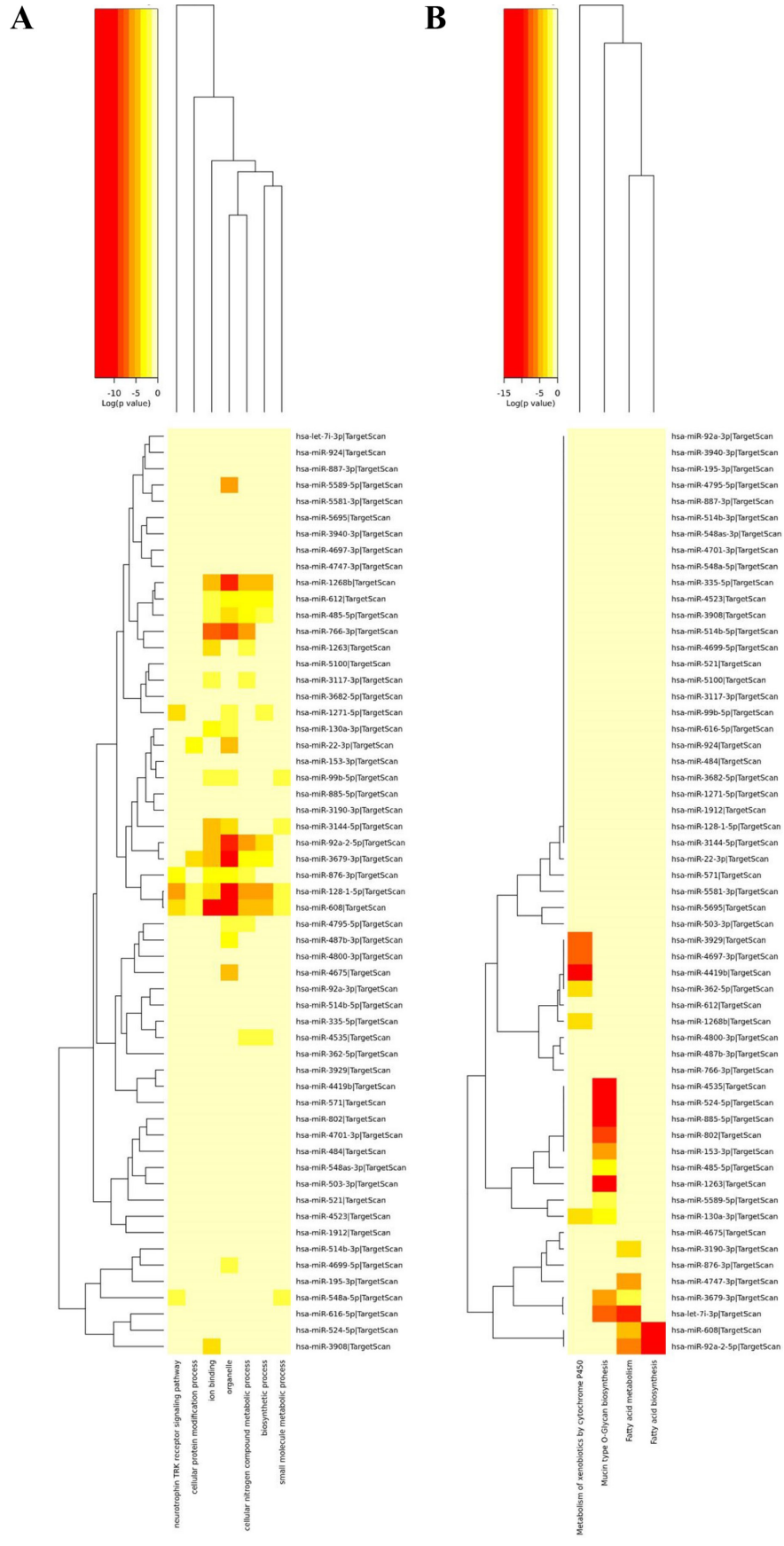

Figure 2 Top gene ontology (A) and Kyoto Encyclopedia of Genes and Genomes pathways (B) influenced by the relevant genes for the miRNAs of interest.

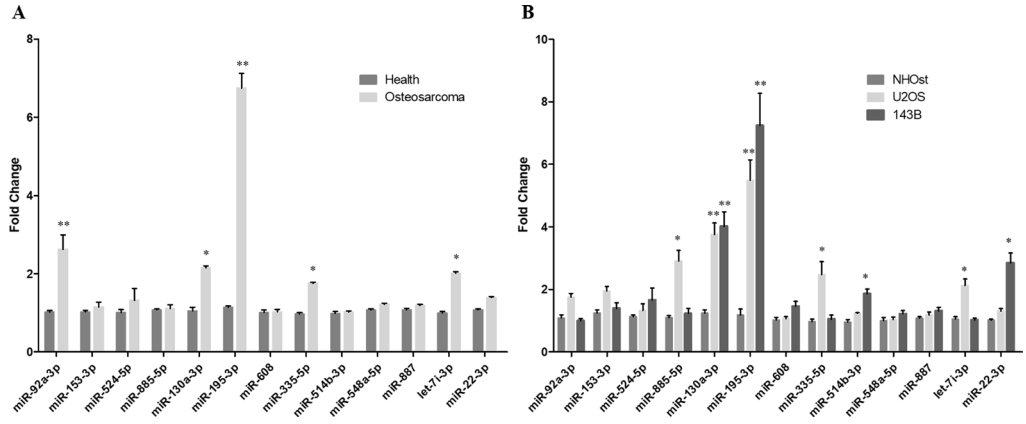

Figure 3 Validation for the differential expression of 13 miRNAs in exosome (A) and osteosarcoma cell lines (B) with reverse trancription-quantitative PCR. The plasma exosomal miRNAs were compared for patients with osteosarcoma (n=25) and normal controls (n=10) with RT-qPCR. *p<0.05 and **p<0.01.

Target gene prediction and pathway analysis

The target genes of the miRNAs of interest were predicted using three databases, TargetScan,[13] miRanda[14] and RNAhybrid.[15] The pathway analysis of the target genes was performed using Gene Ontology (GO) functional annotation[16] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis[17] with DIANA TOOLS (http://snf-515788.vm.okeanos.grnet.gr/).

Plasma RNA extraction

Total RNAs were extracted from plasma samples using miRNeasy Serum/Plasma kit in accordance with the manufacturer's instructions. The total RNAs were suspended in 14 µL nuclease-free water. The RNA concentration was detected using a NanoDrop ND-2000 spectrophotometer.

Reverse transcription-quantitative PCR

Total RNAs were reverse transcribed to cDNA using Qiagen miScript II RT kit in accordance with the instructions of the manufacturer. Quantitative PCR was performed using Qiagen miScript SYBR Green PCR kit following the instructions. The miScript primers used in this experiment are shown in online supplementary table 1. The Cq values were normalized to the synthetic spike as previously described.[18] The relative fold-changes were estimated by the $2-\Delta\Delta Cq$ method.[19]

Cell proliferation assay

Osteosarcoma 143B cells stimulated with osteosarcoma cells-secreted exosomes (143B osteosarcoma cells transfected with miR-195–3 p mimic), health control (HC)-secreted exosomes or normal control (NC) were seeded in 96-well plates. At the time points of 24, 48, and 72 hours, cell proliferation was assessed using the CCK-8 kit (Dojindo, Japan) following the manufacturer's protocols.

Flow cytometry

Annexin V-FITC kit was used for the detection of apoptosis as previously described. In brief, 143B cells treated with osteosarcoma cells-secreted exosomes (143B cells transfected with miR-195–3 p mimic), HC-secreted exosomes or NC were harvested for flow cytometry analysis, and the results were analyzed using CellQuest software (BD Biosciences).

Transwell assay

Cellular mobility was evaluated using transwell chambers purchased from Corning costar (8 µm pore size; Cambridge, USA). In brief, cells were incubated in the upper chamber, and 20% serum as the chemoattractant was added into the lower chamber. After 48 hours of incubation, the filter was fixed and stained using methanol and 0.1% crystal violet. The number of cells was counted under a microscope. This experiment was performed in triplicate.

Wound-healing assay

An equal number of 143B cells stimulated with osteosarcoma cells-secreted exosomes (143B cells transfected with miR-195–3 p mimic), HC-secreted exosomes and NC were seeded into six-well plates. A straight scratch was made on cell monolayer with a pipette tip. Cell migration was observed under a microscope at designated time points.

Animal studies

All animal experiments were supervised and authorized by the Animal Care and Use Committees of The Sixth Hospital of Ningbo, and performed in compliance with the National Institutes of Health principles of laboratory animal care. The BALB/c-nude mice (4-week to 5-week old) were obtained from SLAC (Shanghai, China). Tumor growth was evaluated every 5 days. The volume-based tumor growth curve was drawn. One month later, all mice were executed and their tumor tissues were collected and weighed.

To establish the abdominal metastasis model, mouse spleen tissues were exteriorized under anesthesia. Approximately 1×10^6 cells transfected with 143B-Luc-vector, 143B-Luc-vector treated with miR-195–3 p-mimic-143B exosomes or HC exosomes were injected into the spleen parenchyma. Then the spleen was repositioned into the peritoneal cavity. Three weeks later, bioluminescence images were taken by the Interactive Video Information System.

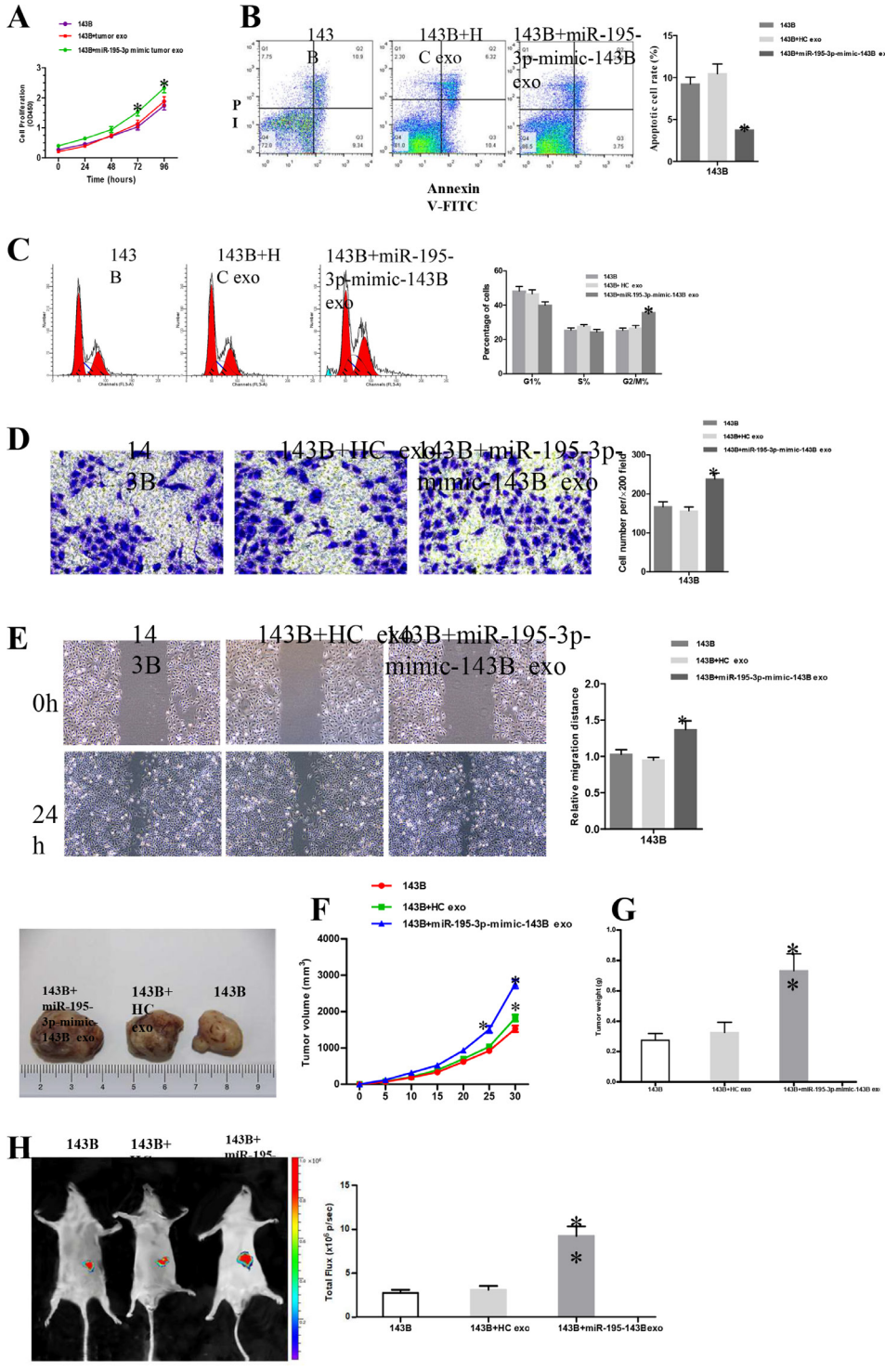

Figure 4 143B cells underwent treatment with NC, miR-195–3 p-mimic-143B exosomes and HC exosomes. CCK8 (A) assay was for measuring cell proliferation. *p<0.05. (B) Cell apoptosis rate was measured by annexin V assay. treating with miR-195–3 p-mimic-143B exosomes promoted apoptosis of osteosarcoma cells. *p<0.05. (C) The phase of the cell cycle was investigated by FACS analysis. The ratio of cells that treated with miR-195–3 p-mimic-143B exosomes in G2/M phase was declined. *p<0.05. n=3 independent experiments. The patterns selected from trans-well assay (D) and wound-healing assay (E) of 143B that treated with NC, miR-195–3 p-mimic-143B exosomes and HC exosomes were listed. Treating with miR-195–3 p-mimic-143B exosomes enhanced the invasion ability of osteosarcoma cells. *p<0.05. 143B cells treated with NC, miR-195–3 p-mimic-143B exosomes and HC exosomes were applied onto the nude mice with subcutaneous injection. After 4 weeks, the execution was conducted, and the tumor tissue was taken out. The features of the tumors were recorded such as the size (F) and weight (G). *p<0.05, **p<0.01. (H) Luciferase-expressed 143 cells with different treatments that placed in the spleen parenchyma were imaged by IVIS bioluminescence imaging system. **p<0.01. IVIS, interactive video information system; FACS, fluorescent-activated cell sorting.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The comparison between two groups was evaluated using unpaired one-tailed t-test. $p<0.05$ was considered statistically significant.

RESULT

Clinical characteristics

The clinical characteristics of 25 patients with osteosarcoma are summarized in table 1. All patients showed positive histopathological results and did not receive any chemotherapy, radiotherapy or radical surgery. In all, 10 healthy subjects were recruited as the control group. The age of the patients was comparable between the two groups with no intergroup significance ($p>0.05$).

Identification of plasma exosomes from patients with osteosarcoma

The diameter of the EVs from the two groups ranged between 50 and 100 nm (figure 1A,B). TEM image analysis showed that the EVs were spherical vesicles with a diameter range similar to that of exosomes (figure 1C,D). Moreover, three previously identified exosome markers, HSP90, TSG101 and CD63 (23), were observed in the EVs from both patients with osteosarcoma and healthy subjects (figure 1E).

Differentially expressed miRNAs in plasma exosomes

The expressions of 57 miRNAs in the exosomes from patients with osteosarcoma and the control group were compared and the p values were shown. The cut-off value was set at threefold change and $p<0.01$. Among all tested miRNAs, 20 exhibited upregulated expression and 37 showed downregulated expression (online supplementary table 2) in the exosomes obtained from patients with osteosarcoma in comparison to the control group. In all, 10 miRNAs showed the highest upregulation of expression: hsa-miR-3190–3 p, hsa-miR-153, hsa-miR-3940–3 p, hsa-miR-92a-3p, hsa-let-7i-3p, hsa-miR-524–5 p, hsa-miR-195–3 p, hsa-miR-608, hsa-miR-4795–5 p and hsa-miR-1268b. The heat map and volcano plot indicated the expression change of each miRNA (figure 1F&G).

Enriched biological processes and molecular functions of miRNA targeted genes

GO analysis on the target genes of these miRNAs suggested that most of them might be involved in the neurotrophin TRK receptor signaling pathway by regulating the modification of cellular proteins and organelle, the metabolic and ion binding processes of cellular nitrogen compounds, the synthesis of biological substances and the metabolic process of small molecules. The investigation on KEGG pathway revealed that most of the relevant genes were primarily involved in cytochrome P450-mediated xenobiotic metabolism, Mucin O-Glycan biosynthesis, and fatty acids metabolism and biosynthesis (figure 2).

Validation of differentially expressed miRNAs by RT-qPCR

The relative expression levels of miR-92a-3p, miR-153–3 p, miR-524–5 p, miR-885–5 p, miR-130a-3p, miR-195–3 p, miR-608, miR-335–5 p, miR-514b-3p, miR-548a-5p, miR-887, let-7i-3p, miR-22–3 p were investigated. In comparison to the controls, the expression levels of miR-92a-3p, miR-130a-3p, miR-195–3 p, miR-335–5 p, let-7i-3p were significantly upregulated in the exosomes from patients with osteosarcoma (figure 3A). However, only miR-130a-3p and miR-195–3 p showed elevated expression levels in both osteosarcoma cell lines (figure 3B).

EXOSOMES SECRETED FROM MIR-195-3P-OVEREXPRESSING OSTEOSARCOMA CELLS PROMOTED CELL PROLIFERATION AND INVASION

To verify the carcinogenic role of miR-195–3 p in osteosarcoma cells-secreted exosomes, the gain-of-function assay was performed. Osteosarcoma 143B cells were treated with miR-195–3 p-mimic-143B exosomes, HC exosomes or NC. Cells in the group of miR-195–3 p-mimic-143B exosomes showed increased proliferation (figure 4A), inhibited apoptosis (figure 4B) and induced G2/M cycle arrest (figure 4C) compared with the other two groups. Data obtained from the wound-healing assay showed that the treatment with miR-195–3 p-mimic-143B exosomes significantly enhanced the invasive capacity of osteosarcoma cells ($p<0.05$) (figure 4D,E).

Animal study

Osteosarcoma 143B cells treated with miR-195–3 p-mimic-143B exosomes, HC exosomes or NC were subcutaneously injected in BALB/c-nude mice. The transplanted tumor derived from miR-195–3 p-mimic-143B exosomes-treated osteosarcoma cells showed significantly larger volume and heavier weight as compared with other two groups ($p<0.05$) (figure 4F,G). In the abdominal metastasis model, the invasive capability of 143B cells treated with miR-195–3 p-mimic-143B exosomes was significantly enhanced in comparison to the other groups ($p<0.05$) (figure 4H).

DISCUSSION

Previous studies have shown that tumor cells secrete exosomes to regulate tumor microenvironment, thereby promoting tumor growth, angiogenesis and metastasis.[20 21] Garimella *et al*[22] constructed a bioluminescent osteosarcoma orthotopic mouse model to detect exosomes in osteosarcoma microenvironment using a TEM. They found that the vesicle globule contained multiple contents, such as matrix metalloproteinases (MMPs), that play potential regulatory roles in tumor microenvironment. MMPs have been identified as prognostic markers for osteosarcoma. Evidence showed that MMP-1 and MMP-13 were responsible for the reconstruction of extracellular matrix. It has been reported that RANKL in osteosarcoma-derived exosomes is involved in the activation of MMPs and the stimulation of osteoclasts. In addition, TGF-β in the exosomes of osteosarcoma cells was proved to regulate chemotactic factor ligand 16 (CXCL16) through regulating the metastasis of osteoblasts precursor cells and osteosarcoma cells.[23] Meanwhile, TGF-β induced the differentiation of monocytes and the accumulation of immature bone marrow cells (myeloid-derived suppressor cells), which promoted bone resorption of osteoclast in tumor microenvironment.[24] Transmembrane 4 superfamily protein CD-9 is a specific biomarker of

exosomes in ExoCarta database and can also be detected in the tumor microenvironment of human 143B osteosarcoma cells. Thus, the inhibition of exosome production may be an effective way to regulate osteosarcoma microenvironment and block the progression of osteoclasia.

Exosomes also participate in the regulation of some signaling pathways in osteosarcoma, such as Wnt and TGF-β pathways. Kansara *et al*[25] found that the knockdown of Wif1 (Wnt inhibitor 1) improved the incidence of osteosarcoma in laboratory mice, implying that the activation of Wnt signaling pathway might promote osteosarcoma occurrence. However, by screening the full-gene profiles of osteoblastoma samples, mesenchymal stem cells and osteogenic cells. Cleton-Jansen *et al* observed reduced Wnt signaling during the formation of osteosarcoma.[26] Korkut *et al*[27] reported the intercellular transportation of Wnt and Wnt gap-associated proteins via exosome vesicles. This study also found that the overexpression of tetramolecular crosslinking family members C82 and CD9 stimulated exosome secretion and the discharge of β-catenin, thereby significantly suppressing the Wnt signaling pathway.[28] Moreover, exosomes upregulated the expressions of inflammatory factors in macrophages and induced the activation of the Wnt5A-Ca^{2+} signaling pathway to promote the release of inflammatory factors. Li *et al*[29] demonstrated that TGF-β1 suppressed the level of miR-143 to increase the expression of proteoglycan, thereby enhancing the metastasis of osteosarcoma. TGF-β1 is highly expressed in osteosarcoma tissues, and its expression is positively correlated with the poor prognosis. Tumor cells can activate downstream effector modules (ie, ELK1) and enhance self-induced amplification by expressing TGF-β, the so-called 'autocrine loop' mechanism. In addition, Clayton *et al*[30] found that TGF-β1 in exosomes secreted from tumor cells significantly decreased NKG 2D expression on the surface of $CD8^+$ T cells and natural killer (NK) cells, resulting in a suppressed activation of lymphocytes and immunological recognition of tumor cells.

In this study, the diameter of the vesicles in the two groups was 50–100 nm. These EVs were abundant with TSG101, CD63 and HSP90, the classical markers of exosomes,[31] suggesting that they might be exosome-like EVs. Xu *et al* demonstrated that miR-124, miR1331, miR-199a-3p, miR-9, and the source of exosomes might be related to the chemosensitivity of patients with osteosarcoma.[32] Also, the plasma exosomes from patients with osteosarcoma exhibited highly expressed levels of miR-135b, miR-148a, miR-27a and miR-9, which were enriched in proteoglycans in cancer. They were also found to be involved in hippo signaling pathway, transcriptional dysregulation pathway, PI3K-Akt signaling pathway, Ras signaling pathway, ubiquitin-mediated proteolysis and choline metabolism in cancer.[33–35] Recent studies indicated that miR-675 in the plasma exosomes of metastatic osteosarcoma promoted the migration and invasion of tumor cells via Calneuron 1 and might be used as an important biomarker for osteosarcoma. Here, we identified 57 abnormally expressed miRNAs in the plasma exosomes of patients with osteosarcoma using high-throughput sequencing, with 20 of them upregulated and 37 downregulated. It has been shown that the target genes of these miRNAs mainly participate in the neurotrophin TRK receptor signaling pathway, the modification of cellular proteins and organelle, the metabolic and ion binding processes of cellular nitrogen compounds, the synthesis of biological substances, the metabolic process of small molecules, cytochrome P450-mediated metabolism of xenobiotics, Mucin O-Glycan biosynthesis, and the pathways related to fatty acid metabolism and biosynthesis. Notably, the differentially expressed miRNA in this study showed no overlaps with previous reports, suggesting that a high heterogeneity may exist in patients with osteosarcoma. Based on limited samples, different conclusions might be drawn. Further investigations using validation assays both in vitro and in vivo will be needed. Next, to further explore the co-carcinogenic factors in the exosomes of osteosarcoma, the aberrantly upregulated miRNAs were chosen for subsequent experiments. Quantitative RT-PCR analysis demonstrated that 13 miRNAs were significantly upregulated in osteosarcoma, in which miR-195–3 p showed the highest fold-change. It has been shown that miR-195–3 p acted as a target for CCL4 in oral squamous cell carcinoma and suppressed lymphangion genesis.[36] In our study, miR-195–3 p appeared to be a phenotype during the progression of cancer and the overexpression of miR-195–3 p was observed in osteosarcoma 143B cells. Furthermore, 143B cells stimulated by exosomes secreted from miR-195–3 p-transfected osteosarcoma cells showed enhanced proliferation and invasive capacities compared with the ones treated by normal exosome. These data were further verified in a plant tumor model. Our results suggested that the miRNAs derived from plasma exosomes of osteosarcoma cells could be used as novel diagnostic biomarkers and therapeutic options in the treatment of osteosarcoma.

This research has some limitations including small sample size and a limited number of tested miRNAs. The biological roles of miR-130a-3p, miR-195–3 p, miR-335–5 p and let-7i-3p in osteosarcoma could be further investigated. The effects and mechanisms of the downregulated miRNAs in osteosarcoma need to be explored. Multicenter studies would be necessary for a better generalization of the results.

To sum up, the miRNA profile in plasma exosomes can be greatly influenced by tumor microenvironment, which may provide us diagnostic and therapeutic evidence. It is of significant importance to further investigate the role of miRNAs in exosomes derived from osteosarcoma cells.

Contributors ZY and ZJ wrote the manuscript and prepared figures, LP edited the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval All experiments in this study were supervised and authorized by the Ethics Committees for Clinical Research on Animal and Human of The Sixth Hospital of Ningbo and conducted in conformity with the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. ANY conditions reuse is NOT permitted.

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