Adipose-derived mesenchymal stem cells (ADSCs) are an important component of the tumor microenvironment. However, whether ADSCs contribute to ovarian cancer progression remains unclear. In this study, we investigated the impact of human ADSCs derived from the omentum of normal donors on human epithelial ovarian cancer (EOC) cells in vitro and in vivo. Direct and indirect co-culture models including ADSCs and human EOC cell lines were established, and the effects of ADSCs on EOC cell proliferation were evaluated by EdU incorporation and flow cytometry. Transwell migration assays and detection of matrix metalloproteinases (MMPs) were performed to assess the invasion activity of EOC cells in vitro. Mouse models were established by intraperitoneal injection of EOC cells with or without concomitant ADSCs to investigate the role of ADSCs in tumor progression in vivo. We found that ADSCs significantly promoted proliferation and invasion of EOC cells in both direct and indirect co-culture assays. In addition, after co-culture with ADSCs, EOC cells secreted higher levels of matrix metalloproteinases (MMPs), and inhibition of MMP2 and MMP9 partially relieved the tumor-promoting effects of ADSCs in vitro. In mouse xenograft models, we confirmed that ADSCs promoted EOC growth and metastasis and elevated the expression of MMP2 and MMP9. Our findings indicate that omental ADSCs play a promotive role during ovarian cancer progression.

1. Introduction

Ovarian cancer has the highest mortality rate of all gynecological malignancies due to early occurrence of metastasis in pelvic and abdominal cavities. Peritoneum is the most common site of ovarian cancer metastasis, and the metastases and malignant ascites accelerate the progress of tumors [1]. Patients without peritoneal metastasis enjoy a 90% five-year survival rate, while those with peritoneal metastasis have a 5-year survival rate of 27% [2].

Tumor microenvironment (TME) defined as a special kind of mesenchymal microenvironment that regulates many aspects of tumorigenesis and plays an important role in invasion and metastasis of ovarian cancer [3]. In the TME, tumor-secreted factors induce local multipotent stromal cells (MSCs) to differentiate into cancer-associated myofibroblasts, which are critical for tumor development [4]. Omental adipose tissue is a multifunctional organ containing different types of cells, and it has been identified as a source of adult multipotent adipose-derived stem cells (ADSCs), which may undergo adipogenic, chondrogenic, myogenic, osteogenic, cardiomyogenic and endothelial differentiation [5]. As an important member of MSCs, ADSCs secrete numerous proangiogenic and immunosuppressive factors, but their roles in tumor growth and progression are still controversial [6,7].

Numerous studies have confirmed the complex and dynamic interplay between cancer cells and resident ADSCs. Muehlberg et al. demonstrated that intravenously injection of ADSCs leaded to their homing to tumor sites in a breast cancer murine model, and promoted the growth and metastasis of breast cancer [8]. Similarly, tumor-homing and tumor promotion effects of ADSCs have been reported in several different mouse models of cancer, such as prostate tumors, lung adenocarcinoma, endometrial tumors, pancreatic cancer and glioblastoma multiforme [9–14]. However, Sun et al. reported inhibition of breast cancer metastasis and growth by implanted ADSCs [15], and Takahara et al. found ADSCs inhibited proliferation of prostate cancer cells by inducing apoptosis [16]. Furthermore, ADSCs could be converted into natural killer-like cells and kill MCF7 breast cancer cells and prostate cancer cells [17].

In the present study, we sought to decipher the role of ADSCs in
ovarian cancer progression. We established direct and indirect co-culture systems as well as tumor xenografts to determine the effect of ADSCs on the cell proliferation and invasion of ovarian cancer cells and to explore the underlying mechanisms.

2. Materials and methods

2.1. Isolation and culture of ADSCs from omentum tissue

Omentum tissues were processed from three healthy adult female donors (BMI range: 22.8–25.6; age range: 39–45 years) who underwent abdominal surgery because of benign gynecologic disease. The characteristics of female donors (N=3) whose omentum tissue was used for ADSC isolation are described in supporting information Table S1. All donors provided written informed consent, and this study was conducted according to institutional guidelines under an approved protocol. ADSCs were isolated from the omentum tissues as previously described [18,19]. In brief, the freshly collected omentum tissues were washed with sterile phosphate-buffered saline (PBS) for several times, and then they were minced into small pieces. Tissue pieces were incubated with 0.1% collagenase (type I; Sigma-Aldrich, St. Louis, MO) in DMEM/F12 (Gibco, Grand Island, USA) for 1 h at 37 °C and agitated gently followed by adding an equal volume of DMEM/ F12 with 10% fetal bovine serum (FBS, Gibco, Australia) to inactivate the collagenase. After centrifugation for 10 min at 300 g, the cellular pellet was resuspended in PBS and filtered through a 100-μm mesh filter. The filtrate was centrifuged again, seeded in culture plates, and cultured in culture medium (DMEM/F12 containing 10% FBS) at 37 °C in a CO2 incubator. ADSCs of 5–6 passages were used in this study.

2.2. Identification of ADSCs

The capacity of ADSCs to differentiate into osteoblasts and adipocytes was assessed. In brief, ADSCs (5 x 10^5/well) were seeded in a six-well plate (Corning, Life Sciences, Amsterdam, the Netherlands). After 12 h, cells were treated with Adipogenesis Differentiation Kit or Osteogenic Differentiation Kit (both from Gibco, Invitrogen Corporation, