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Effects of bone marrow mesenchymal stem cell-conditioned medium on the proliferation and migration of liposarcoma cells

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Abstract

Introduction. Liposarcoma constitutes a prevalent subtype of soft tissue sarcoma, represents approximately 20% of all sarcomas. However, conventional chemotherapeutic agents have shown restricted effectiveness in treating liposarcoma patients. Accumulating evidence indicates that mesenchymal stem cells (MSCs) have the characteristic of migration to tumor site, promote or suppress tumors. How human bone marrow mesenchymal stem cells (BMSCs) contribute to liposarcoma phenotype remains poorly understood. This study aims to investigate the effects of human bone marrow mesenchymal stem cell-conditioned medium (BMSC-CM) on the proliferation and migration of liposarcoma cell lines 93T449 and SW872, as well as explore potential underlying mechanisms of BMSC-CM action on these cells.

Materials and methods. We transfected BMSCs with lentiviral constructs to knock down the transcriptional co-activator Yes-associated protein 1 (YAP1), conditioned medium (CM) obtained from BMSCs and shYAP1-BMSC, respectively. Liposarcoma cell lines 93T449 and SW872 were co-cultured with BMSC-CM or shYAP1-BMSC-CM. Cell proliferation ability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cell apoptosis was evaluated using flow cytometric assay. A wound healing assay was used to analyze cell migration. The expression levels of YAP1, Bcl-2, and matrix metalloproteinase-2 (MMP-2) were determined by western blot assay.

Results. Co-culturing liposarcoma cell lines 93T449 and SW872 with BMSC-CM promoted tumor cell proliferation, while shYAP1-BMSC-CM significantly inhibited cell viability and migration, induced apoptosis, and downregulated Bcl-2 and MMP-2 expression.

Conclusions. These findings provide new insights into the impact of BMSC-CM on liposarcoma and suggest its possible involvement in liposarcoma cell growth. *(Folia Histochemica et Cytobiologica 2024, Vol. 62, No. 1, 50–60)*

Keywords: human BMSC; conditioned medium; liposarcoma; proliferation; migration; YAP1 silencing

Introduction

Liposarcoma is a malignant tumor originating from primitive mesenchymal cells and represents one of the most common soft tissue sarcomas in adults, accounting for about 20% of all sarcoma cases. Liposarcoma is classified into four pathological subtypes: atypical lipomatous tumor/well-differentiated, dedifferentiated, myxoid, and pleomorphic liposarcoma [1]. These subtypes vary in their degree of malignancy and clinical characteristics, posing challenges to the understanding and treatment of liposarcoma. The five-year survival rate for patients with high-grade liposarcoma is only 25% [2]. Complete surgical resection is challenging,

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especially for patients with retroperitoneal liposarcoma, and is often associated with a high postoperative recurrence rate. There is still a lack of clear evidence whether radiotherapy can improve the prognosis of patients with liposarcoma [3–5]. Doxorubicin and ifosfamide continue to be the first-line cytotoxic chemotherapy drugs treatment options for patients with metastatic or advanced liposarcoma. However, the response rates to these therapies remain a subject of controversy [6, 7]. Eribulin and trabectedin are both marine-derived anticancer agents used in the treatment of advanced liposarcoma. Indeed, current evidence has not established a significant improvement for patients when they are used as second-line chemotherapy [8, 9]. Various therapeutic targets have been identified, and clinical trials are being designed based on the specific genetic features of different liposarcoma types. These targets include CDK4/CDK6 inhibitors, MDM2 antagonists, the pan-tyrosine kinase inhibitor pazopanib, immune checkpoint inhibitors such as pembrolizumab, and the exportin 1 (XPO1) inhibitor selinexor [10–14].

Mesenchymal stem cells (MSCs) exhibit strong tropism, being recruited to the tumor microenvironment in response to multiple growth factors and cytokines secreted from tumors as well as surrounding tissues [15]. MSCs constitute a portion of the tumor stroma and exert regulatory effects on tumor progression, either promoting or inhibiting processes such as cell proliferation, angiogenesis, invasion, metastasis, epithelial-mesenchymal transition (EMT), metabolism, and drug resistance [16]. So far, there are relatively few reports regarding the role of MSCs in liposarcoma. Human bone marrow mesenchymal stem cells (BMSCs) harboring oncogenic gene mutations, which express the FUS-CHOP fusion protein, have the ability to undergo transformation into liposarcoma [17]. In pre-transformed BMSCs with oncogenes hits, overexpression of MDM2 and CDK4 can induce liposarcoma-like changes and promote cell proliferation and metastasis [18]. Utilizing the intrinsic migratory properties of MSCs towards tumor sites, engineered MSCs co-expressing dodecameric tumor necrosis factor receptor apoptosis-inducing ligand (TRAIL) and herpes simplex virus thymidine kinase were able to enhance ganciclovir-induced inhibition of tumor cell growth, reducing recurrence and metastasis [19]. These studies suggest a close relationship between BMSCs and liposarcoma.

Yes-associated protein 1 (YAP1) is one of the two main downstream effectors of the Hippo signaling pathway, an evolutionarily conserved pathway that regulates organ size by controlling cell proliferation, apoptosis, and stem cell self-renewal. When the Hippo pathway is activated, YAP1 and transcriptional coactivator with PDZ-binding motif (TAZ) will be sequestered in the cytoplasm and undergo ubiquitination and degradation. Conversely, when the Hippo pathway is inhibited, YAP1/TAZ can translocate into the nucleus and interact with transcriptional enhanced associate domain (TEAD) 1–4 and other transcription factors to exert its transcriptional co-activator function [20]. Overexpression and activation of YAP1 has been implicated in various tumors, including liposarcoma, osteosarcoma, hepatocellular carcinoma, lung and prostate cancer, serving as a crucial regulatory factor in tumorigenesis and mostly considered as an oncogene [21–26]. Furthermore, aberrant hyperexpression of YAP1 is associated with progression and poor prognosis in patients with clear cell renal cell carcinoma, breast and ovarian cancer, esophageal squamous cell and oral squamous cell carcinoma [27–31]. Although YAP1 overexpression or activation has been proven to promote tumor progression, accumulating evidence shows that YAP1 also exerts tumor-suppressive functions in a context-dependent manner. Overexpression of YAP1 suppresses colorectal cancer tumor growth and metastasis, while deletion of YAP1 promotes tumor growth [32]. In breast cancer, YAP1 protein expression is decreased or lost, and inhibition of YAP1 promotes tumorigenesis *in vitro* and *in vivo*, highlighting its role as a tumor suppressor [33]. Another study indicated that high levels of YAP1 correlate with improved survival in estrogen receptor-positive (ER+) breast cancer patients, as YAP1 inhibits ERα-positive breast cancer cell growth [34]. The tumor suppressor function of YAP1 in androgen receptor (AR)-positive prostate cancer (PCa) has been demonstrated, showing that YAP1 impedes AR+ PCa growth by disrupting the AR-TEAD interaction and preventing TEAD from promoting AR signaling [35]. YAP1 suppresses lung squamous cell carcinoma *in vitro* and *in vivo via* the accumulation of reactive oxygen species (ROS) [36]. YAP1 expression is strongly negatively correlated with neuroendocrine markers in Merkel cell carcinoma patient tumor samples as well as in established and patient-derived cell lines. Expression of YAP1 suppresses tumor cell growth and cell-cycle progression [37]. Similarly, reports support a role for YAP1 as a tumor suppressor gene in hematological cancers [38, 39]. Moreover, YAP1 plays a significant role as a hub of pathway transducer in the tumor microenvironment, function within tumor cells to orchestrate responses in stromal cells [40]. However, it remains unclear whether BMSCs are involved in the regulation of liposarcoma through YAP1 signaling. Therefore, in the present study, we conducted a cell viability assay to investigate the effects of BMSCs on liposarcoma. We also evaluated the impact of YAP1 knockdown in

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BMSCs on the proliferation and motility of liposarcoma cells. In addition, we assessed a potential mechanism for YAP1 inhibition suppresses cell proliferation.

Materials and methods

Cell lines and cell culture. Human liposarcoma cell lines 93T449 and SW872 (ATCC, Manassas, VA, USA) were maintained in DMEM (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), penicillin (100 mg/mL), and streptomycin (100 mg/mL; Invitrogen, Grand Island, NY, USA). Cell lines were short tandem repeat authenticated within 6 months of use. Human bone marrow Mesenchymal Stem Cells (HUXMA-01001, Cyagen Biosciences, Guangzhou, China) were cultured in OriCellTM Human Mesenchymal Stem Cell Growth Medium (HUXMX-90011, Cyagen Biosciences). BMSCs were maintained following the manufacturer's instructions. All experiments were conducted using BMSCs cells from passages 3 to 8. All cells were maintained at 37°C in a humidified incubator containing 5% CO_2 and 95% air atmosphere.

Preparation of BMSC-CM. For the preparation of BMSC--conditioned media, we grew BMSCs to 70–80% confluence in a T-175 flask containing their growth medium. The culture medium was removed, and the cells were gently washed three times with phosphate-buffered saline (PBS) to eliminate residual serum. The cells were then incubated for 24 h in serum-free medium (DMEM supplemented with 1% penicillin/streptomycin). The conditioned medium was collected using a sterile pipette and centrifuged at 1,500 rpm for 5 min. The supernatant (CM) was filtered through a 0.22 μm pore filter and stored at –80°C for subsequent experiments.

Lentiviral transfection. BMSCs were stably transfected with lentiviral vectors expressing YAP1 shRNA to achieve YAP1 knockdown. The pLVX-shRNA2-Puro vector (Viraltherapy Technologies, Wuhan, China) harboring a specific shRNA sequence targeting the YAP1 coding region (NM_001130145.3: 5'-GTGGATGAGATGGATACAGGT-3') was used. Lentiviral particle packaging followed the manufacturer's instructions. Briefly, BMSCs were seeded into a 6-well plate at 1×10^5 cells *per* well and incubated with lentivirus particles engineered expressin g YAP1 shRNA for 24 h at 37°C in a humidified incubator with 5% CO_2. The empty vector was used as the negative control. The media containing lentiviral particles was replaced with fresh media and incubated for a further 48 h. Stably transfected cells were selected by the addition of fresh medium containing 2 μg/mL puromycin, a concentration lethal to most lentivirus-untransduced cells. The medium was changed every 2 days until stable colonies were established. The efficiency of YAP1 knockdown in BMSCs was observed using an inverted fluorescence microscope. The knockdown efficiency vas validated by performing quantitative real-time PCR (RT-qPCR) and western blot analysis to measure YAP1 gene mRNA expression and protein levels, respectively.

Quantitative Real-time PCR. To analyze YAP1 mRNA expression, RT-qPCR was performed. Total RNA was isolated using TRIzol reagent (Invitrogen) following the manufacturer's instructions. The RNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Full-length cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific,). The reaction mix contained 2 μL cDNA sample, 10 μL SYBR Green PCR Master Mix, 0.6 μL forward and reverse primers, and 7.4 μL of H_2O. The primer sequences were provided as following: YAP1 forward, 5′-TGACCCTCGTTTTGCCATGA-3 and reverse, 5′-GCCTCTCCTTCTCCATCTGC-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward, 5′-TTCCTTCCTGGGCATGGAGTC-3′ and reverse, 5′-TCTTCATTGTGCTGGGTGCC-3′. RT-qPCR was performed by the ABI PRISM® 7500 FAST Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The reaction conditions were as follows: pre-denaturation at 95°C for 2 min, denaturation at 95°C for 15 s, annealing at 58°C for 20 s, and extension at 72°C for 30 s. A total of 40 cycles were performed. After completing the PCR cycles, a final extension was performed at 95°C for 10 s, followed by a hold at 4°C. The PCR-amplified mRNA was quantified and the results were normalized to the GAPDH mRNA level. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative mRNA expression level. Each experiment was performed in triplicate.

Western blot. Protein lysates of the cells were extracted with 1× RIPA lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA) supplemented with complete protease inhibitor cocktail tablets (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. The protein concentration was determined using a BCA protein assay kit (Thermo Scientific). Protein samples were mixed with 4 × loading buffer and denatured at 95°C for 5 min. The denatured proteins were separated by SDS-PAGE polyacrylamide gels and transferred onto a PVDF membrane. The membranes were blocked in 5% nonfat milk for 1 h at room temperature. and incubated with specific primary antibodies YAP1 (13584-1-AP, Proteintech, Wuhan, China, dilution 1:1000), Bcl-2 (sc-7382, Santa Cruz Biotechnology, dilution 1:500), MMP-2 (sc-13595, Santa Cruz Biotechnology, dilution 1:500), and GAPDH (sc-47724, Santa Cruz Biotechnology, dilution 1:1000) overnight at 4°C with gentle shaking. The membranes were further probed with respective secondary antibodies (Santa Cruz Biotechnology). The bands were visualized using an enhanced chemiluminescence (ECL) substrate and imaging system (Thermo Scientific). Band intensity was quantified using Image J software (National Institutes of Health, Bethesda, MD, USA). The western blot assays were performed in duplicates.

Cell proliferation assay. Cell proliferation ability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich). In brief, liposarcoma cells were seeded in 3×10^3 per well in a 96-well plate with complete growth medium without antibiotics in triplicate and treated with

BMSC-CM or shYAP1-BMSC-CM. Untreated cells served as the control. After the indicated time periods of incubation, 10 μL of MTT solution was added to each well and the cells were cultured for 4 h at 37°C and 5% CO_2. Subsequently, the MTT formazan product was solubilized using acid-isopropanol The absorbance was measured at 590 nm using a microplate reader (Azure Biosystems Scientific, Dublin, CA, USA). Experiments were performed in triplicates.

Flow cytometry assay. Liposarcoma cells (93T449 and SW872 lines) were harvested following the manufacturer's protocol. Briefly, cells were washed twice with cold PBS and then re-suspended in 1 × Binding Buffer (BD Biosciences, San Diego, CA, USA) at a concentration of 1×10^6 cells/mL. A total of 1×10^5 cells in 100 μL of solution were transferred to a 5 mL culture tube, followed by the addition of 5 μL of FITC Annexin V and 10 μL of propidium iodide (PI) (BD Biosciences). The cells were vortexed and subsequently incubated at 25°C for 15 min in the dark. Next, 400 μL of 1 × Binding Buffer was added to each tube, and the cells were analyzed using BD FACSCalibur™ Flow Cytometer System (BD Biosciences). Experiments were performed in duplicates.

Wound healing assay. Cell migration was assessed using a wound healing assay. Liposarcoma cells were seeded in 6-well plates at 4×10^5 cells/well and treated as described. Once cells reached 100% confluency, a sterile pipette tip was used to create a scratch of consistent width across the cell monolayer. Cells were then washed with serum-free DMEM medium and incubated with regular culture medium. Wound closure was monitored at 0, 8, 24, and 48 h using microscopy. Three images were captured *per* well at each time point, and the cell repair process was monitored by microscopy. Wound width was measured at five random locations per image, and the migration distance was calculated by subtracting the wound width at 0 h from each subsequent time point. Cell migration ability was determined using the formula: Percentage of wound healing (%) = [(wound width at 0-h time point — wound width at 8, 24, 48-h time point) / wound width at the 0-h time point] × 100%. The wound-healing assay was conducted in triplicate.

Statistical analysis. Data were analyzed using Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) and presented as mean ± SD. Statistical significance was determined using independent two-tailed Student's *t*-tests for independent data, and one-way ANOVA followed by Tukey's test for multiple comparisons. Differences were considered significant for all statistical tests when the P-value < 0.05.

Results

Establishment of BMSCs with stable YAP1 knockdown

We established a stable BMSC line with reduced YAP1 expression using lentiviral transduction. The efficiency of YAP1 knockdown was confirmed through multiple assays. Green fluorescent protein (GFP) fluorescence staining (Fig. 1A) was utilized, followed by validation using RT-qPCR to detect changes in YAP1 mRNA expression (Fig. 1B). Additionally, western blot analysis was performed to assess alterations in YAP1 protein levels (Fig. 1C, D). Empty lentiviral vector (shNC BMSC) served as the negative control. Collectively, these experiments demonstrated the effectiveness of the shRNA in downregulating YAP1 expression in BMSCs.

Effect of BMSC-CM or shYAP1-BMSC-CM on liposarcoma cell proliferation

To investigate the impact of BMSCs on liposarcoma cell proliferation, we utilized untreated liposarcoma cells as the control group, while both 93T449 and SW872 cell lines were treated with either BMSC-CM or shYAP1-BMSC-CM as the experimental groups. The relative cell proliferation activity of both cell lines was assessed using the MTT assay at 24, 48, and 72 h post-treatment, as depicted in Fig. 2. Treatment with BMSC-CM significantly increased the relative proliferation rate of 93T449 and SW872 cell lines ($P < 0.05$). Conversely, treatment with shYAP1-BMSC-CM led to a significant suppression of cell proliferation compared to the control group ($P < 0.01$). These results suggest that BMSCs promote the growth of liposarcoma cells, while knocking down YAP1 in BMSCs inhibits tumor cell proliferation.

Knockdown of YAP1 in BMSCs induces apoptosis in liposarcoma cell

To examine whether the observed growth inhibitory effect induced by YAP1 knockdown in BMSCs on liposarcoma cells was attributed to apoptosis, flow cytometric analysis assays were conducted. Following 48 h of treatment with shYAP1-BMSC-CM as described, both 93T449 and SW872 cell lines showed significantly increased rates of apoptosis compared to untreated cells, which served as the control group (Fig. 3).

Knockdown of YAP1 in BMSCs suppresses liposarcoma cell migration in vitro

To assess the impact of YAP1 knockdown in BMSCs on liposarcoma cell migration, we conducted wound healing assays. Given the aggressive nature of liposarcoma and its propensity for recurrence and distant metastasis, understanding its migratory abilities is crucial. After treatment with shYAP1-BMSC-CM, relative cell migration distances were evaluated at 0, 8, 24, and 48 h post-treatment using scratch assays. Our results revealed a significant inhibition of migratory potential in both 93T449 and SW872 cell lines compared to the control group (Fig. 4). Wounds were almost fully

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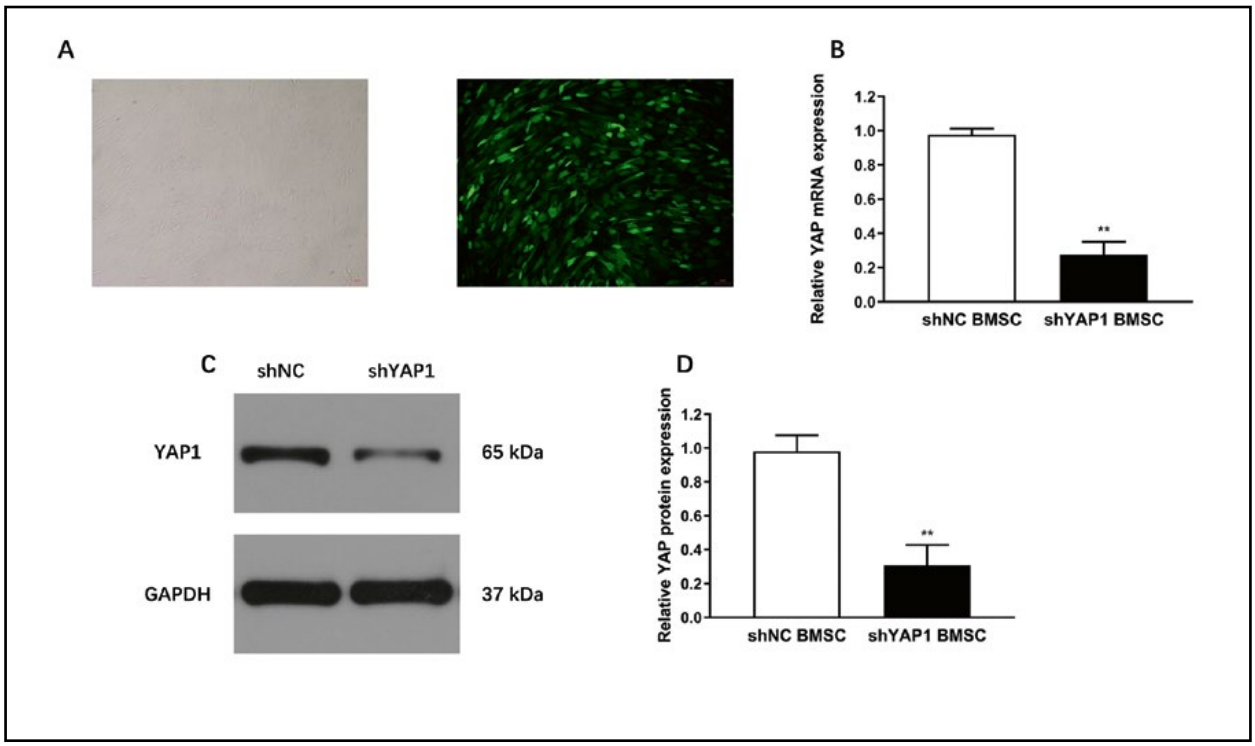

Figure 1. Lentiviral transfection for stable knockdown of YAP1 expression in BMSCs. **A.** Microscopic image captured 48 hours after transfection with recombinant virus vector. Representative bright field (left panel) and fluorescent (right panel) images. Original magnification: 100 ×, scale bar = 100 μm. YAP1 expression was knocked down in BMSCs after YAP1 shRNA transfection, as determined by RT-qPCR analysis (**B**), western blot (**C**), and semi-quantitative analysis (**D**). These levels were compared in the knockdown BMSCs to control group transduced with an empty vector shRNA (shNC). The experiment was repeated three times. **P < 0.01 *vs*. control.

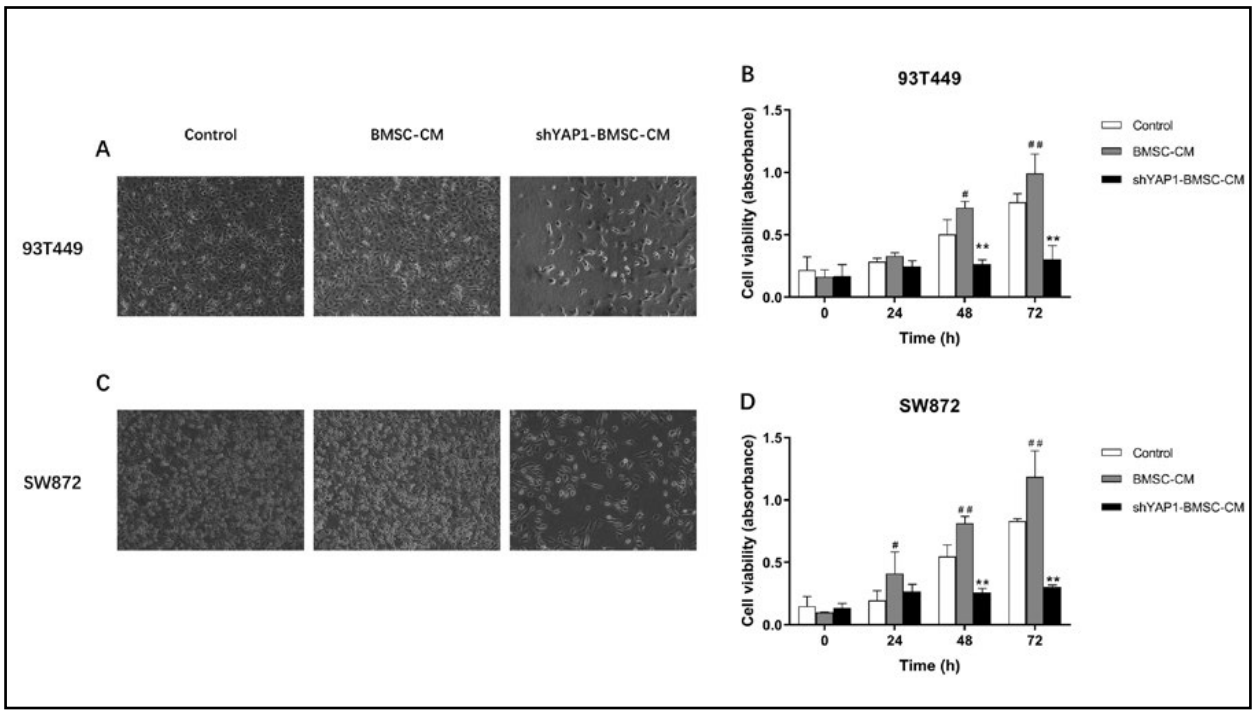

Figure 2. Effect of BMSC-conditioned medium (CM) or shYAP1-BMSC-CM on liposarcoma cell proliferation analyzed by MTT assay. **A, C.** Representative images of liposarcoma cells after treatment with BMSC-CM or shYAP1-BMSC-CM. Original magnification: 100×, Scale bar = 100 μm. **B, D.** Proliferation of liposarcoma cells after treatment with BMSC-CM or shYAP-1-BMSC-CM as determined by the MTT assay at OD 590 nm. The experiment was repeated three times. **P < 0.01, #P < 0.05, ##P < 0.01 *vs*. control.

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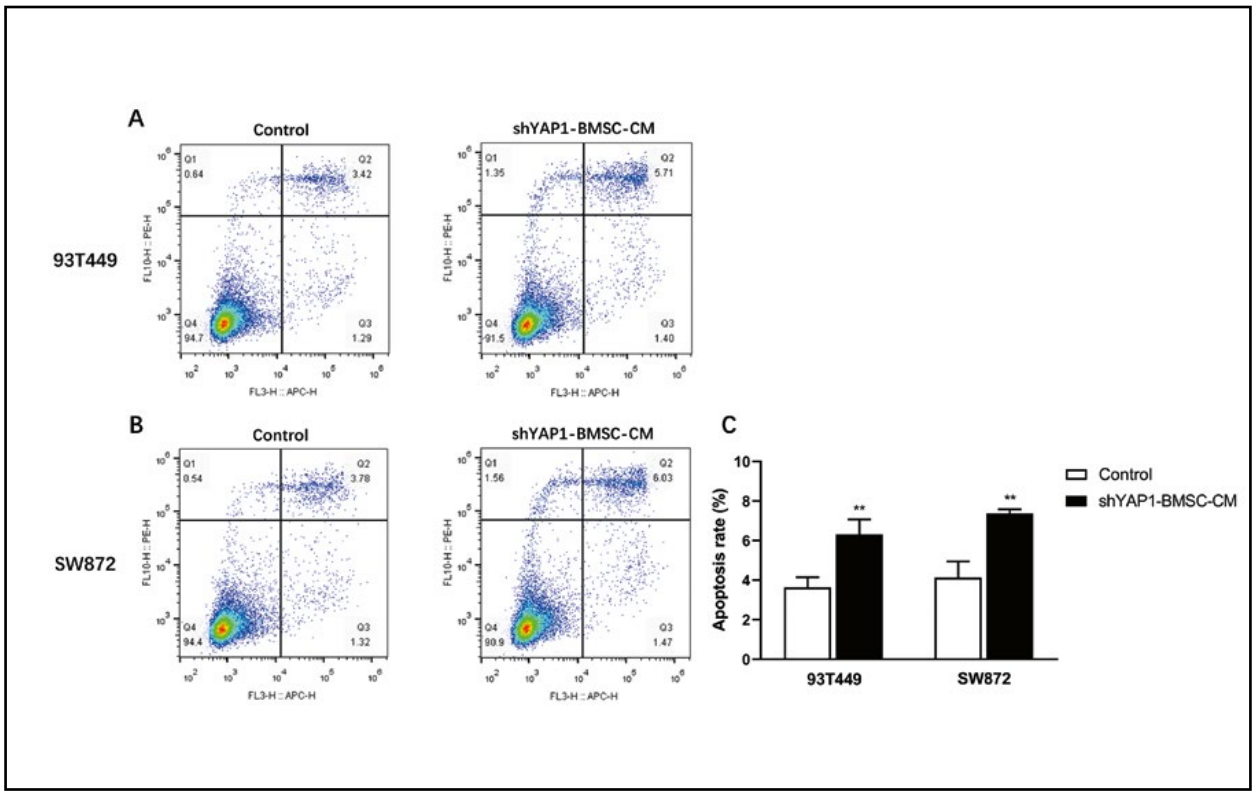

Figure 3. Knockdown of YAP1 in BMSCs induces cell apoptosis in liposarcoma cell lines. **A, B.** Representative results of flow cytometric analysis of cell apoptosis in 93T449 and SW872 cells treated with shYAP1-BMSC-CM. **C.** Analysis of the rate of apoptosis in 93T449 and SW872 cells treated with shYAP1-BMSC-CM. The experiment was repeated twice. **P < 0.01 *vs*. control.

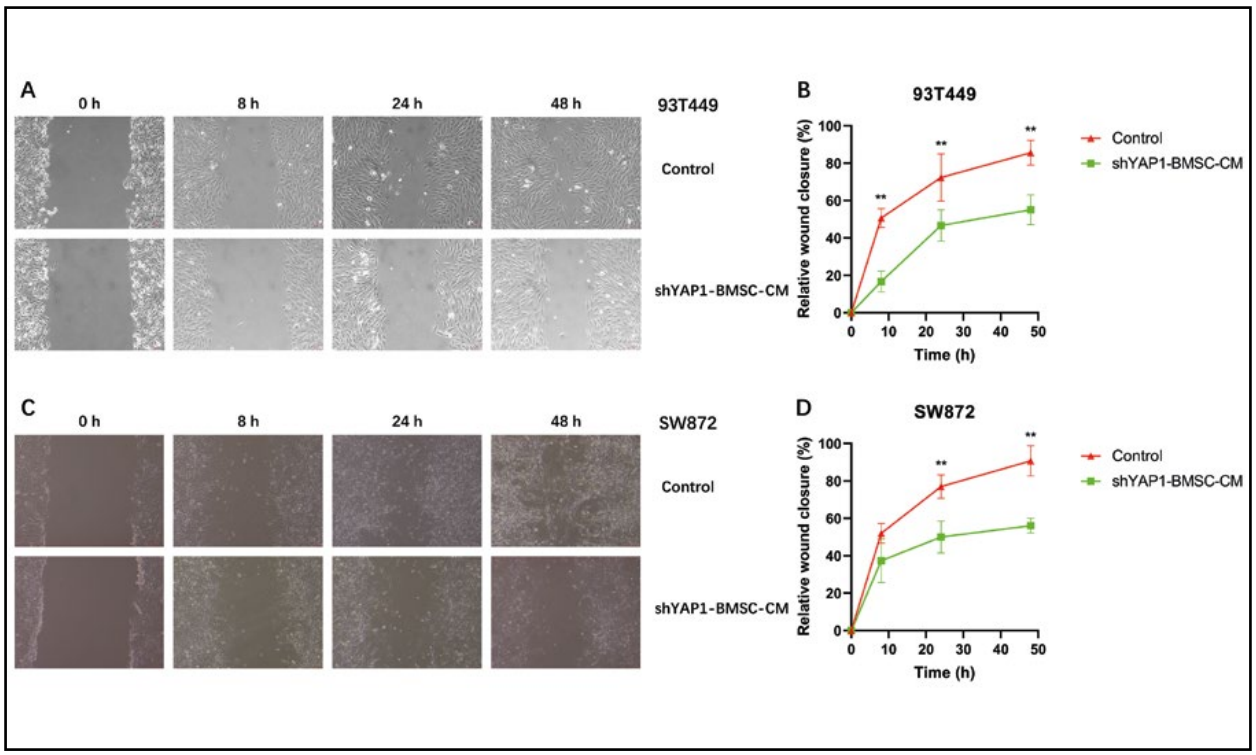

Figure 4. Knockdown of YAP1 in BMSCs impairs migration in liposarcoma cell lines. **A, C.** Representative migration images of 93T449 and SW872 cells treated at different time points (0, 8, 24, and 48 h) with shYAP1-BMSC-CM. Original magnification: 100×, Scale bar = 100 μm. **B, D.** Relative wound closure of cultured 93T449 and SW872 cells treated at different time points (0, 8, 24, and 48 h) with shYAP1-BMSC-CM. The experiment was repeated three times. **P < 0.01 *vs*. control.

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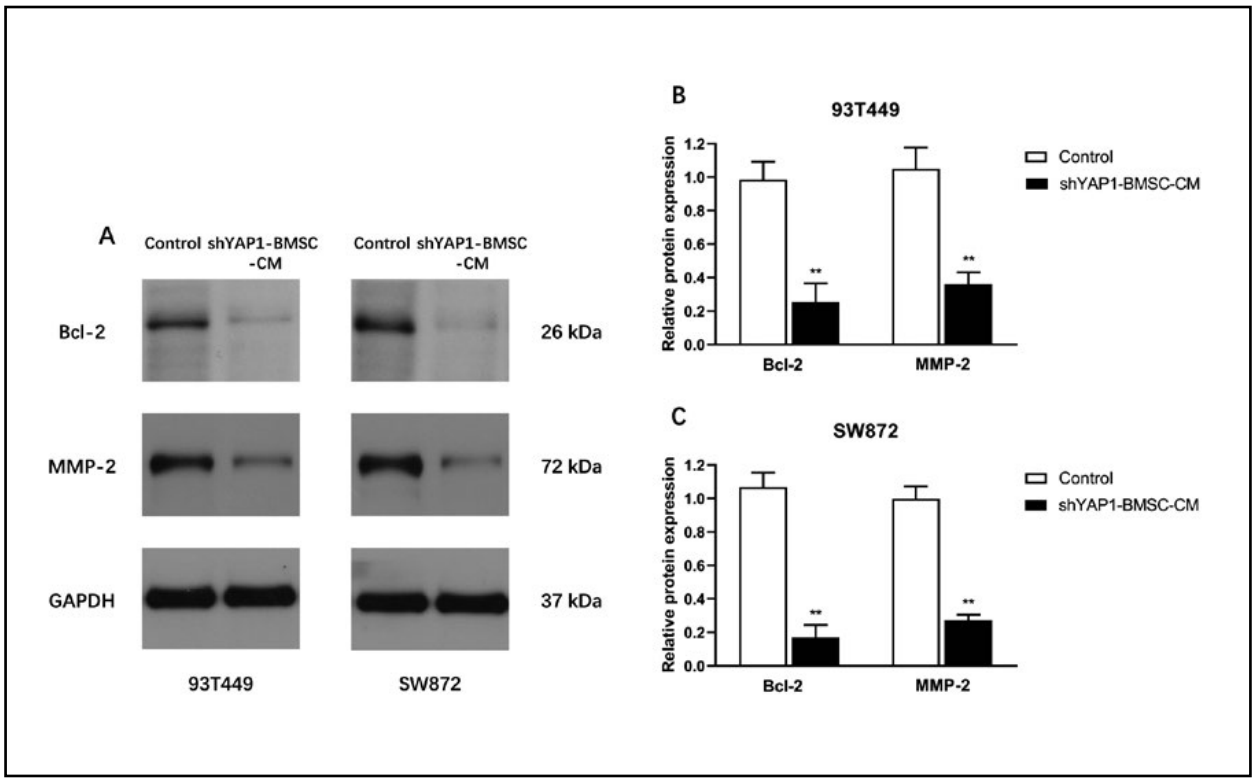

Figure 5. Knockdown of YAP1 in BMSCs results in the altered expression of related protein in liposarcoma cell lines. **A.** Representative western blot analysis after treatment of 93T449 and SW872 cells with shYAP1-BMSC-CM. **B, C.** Downregulation of Bcl-2 and MMP-2 expression after treatment with shYAP1-BMSC-CM in 93T449 and SW872 cell lines as determined by semi-quantitative analysis. The experiment was repeated twice. **P < 0.01 *vs*. control.

recovered after the 48-h migration in control cells, indicating the inhibitory effect of YAP1 knockdown in BMSCs on the migration of liposarcoma cells.

Knockdown of YAP1 in BMSCs reduces Bcl-2 and MMP-2 expression in liposarcoma cell

To investigate the mechanisms underlying the reduced proliferation and migration of liposarcoma cells observed upon YAP1 knockdown in BMSCs, we examined the expression of proteins associated with apoptosis and cell motility in liposarcoma cells. As depicted in Fig. 5, treatment with shYAP1-BMSC-CM led to the downregulation of Bcl-2 and MMP-2 in both 93T449 and SW872 cell lines compared to the control.

