

Effect of umbilical cord mesenchymal stem cells in human ovarian cancer SKOV3 cells

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Research

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Abstract

Objective: To investigate the effects of human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) on apoptosis and proliferation of human ovarian cancer SKOV3 cells and to explore mechanism.

Methods: hUC-MSCs were isolated and cultured by tissue block adherent culture method. The hUC-MSCs phenotype were identified by flow cytometry. The hUC-MSCs lysate and conditioned medium, directly combine were used to treat SKOV3 cells. The effects on the proliferation/apoptosis/mechanism of SKOV3 cells were examined by cell counting kit-8(CCK-8)/Annexin V-FITC/PI/quantitative real time polymerase chain reaction(RT-qPCR) and spheroid formation assays. Establish ovarian cancer xenograft models. 1×10^6 hUC-MSCs and 2×10^6 hUC-MSCs were administrated into the mice t rear back tumor tissue. After three injections of hUC-MSCs, the nude mice were sacrificed after 1 week of observation. Remove tumor tissue. Observed tumor volume changes every 3 days after the start of the experiment. The expression of CD34 and VEGF were detected by immunohistochemistry.

Results: Human umbilical cord mesenchymal stem cells were cultured and isolated from tissue block. Flow cytometry results revealed that the hUC-MSCs marks CD44 and CD29, but not CD45 and CD34 were expressed on obtained cells. The apoptosis of SKOV3 cells was induced by hUC-MSCs lysate, conditioned medium and Transwell co-culture method in SKOV3 cells, and the apoptosis rate was higher with increasing concentration. hUC-MSCs conditioned medium and Transwell co-culture method can inhibit cell proliferation. After adding experimental factors, the conditioned medium and Transwell co-culture method can down-regulate the transcription of PI3KCA, AKT and BCL-2 genes in SKOV3 cells, and up-regulate the Caspase-3 gene. The tumor volume of the experimental group was smaller than that of the control group during the observation period. The expression levels of CD34 and VEGF in the experimental group were significantly lower than those in the control group ($P < 0.05$).

Conclusion: The conditioned medium of hUC-MSCs and the co-culture method of hUC-MSCs and SKOV3 can significantly inhibit the proliferation of SKOV3 cells, which is mainly achieved by inhibiting PI3K/AKT signaling pathway. hUC-MSCs can inhibit the growth of subcutaneous subcutaneous transplantation and the expression of CD34 and VEGF in ovarian cancer. It provides a new idea for the treatment of ovarian cancer.

Introduction

Ovarian cancer (OvCa), one of the most common gynecological malignancies in women worldwide, which the incidence is second only to cervical and uterine cancer^[1-2]. According to statistics, there were nearly 300,000 new cases of OvCa in 2018^[3]. Because the anatomical part of the ovary is located deep in the pelvis and the lack of specific symptoms in patients with OvCa makes early diagnosis difficult, initial diagnosis typically occurs when the tumor has reached an advanced stage. About 70% -75% of patients are in advanced stage at the time of onset; their 5-year survival rate is only about 20%^[4]. Although application of treatments such as surgical resection, chemotherapy, radiotherapy and targeted therapy

significantly made substantial progress, many OvCa patients still have no improve overall survival (OS) time^[5]. Therefore, in order to improve the survival rate of OvCa patients, new methods need to be explored. MSCs were originally found in bone marrow and are pluripotent stem cells derived from mesoderm. MSCs have low immunogenicity, tumor orientation and targeted migration ability^[6-7]. Many studies have shown that when MSCs aggregate or contact tumor tissues, they can directly inhibit tumor growth or play a dual role by directly acting or secreting certain cytokines; Therefore, there is no unified understanding of the relationship between hUC-MSCs and tumorigenesis and tumor progression. In this study, we applied hUC-MSCs lysate and conditioned medium to ovarian cancer SKOV3 cells, and co-cultured hUC-MSCs with human ovarian cancer SKOV3 cancer cells, and observed the effects on the proliferation and apoptosis of human ovarian cancer SKOV3 and its occurrence Preliminary investigation of the mechanism, establishment of a model of OvCa xenograft, the application of hUC-MSCs to the xenograft model, and observation of its effect on the xenograft model.

Materials And Methods

hUC-MSC isolation,culture and treatment

We isolated hUC-MSCs using an adherent tissue method. Briefly, a 10 cm umbilical cord from a full-term healthy newborn(The umbilical cord was obtained from a healthy full-term Caesarean section in the Department of Obstetrics and Gynecology, Henan Provincial People's Hospital.)was washed with PBS (containing 1% penicillin-streptomycin double-resistant solution, Beijing Solarbio Science & Technology Co.,Ltd) 3 times. Then, we cut the cord into small pieces, dislodged the umbilical vein and umbilical artery, and left Wharton's jelly at last. Wharton's jelly was then cut into 1 mm × 1 mm × 1 mm and cultured in DMEM/F12 medium (Gibco, Grand Island, NY) containing 10% FBS (Gibco, Grand Island, NY) ; then cultured in 25 cm² cell culture flask,The umbilical cordexplants were cultured in 37°C, 5% CO₂ humidified atmosphere, the medium was changed every 72 hours, and the cell fusion degree reached 80% before 1: 3 passage.3–6 passages (P3- 6) were selected for the following experiments.

Cell lines culture and treatment

SKOV3 cell lines of human origin were purchased from Cyagen Biosciences Inc. (Guangdong,China)and cultured in DMEM/F 12 medium containing 10% FBS. then cultured in 25 mm cell culture flask□The umbilical cordexplants were cultured in 37°C, 5% CO₂ humidified atmosphere, the medium was changed every 72 hours, and the cell fusion degree reached 80%.

Flow cytometry and hUC-MSCs identification

hUC-MSCs were trypsinized, and the cell concentration was adjusted to 1 × 10⁶ /mL in PBS. Then, 200 μl of the suspension was incubated with 5 μl of antibodies against CD29□CD45□CD44 and CD34 without light for 30 min. Primary antibodies were directly conjugated with FITC and phycoerythrin. For isotype control, non-specific FITC-conjugated IgG was substituted for the primary antibodies. Lastly, the samples were analyzed using fflow cytometry.

Lysate preparation

Select 3–6 passages hUC-MSCs with cell fusion to 80% and trypsinize them. After washing 3 times with PBS, resuspend them in DMEM medium without FBS, Count on the cell counting plate and adjust the cell concentration to 1×10^6 cells / ml. The cells were lysed by repeated freeze-thaw methods, and the collected cells were quickly frozen under liquid nitrogen for 10 minutes, taken out and placed at room temperature to slowly thaw, repeatedly frozen and thawed for more than 10 times, and stored in a refrigerator at -20°C for later use. Add 0.5×10^6 SKOV3 cells to four 25 cm² culture flasks, add the culture medium to 4 ml, numbered as lysate control group, 1 / 2 times group, 1times Group, 2 times group, and then add 1 ml DMEM / F12, 0.25 ml lysate + 0.75DMEM / F12, 0.5 ml lysate + 0.5 ml DMEM / F12, 1 ml lysate, and incubate for 48 h. Each experiment was repeated 3 times.

Conditioned media (CM) preparation

Select 3–6 passages hUC-MSCs with cell fusion to 80%, discard the spent culture medium, and wash 3 times with PBS. Add 10 ml DMEM / F12 medium in the 75 cm² cell culture flask. After 48 hours, the supernatant, containing all released cytokines and chemokines to be studied, was collected. Centrifuge at 1500 rpm for 5 minutes, aspirate the supernatant, filter through a 0.22 μm sterile filter to obtain hUC-MSCs CM, store at -20°C , and use it within a week. Configure 50%, 75%, and 100% CM according to the following ratios: 45 ml CM + 45 ml DMEM/F12 + 10 ml FBS (50%); 67.5 ml CM + 22.5 ml DMEM/F12 + 10 ml FBS (75%); 90 ml CM + 10 ml FBS (100%). Add 0.5×10^6 SKOV3 cells to four 25 cm² culture flask, numbered as CM control group, 50% group, 75% group, and 100% group. The control group was 5 ml of SKOV3 complete medium (CM concentration was 0%) and cultured in an incubator for 48 h. Each experiment was repeated 3 times.

Cell viability assay

Cell Counting Kit-8 (CCK-8, Dojindo) was used to the viability of SKOV3 cells. Cells were seeded in 96-well plates and cultured for 48 hours. After different treatment by group, added 10 μl CCK-8 solution in each well. The optical density values were measured by microplate reader at 450 nm.

Apoptosis analysis

annexin V-FITC/PI Kit (Keygen Biotechnology, Nanjing, China) were used to assess cells apoptosis of SKOV3 cells: After SKOV3 cells were treated respectively, collect all cells in the culture flask, 4°C Centrifuge at 300 g for 5 min. Cells were washed twice with pre-chilled PBS, and $(1-5) \times 10^5$ cells were collected. Resuspended cells in 100 μl binding buffer, added 5 μl Annexin V-FITC and 5 μl PI and shake gently. Incubated for 10 minutes at room temperature in the dark. Add 400 μl binding buffer and mix well. Finally, analyzed by flow cytometer within 1 hour.

coculture system

Co-culture of hUC-MSCs and SKOV3 cells in direct contact: Preparation of hUC-MSCs cell suspension. The Transwell cell with a pore size of 0.4 μm was placed in a medical sterile measuring cup, and the hUC-

MSCs suspension was added dropwise to the surface of the polycarbonate membrane in the cell. The number of MSCs cells was 0.5×10^6 , 1×10^6 , and 2×10^6 respectively. Place the lid of the sterile measuring cup on the measuring cup, and place the measuring cup in the incubator for 6 hours. Take out the Transwell chamber, aspirate the residual liquid on the surface of the polycarbonate membrane, and place the chamber in a 6-well plate. 2 ml of hUC-MSCs complete medium is added to the lower layer of each group of Transwell chambers. 2 ml of SKOV3 cell complete medium with a density of 0.5×10^6 cells / ml of SKOV3 cell complete medium was placed in an incubator for 60 hours. In the control group, the hUC-MSCs suspension was not added to the lower surface of the polycarbonate membrane of the Transwell chamber, and the rest of the treatment was the same as the above groups. Each experiment was repeated 3 times. Collect all cells in the upper layer of the Transwell chamber and perform Cell viability assay and Apoptosis analysis.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR)

RNA extraction Kit (Qiagen Inc., Valencia, CA, USA). RNA quality and yields were analyzed using nanodrop. Complementary DNA (cDNA) was synthesized from 500 ng of total RNA in a 20 μ L reaction solution using iScript™ Cdna-synthesis Kit (Bio-Rad Laboratories, CA). Quantitative real-time PCR was performed with the iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, CA) in triplicate. The reaction conditions were: polymerase activation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 20 s, and annealing and extension at 62 °C for 20 s. The relative quantification of gene expression was normalized to the expression of endogenous GAPDH. Real-time PCR was performed with multiple sequences as indicated in Table 1.

Total RNA was extracted from treated SKOV3 cells using TRIzol reagent (TaKaRa, Japan). 1 μ g RNA was reverse transcribed using the MMLV reverse transcriptase kit according to the manufacturer's instructions (TaKaRa, Japan). The GAPDH, PI3KCA, AKT, bcl-2 and caspase-3 primers were provided directly and the sequences were kept secreted by Sangon Biotech (Shanghai) Co.,Ltd. All data were normalized using the internal control GAPDH.

Genes	Primers sequences (5'-3')
GAPDH	F 5' AAGAAGGTGGTGAAGCAGG 3' R 5' GTCAAAGGTGGAGGAGTGG 3'
PIK3CA	F 5' TCTGTCTCCTCTAAACCCTG 3' R 5' TAGCATATCTTGCCGTAAT 3'
AKT	F 5' CTGGGCAAGGGCACTTTTCGG 3' R 5' CAGAGGCGGTCGTGGGTCTG 3'
bcl-2	F 5' CGACTTCTCCCGCCGCTACC 3' R 5' CCCAGTTCACCCCGTCCCT 3'
caspase-3	F 5' GGTTTCATCCAGTCGCTTTGT 3' R 5' AATTCTGTTGCCACCTTTTCG 3'

Table 1. Sequences of primers used for RT-PCR

Animal model

BALB/c nude mice (4–5 weeks old) were purchased from the BEIJING VITAL RIVER LABORATORY ANIMAL TECHNOLOGY CO., LTD. (Beijing, China). The animals were maintained in accordance with institutional policies, and all experiments were performed with approval of the Committee on the Use and Care of Animals of the HENAN PROVINCE PEOPLE'S HOSPITAL. 5×10^6 cells were subcutaneously inoculated on the back of nude mice. The next diary was the first day of vaccination, and the subcutaneous tumors were checked every other day. The experiments will begin when the tumors have a diameter of about 0.5 cm. OvCa cells nude mice were randomly divided into 3 groups of 5 mice each, which were respectively the normal control group, the 1×10^6 hUCMSCs group, and the 2×10^6 hUCMSCs group. 1×10^6 and 2×10^6 hUCMSCs were injected into the dorsal tumors of ovarian cancer-bearing nude mice, respectively, for 3 consecutive weeks and once a week. The control group was injected with physiological saline, and hUCMSCs were injected 3 times. The nude mice were sacrificed and the tumor tissues were removed after 1 week.

Immunohistochemistry

Formalin-fixed paraffin-embedded mouse tumor tissue sections were first deparaffinized in xylene and rehydrated through graded ethanol. Subsequently, the sections were boiled for 10 min in citrate buffer (pH 6.0, 10 mM) for antigen retrieval. Endogenous peroxidase activity was then inhibited by exposure to 3% hydrogen peroxide for 10 min. The sections were then blocked with 5% BSA and incubated with properly diluted VEGF and CD34 primary antibody at 37°C for 1 h. After the sections were washed with PBS, they were then incubated with diluted secondary antibody for 20 min. Finally, the sections were visualized with 3,3'-diaminobenzidine (DAB) and then counterstained with hematoxylin for examination under a light microscope (x100, SN:9G15626; Olympus). Immunohistochemistry experiments were performed in strict accordance with the kit instructions, and images were collected under a microscope.

Statistical analysis

All experiments were conducted at least in triplicate. Data were presented as the means \pm standard error. Statistical analysis was performed using SPSS 25.0 software. Potential differences between groups with different treatments were determined using one-way ANOVA or an independent sample t-test. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Characterization of hUC-MSCs

Adherent spindle-shaped cells and classical MSCs colonies were observed under inverted microscope. We chose P6 hUC-MSCs to identify the cell phenotype by flow cytometry. Inverted phase contrast microscopy showed that P3 hUC-MSCs and P6 hUC-MSCs, as shown in Fig. 1A and B. Flow cytometry analysis of the cell phenotype showed that the hUC-MSCs were positive for CD44 ($98.4067 \pm 0.1332\%$) and CD29 ($98.3933 \pm 0.3669\%$), but were negative for CD34 ($0.0133 \pm 0.0058\%$) and CD45 ($0.0133 \pm 0.0058\%$) (Fig. 1C and D)

hUC-MSCs lysate promotes SKOV3 cells apoptosis but does not promote proliferation at same times

Flow cytometry was conducted to identify changes in the apoptosis rate of SKOV3 cells treated with hUC-MSCs lysate according to Annexin V staining. The results indicated that the apoptosis rates of SKOV3 cells did not differ markedly between the control and 1 / 2 times group, 1 times group, 2 times group at either 48 h ($1.95 \pm 0.17\%$, $6.74 \pm 0.38\%$, $11.94 \pm 0.16\%$, $19.52 \pm 0.49\%$). $P > 0.05$. Microplate reader was used to measure the relative proliferation rate of SKOV3 cells treated with hUC-MSCs lysate according to CCK-8. However, the relative proliferation rate of SKOV3 cells between the control and 1 / 2 times group, 1 times group, 2 times group at either 48 h ($0 \pm 0\%$, $0.83 \pm 0.25\%$, $2.63 \pm 0.15\%$, $17.70 \pm 0.92\%$) (Fig. 2A-F). $P > 0.05$.

hUC-MSCs CM promotes SKOV3 cells apoptosis and inhibits proliferation

Flow cytometry was conducted to identify changes in the apoptosis rate of SKOV3 cells treated with hUC-MSCs CM according to Annexin V staining. The results indicated that the apoptosis rates of SKOV3 cells did not differ markedly between the control and 50% group, 75% group, and 100% group at either 48 h ($2.44 \pm 0.28\%$, $6.95 \pm 0.48\%$, $10.52 \pm 0.30\%$, $15.57 \pm 0.57\%$). $P > 0.05$. Microplate reader was used to measure the relative proliferation rate of SKOV3 cells treated with hUC-MSCs CM according to CCK-8. However, the relative proliferation rate of SKOV3 cells between the control and 50% group, 75% group, and 100% group at either 48 h ($0 \pm 0\%$, $12.00 \pm 0.32\%$, $16.80 \pm 0.76\%$, $26.29 \pm 1.85\%$) (Fig. 3A-F). $P > 0.05$.

Co-culture of hUC-MSC and SKOV3 cells promotes SKOV3 cells apoptosis and inhibits proliferation

Flow cytometry was conducted to identify changes in the apoptosis rate of SKOV3 cells treated with co-culture of hUC-MSC according to Annexin V staining. The results indicated that the apoptosis rates of SKOV3 cells did not differ markedly between the control group and 0.5×10^6 group, 1×10^6 group, and 2

$\times 10^6$ group at either 60 h ($31.60 \pm 0.53\%$, $40.63 \pm 0.74\%$, $45.53 \pm 0.67\%$, $56.53 \pm 0.57\%$). (P ≥ 0.05). Microplate reader was measured in the relative inhibits proliferation rate of SKOV3 cells treat with Co-culture of hUC-MSC according to CCK-8. However, the relative inhibits proliferation rate of SKOV3 cells between the control group and 0.5×10^6 group, 1×10^6 group, and 2×10^6 group at either 60 h ($0 \pm 0\%$, $23.96 \pm 1.55\%$, $40.35 \pm 1.34\%$, $46.25 \pm 2.41\%$). (P ≥ 0.05).

hUC-MSCs inhibited PI3K/AKT kinase and BCL-2 expression but promotes Caspase-3 in SKOV3 cells.

To verify the underlying pathway, we detected PI3K/AKT signaling pathway related genes and related apoptotic genes in SKOV3 cells treat with hUC-MSCs CM or in the hUC-MSCs co-culture system. The RT-PCR results showed that the expression of PI3KCA in SKOV3 cells treat with hUC-MSCs CM relative to the control group in the 50% group, 75% group, and 100% group was 0.74 ± 0.07 , 0.60 ± 0.07 , 0.40 ± 0.08 , respectively; the relative expression levels of AKT in each group were 0.80 ± 0.03 , 0.60 ± 0.04 , 0.44 ± 0.05 , respectively; the relative expression levels of BCL-2 in each group were 0.63 ± 0.02 , 0.40 ± 0.04 , 0.25 ± 0.09 , respectively; the relative expression levels of Caspase-3 in each group were 1.52 ± 0.11 , 2.02 ± 0.08 , 2.58 ± 0.29 , respectively (P ≥ 0.05) (Fig.5 A); The RT-PCR results showed that the expression of PI3KCA in SKOV3 cells in the hUC-MSCs co-culture system relative to the control group in the 0.5×10^6 group, 1×10^6 group, and 2×10^6 group was 0.6 ± 0.02 , 0.46 ± 0.02 , 0.34 ± 0.04 , respectively; the relative expression levels of AKT in each group were 0.85 ± 0.04 , 0.66 ± 0.03 , 0.46 ± 0.05 , respectively; the relative expression levels of BCL-2 in each group were 0.85 ± 0.04 , 0.69 ± 0.04 , 0.39 ± 0.03 , respectively; the relative expression levels of Caspase-3 in each group were 0.85 ± 0.04 , 0.69 ± 0.04 , 0.39 ± 0.03 , respectively (P ≥ 0.05) (Fig.5 B); the relative expressions of PI3KCA, AKT, BCL-2 genes in SKOV3 cells treat with CM or in the hUC-MSCs co-culture system were lower than those in the control group. And the relative expression decreased with the increase of the CM concentration or number of hUC-MSCs; while the relative expression of the Caspase-3 gene of SKOV3 cells cultured in the CM or in the hUC-MSCs co-culture system was higher than that of the control group, and the relative expression increased with the increase of the CM concentration or number of hUC-MSCs. The differences were statistically significant (P < 0.05).

seen subcutaneously on the back of the nude mice. The nodules were hard and had good mobility, and the surrounding skin was free of ulceration and swelling. The tumor formation rate was 100%. The tumor-bearing nude mice had no significant changes in their activities, diet, and mental state before tumor implantation.

Umbilical cord mesenchymal stem cells inhibit the volume of ovarian cancer xenografts

During the entire observation period, the quality of the tumor-bearing nude mice in the control group and the experiment was group generally stable; the tumor volume of the tumor-bearing nude mice in the control group and the 1×10^6 hUCMSCs group and the 2×10^6 hUC-MSCs group showed an overall increase. At the same times, as the number of hUC-MSCs injected increases, the volume of the transplanted tumor decreases (Fig.6).

Umbilical cord mesenchymal stem cells inhibit VEGF and CD34 expression in ovarian cancer xenografts

Observation of the light microscope after HE staining in the control group and the experimental group showed that tumor cells were abundant, densely arranged, obvious cell atypia, large nuclear deep staining, significantly increased nuclear and cytoplasmic ratios, and more abnormal nuclear and mitotic phases. A large number of vacuoles can be seen in the cell, a few nuclei are condensed and lysed, and apoptotic cells are occasionally seen. There was more infiltration of necrotic tissue and inflammatory cells in the tumor tissue, and fibrosis was obvious(Fig.7A).Microscopic observation of VEGF positive signals were all expressed in the cytoplasm of the tissue. The expression of VEGF in tumor tissue of nude mice in control group was 7.68 ± 0.50 , the expression of VEGF in tumor tissue of experimental group 1 was 5.94 ± 0.86 , and the expression of VEGF in tumor tissue of experimental group 2 was 4.44 ± 0.33 . Compared with the control group, the expression of VEGF in the experimental group was significantly reduced, and it decreased with increasing concentration in a certain concentration range, and the difference was statistically significant(Fig.7B-D). The positive expression of CD34 protein localizes the cytoplasm and cells of vascular endothelial cells, and appears as brown particles, which are used to mark microvascular density. The MVD in tumor tissue of nude mice in the control group was 47.80 ± 6.73 , the MVD in tumor tissue of the experimental group 1 was 39.32 ± 6.30 , and the MVD in tumor tissue of the experimental group 2 was 28.68 ± 6.05 . Compared with the control group, the tumor microvessel density in the experimental group was significantly lower, and it decreased with increasing concentration within a certain concentration range, and the difference was statistically significant(Fig.7E-G)(Table 2).

Conclusion

The current research shows that umbilical cord mesenchymal stem cells have the characteristics of targeted migration to tumor tissues. However, there are many disputes about the occurrence and development of umbilical cord mesenchymal stem cells in tumor tissues. Studies have shown that hUC-MSCs can inhibit tumor progression. For example,. Shan CJ et al. concluded in their review that hUC-MSCs can secrete IFN- β and inhibit breast cancer cell growth through apoptosis^[8].Studies by Akimoto, K, et al. Show that UC-MSCs promote apoptosis of primary glioblastoma (GBM) via tumor necrosis factor-related apoptosis inducing ligand (TRAIL)^[9]. Sun B et al. concluded in their review that believe that the hUC-MSCs-derived dickkopf (DKK1) can inhibit cancer cell proliferation and stem cell extracellular matrix (ECM) can prevent cancer cell proliferation by up-regulating PTEN levels in aggressive cancer cells^[10].The

results of Wang Y et al. Showed that the artificial fusion of human mesenchymal stem cells and esophageal cancer resulted in reduced hybrid cell growth, apoptosis and proliferation, and inhibition of tumorigenicity^[11]. Then there are some studies that show that umbilical cord mesenchymal stem cells can promote tumor development. Karnoub et al. Demonstrated that mesenchymal stem cells promote cell metastasis by secreting cell-derived factor-1 (SDF-1), interleukin-6 (IL-6), and vascular endothelial growth factor (VEGF)^[12]. However, there are relatively few reports on the effects of umbilical cord mesenchymal stem cells on ovarian cancer cells, and there are different opinions. Some studies have shown that co-

culture of mesenchymal stem cells and human SKOV3 ovarian cancer cells can promote apoptosis, and The fusion of SKOV3 cells can also significantly reduce tumorigenicity^[13].

The PI3K / AKT signaling pathway is one of the important signal transduction pathways in cells, and plays an important role in inhibiting apoptosis, regulating the cell cycle, and promoting cell invasion and metastasis^[14]. The P13K / AKT signaling pathway plays an important role in the proliferation, invasion, cell cycle progression, angiogenesis, and drug resistance of ovarian cancer, so PI3K / AKT / mTOR signaling pathway inhibitors have become a new direction in the treatment of ovarian cancer^[15].

In this study, an isolation method of hUC – MSCs tissue block adherent culture was used. It was identified as umbilical cord mesenchymal stem cells by flow phenotypic identification. This study found that hUC – MSCs conditioned medium and hUC – MSCs and SKOV3 co – culture method were used. SKOV3 proliferation can be inhibited by inducing apoptosis, while lysates of hUC – MSCs, although capable of inducing apoptosis, do not inhibit their proliferation. In order to further study the mechanism of hUC – MSCs inhibiting ovarian cancer, RT – qPCR was used to detect the expression of related genes on the PI3K / AKT signaling pathway in SKOV3 cells after adding experimental factors. This study found that hUC – MSCs conditioned medium and hUC – MSCs co – cultured with SKOV3 can inhibit the expression of PI3KCA, AKT, BCL – 2 mRNA and increase the expression of Caspase – 3 mRNA. Decreased BCL – 2 expression and increased Caspase – 3 expression will promote cell apoptosis. The results are consistent with the

conclusion that hUC – MSCs conditioned medium and co – culture of hUC – MSCs and SKOV3 can inhibit the proliferation of SKOV3 cells in this experiment; At the same time, hUC – MSCs conditioned medium and hUC – MSCs co – cultured with SKOV3 can inhibit PI3K / AKT expression and the inhibitory effect is related to concentration. However, hUC – MSCs lysate does not have this characteristic in SKOV3 cells. Therefore, we speculated that hUC – MSCs may inhibit the proliferation of SKOV3 cells by paracrine certain cytokines acting on SKOV3 cells and inhibiting their PI3K / AKT cell signaling pathway, down – regulating the BCL – 2 gene, and up – regulating the expression of Caspase – 3 gene.

The growth, invasion and metastasis of a tumor depend on the angiogenesis in the tumor cell mass. The angiogenesis of tumors is regulated by many kinds of active factors. It is the result of the interaction between tumor cells, vascular endothelial cells and the microenvironment. The process is complicated, and the current mechanism is still unclear^[16]. VEGF has the ability to increase capillary permeability, promote tumor cells to enter the vascular wall, and metastasize. Its biological activity is regulated by oncogenes, hypoxia, cytokines, and inflammatory factors^[17]. MVD assay is to study the expression of vascular endothelial cells in solid tumor stroma, but not in parenchymal cells. CD34 can efficiently and specifically label vascular endothelial cells^[18].

This experiment found that the expression of CD34 and VEGF decreased significantly when the umbilical cord mesenchymal stem cells were injected into the tumor compared with the control group. When the injected cells were larger, the expression of CD34 and VEGF decreased more significantly. This is also consistent with the slower growth of umbilical cord mesenchymal stem cell tumors. This indicates that

umbilical cord mesenchymal stem cells may inhibit the expression of CD34 and VEGF to inhibit the growth of ovarian cancer subcutaneous xenografts. However, Otsu et al. Found that direct inoculation of MSCs into subcutaneous melanoma can induce apoptosis and capillary degeneration and inhibit tumor growth. When it is injected locally in tumor tissue, it can be used as an effective anti-angiogenic factor for anti-tumor Treatment^[19]. At the same time, Bexell and other studies have shown that MSC can be integrated into the vascular wall of tumor tissue^[20]. These studies also suggest that implanting MSCs in tumors may serve as a unique carrier to affect tumor angiogenesis. Thereby inhibiting tumor growth. Currently, HUC-MSCs have been tested for their anti-tumor effects in osteosarcoma, ovarian cancer, and breast cancer^[21,22].

However, whether the inhibition of tumor growth through modulation of the PI3K / AKT signaling pathway in this experiment leads to a decrease in the expression of CD34 and VEGF or whether the inhibition of tumor growth by directly down-regulating the expression of CD34 or VEGF needs to be further explored. In conclusion, the down-regulation of VEGF and CD34 expression after hUC-MSC injection provides a new way for us to control ovarian cancer microangiogenesis and control tumor growth and metastasis.

In summary, conditioned medium of hUC-MSCs and co-culture of hUC-MSCs with SKOV3 can significantly inhibit the proliferation of SKOV3 cells, which is mainly achieved by inhibiting the PI3K / AKT signaling pathway. This study provides a certain theoretical basis and experimental basis for the treatment of ovarian cancer by hUC-MSCs. At the same time, hUC-MSCs can inhibit the growth of ovarian cancer subcutaneously transplanted tumors, providing new ideas for the treatment of ovarian cancer and slowing down the progress. However, because the concentration of stem cells selected in this experiment is too limited, the effect of higher doses of umbilical cord mesenchymal stem cells on it is unclear. The mechanism of hUC-MSCs on tumor is very reexamined. We need to further understand the effect of stem cells on cancer progression and provide scientific guidance for clinical application.

Declarations

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Authors' contributions

Xiaoli Lu perform all the experiences, analyzing results and statistics and contribute in writing, Guangzhi Liu designed the experiments and provided guidance on writing. Lenan Cheng contribute to provide cells from patients, Licong Ge and Ziyi Zhao assist in the experience, Qiuyun Yang contribute in experience design and interpretation of the results, All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animals were maintained in accordance with institutional policies, and all experiments were performed with approval of the Committee on the Use and Care of Animals of the Henan Province People's Hospital. and all patients were asked to read and approve/sign informed consent forms prior to any participation. All experiments involving the handling of human tissues were performed in accordance with Tenets of the Declaration of Helsinki.

Consent for publication

Written informed consent for publication was obtained from the participants before the collection of any samples.

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Competing interests

The authors declare that they have no competing interests.

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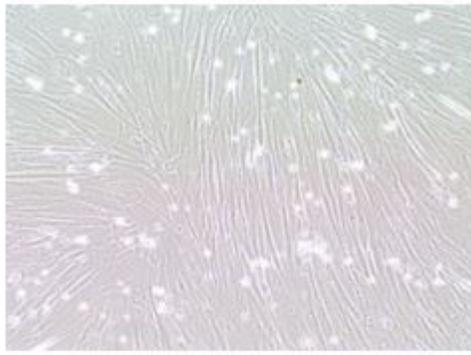
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Table 2

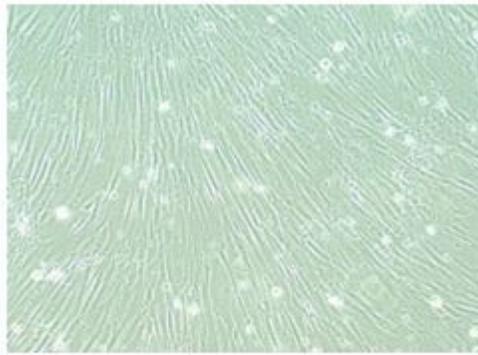
Table 2 Relative expression levels of VEGF and CD34 in tumor tissues when hUC-MSCs were applied to tumor-bearing mice

Group	VEGF	MVD
the control group	7.68±0.50	47.80±6.73
the 1 × 10 ⁶ hUCMSCs group	5.94±0.86	39.32±6.30
the 2 × 10 ⁶ hUCMSCs group	4.44±0.33	28.68±6.05
P-Value	P=0.05	P=0.05

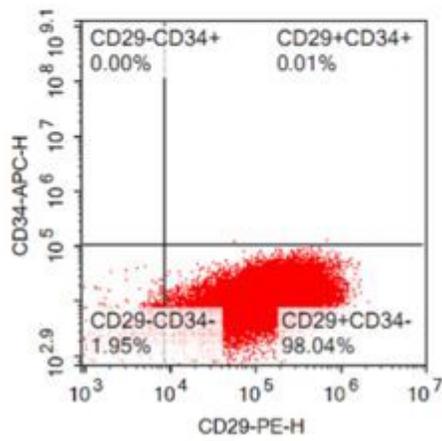
Figures



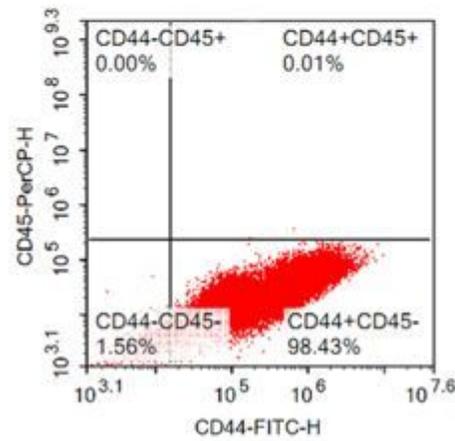
A



B



C



D

Figure 1

hUC-MSCs identification. A: Inverted phase contrast microscope images ($\times 100$) showing that P3 hUC-MSCs ; B: Inverted phase contrast microscope images ($\times 100$) showing that P6 hUC-MSCs; C and D: Flow cytometry showed that hUC-MSCs were positive for CD29, CD44, but were negative for CD34, CD45.

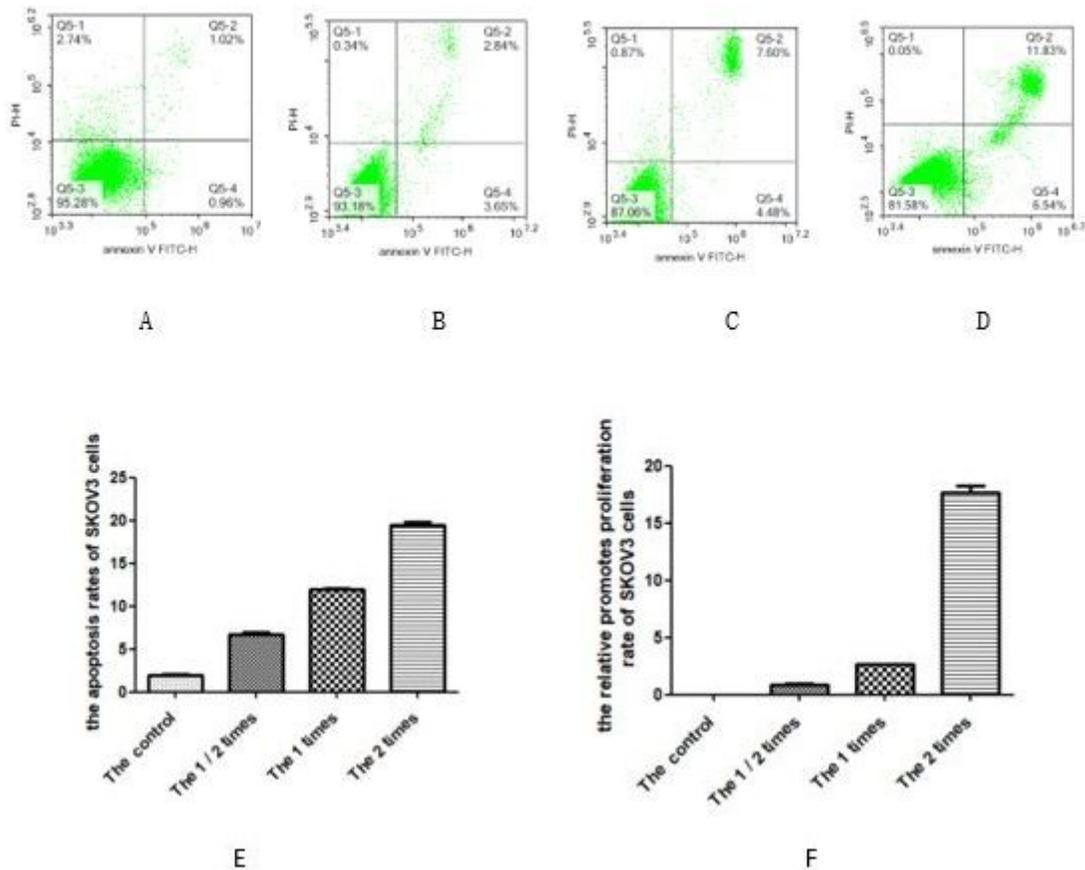


Figure 2

Effect of umbilical cord mesenchymal stem cell lysate on SKOV3 cells: A: The apoptosis of the control group; B: The apoptosis of the 1/2 times group; C: The apoptosis of the 1 times group; D: The apoptosis of the 2 times group; E: The apoptosis rates of SKOV3 cells between the control and 1 / 2 times group, 1 times Group, 2 times group at either 48 h; F: The relative promotes proliferation rate of SKOV3 cells between the control and 1 / 2 times group, 1 times Group, 2 times group at either 48 h

Fig. 3

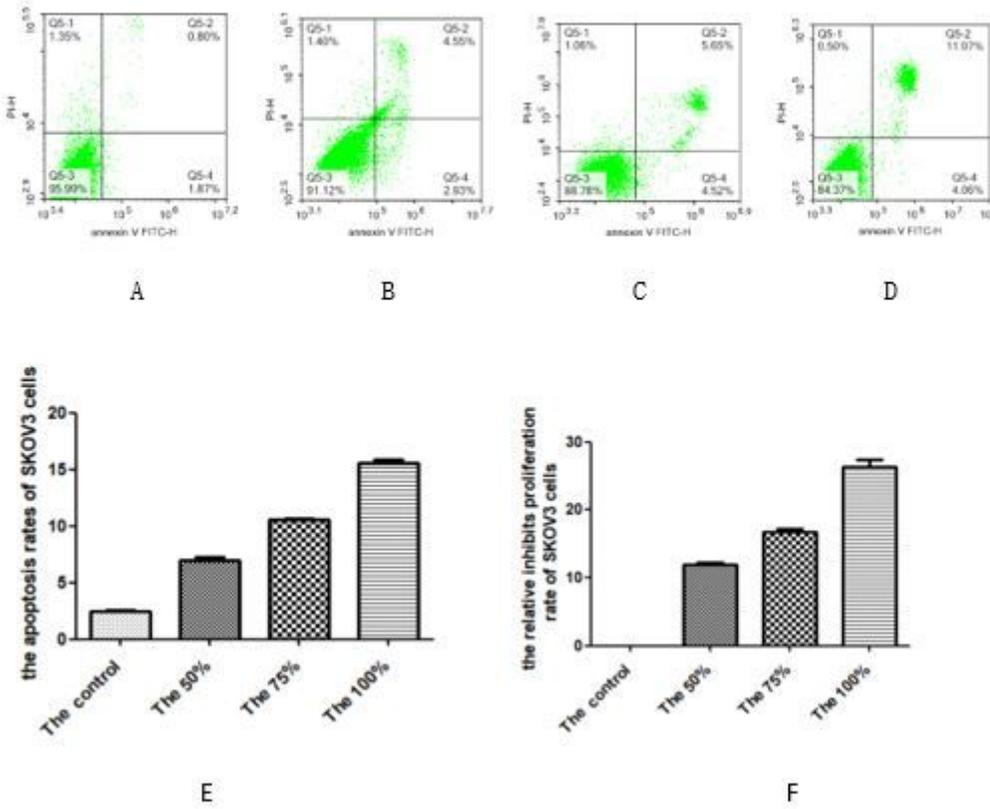


Figure 3

Effect of hUC-MSCs CM on SKOV3 cells: A: The apoptosis of the control group; B: The apoptosis of the 50% group; C: The apoptosis of the 75% group; D: The apoptosis of the 100% group; E: The apoptosis rates of SKOV3 cells between the control and 50% group, 75% Group, 100% group at either 48 h; F: The relative inhibits proliferation rate of SKOV3 cells between the control and 50% group, 75% Group, 100% group at either 48 h

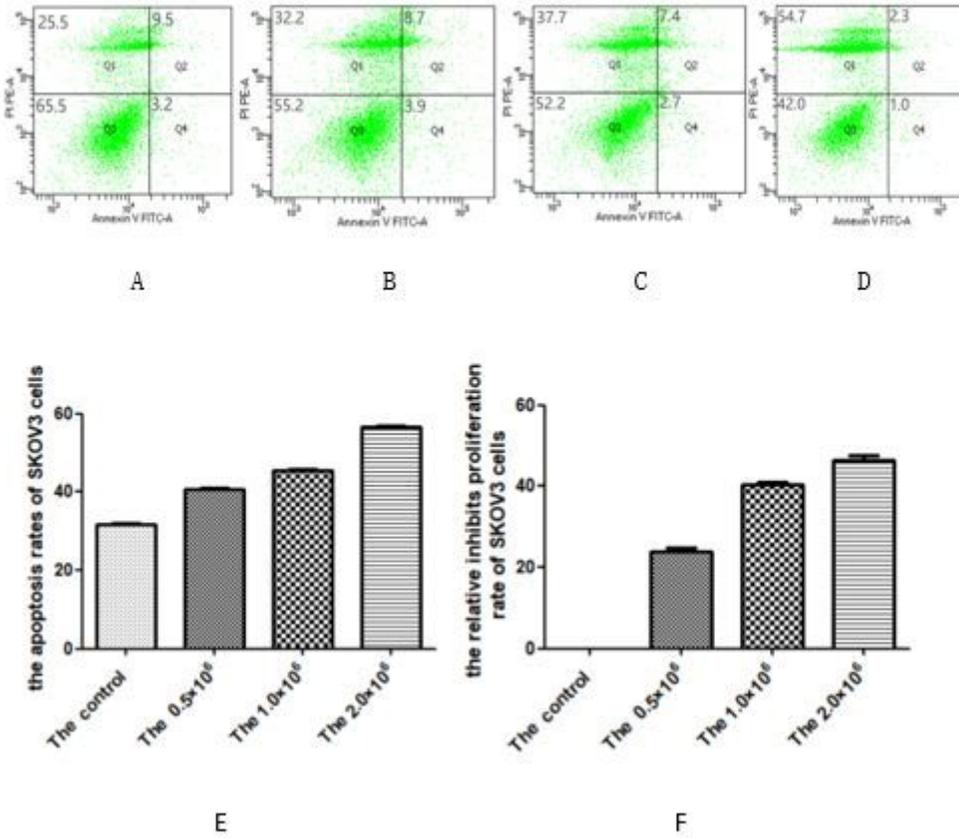
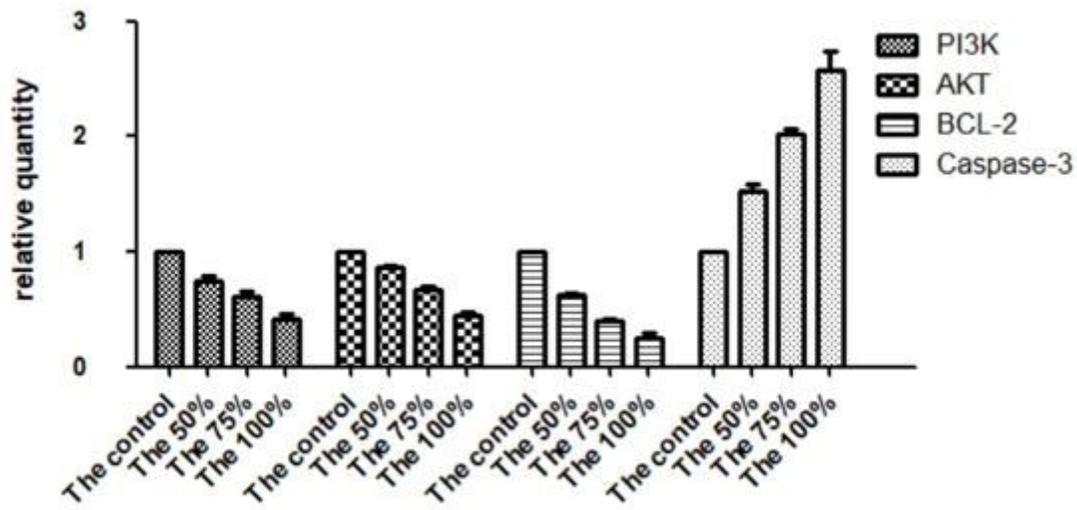
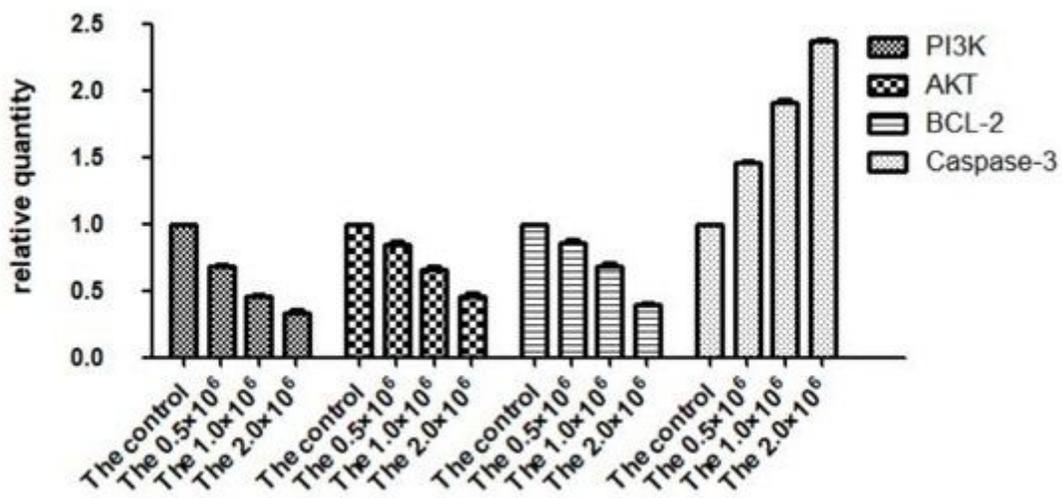


Figure 4

Effect of SKOV3 cells treat with Co-culture of hUC-MSC: A: The apoptosis of the control group; B: The apoptosis of the 0.5 × 10⁶ group; C: The apoptosis of the 1 × 10⁶ group; D: The apoptosis of the 2 × 10⁶ group; E: The apoptosis rates of SKOV3 cells between the control and 0.5 × 10⁶ group, 1 × 10⁶ Group, 2 × 10⁶ group at either 60 h; F: The relative inhibits proliferation rate of SKOV3 cells between the control and 0.5 × 10⁶ group, 1 × 10⁶ Group, 2 × 10⁶ group at either 60 h.



A



B

Figure 5

A,B: The RT-PCR results of conditioned medium and co-culture system on SKOV3 cells

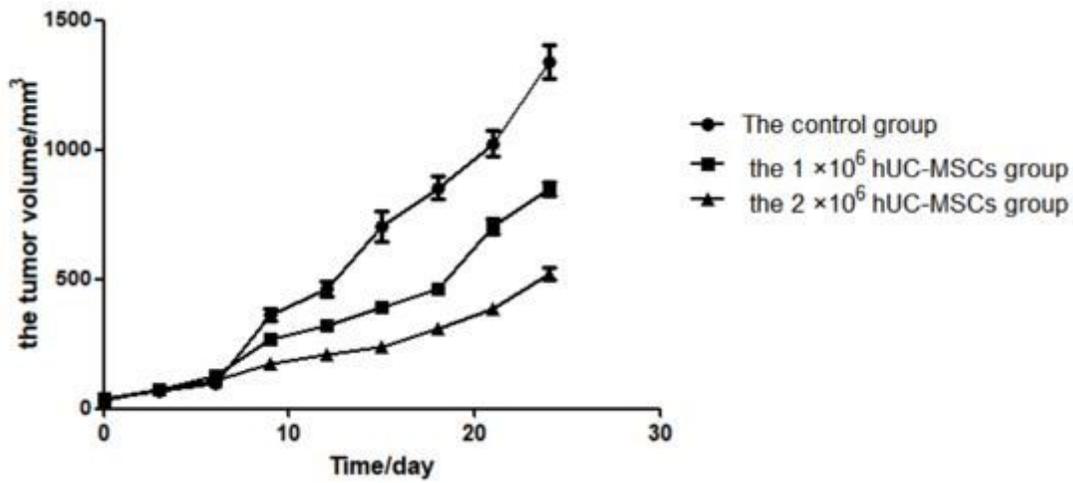
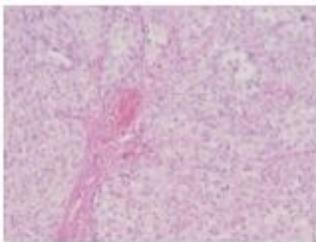


Figure 6

The Volume of xenograft tumors in nude mice bearing each group



A: H&E staining ×200



B: the control group



C: the 1 × 10⁶ hUCMSCs group



D: the 2 × 10⁶ hUC-MSCs group



E: the control group



F: the 1 × 10⁶ hUCMSCs group



G: the 2 × 10⁶ hUC-MSCs group

Figure 7

Role of hUC-MSCs in SKOV3 Ovarian cancer cell-derived tumor xenograft growth in vivo. A:H&E staining of tumor tissue. B,C and D:Immunohistochemical analysis of VEGF expression in tumor tissues($\times 100$). E,G and G:Immunohistochemical analysis of CD34 expression in tumor tissues($\times 100$).