Discussion

In this study, we established stable YAP1 knockdown BMSCs cell lines, collected conditioned medium (CM), and co-cultured with two liposarcoma cell lines. The experimental results demonstrated that BMSC-CM promoted tumor cell proliferation, while knockdown of YAP1 in BMSCs significantly inhibited tumor cell proliferation and migration. Furthermore, YAP1 knockdown led to increased tumor cell apoptosis and reduced expression levels of Bcl-2 and MMP-2. These findings suggest that BMSCs could play a critical role in maintaining liposarcoma cell growth partly through the YAP1 signaling pathway. Previous study has revealed that BMSC-CM activates the STAT3 signaling pathway through interleukin (IL)-6 secretion, promoting invasion in hepatocellular carcinoma cells [41]. In oral squamous cell carcinoma, BMSC-CM has been shown to induce EMT, enhance cell proliferation, migration, and infiltration by activating the Ras/Raf/ERK signaling pathway through the generation of TGF-β [42]. BMSC-CM increases the levels of monocyte chemoattractant protein (MCP)-1, growth-regulated oncogene (GRO)-a, IL-6, and IL-8, thereby facilitating mesenchymal to amoeboid transition (MAT), as well as enhancing the motility, invasiveness, and transendothelial migration of osteosarcoma cells [43]. It was found that BMSC-CM contributes to the metastasis of gastric cancer cells through the secretion of stanniocalcin 1 and IL-8 in response to small extracellular vesicles released by gastric cancer cells [44]. In line with these findings, we show for the first time that BMSC-CM promotes the proliferation of liposarcoma cells.

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We then investigated the potential mechanism of YAP1 involvement in tumor cells growth and migration. Prior research has unveiled that YAP1 serves not only as a key regulatory factor within cellular pathways, but also represents a critical hub mechanotransducer mediating biophysical signals' communication between cells and their microenvironment. This enables YAP1 to convert biomechanical stimuli into intracellular biochemical signals, thereby modulating alterations of corresponding up/downstream molecules [45, 46]. Moreover, YAP1's influence extends to immune cell functionality, cellular metabolism, and contributes to tumor progression [47, 48]. Notably, studies have demonstrated that YAP1 inhibition in BMSCs using the inhibitor verteporfin significantly suppresses its pro-carcinogenic effect on gastric cancer cells [49]. Similarly, YAP1 signaling pathway in BMSCs has been identified to be involved in tumor stromal growth in leukemia [50]. Consistently, our findings align with these studies by revealing that YAP1 knockdown in BMSCs inhibits liposarcoma cell proliferation and migration.

Bcl-2 belongs to the Bcl-2 family, which is a well-documented regulator of the apoptosis gene family. It plays a crucial role in promoting cell survival and counteracting the effects of pro-apoptotic proteins by preventing mitochondrial permeability transition and the release of cytochrome c [51]. Accumulated evidence substantiates that aberrant expression of Bcl-2 is strongly implicated in the progression of diverse cancer types [52]. It was demonstrated that a Bcl-2 inhibitor can effectively suppress the viability of primary tumor cells derived from liposarcoma patients, as well as other types of sarcomas, including chondrosarcoma, leiomyosarcoma, and osteosarcoma [53]. Similar findings have been documented in studies related to hepatocellular carcinoma, breast cancer, melanoma, gastroesophageal carcinoma. These results demonstrated that suppressing Bcl-2 expression can effectively restrain tumor cell growth [54–57]. In accordance with these reports, we observed that liposarcoma cell viability decreased with the downregulation of Bcl-2. MMP-2 (gelatinase A), a member of the matrix metalloproteinases (MMPs) family, contributes significantly to degradation of type IV collagen and gelatin, the two main components of extracellular matrix (ECM) and basement membrane [58]. MMP-2 is centrally involved in cancer invasion and metastasis by influencing ECM remodeling, angiogenesis, lymphangiogenesis, and integrin-cell binding [59]. Downregulation of MMP-2 has been shown to decrease the metastatic potential of various cancer cell types, including lung cancer, glioma, colorectal cancer, nasopharyngeal carcinoma [60–63]. In our study, we noticed that treating liposarcoma cells with shYAP1-BMSC-CM resulted in impeded migration capacity, concurrently with a reduced MMP-2 protein expression. These observations are consistent with the aforementioned reports.

Conclusions

In conclusion, the inhibition of YAP1 in BMSCs caused a significant decrease in both the growth and motility of liposarcoma cell. These results provide new insights into the role of BMSCs in liposarcoma and suggest a promising therapeutic approach for treating this malignant tumor. Considering the wide availability and the ease of isolation of BMSCs, further *in vitro* and *in vivo* investigations of the molecular mechanisms of BMSCs underlying their impact on liposarcoma should be explored.

Article information and declarations

Data availability statement

The data in this study are available from the corresponding author upon reasonable request.

Ethics statement

Not applicable. This article does not contain any studies with human or animal participants.

Author contributions

Hua Chen and Haijun Hu conceived and designed the study. Hua Chen, Na Sha, Ning Liu and Haijun Hu conducted and analyzed the experiments, and performed the statistical analysis. Hua Chen and Haijun Hu drafted the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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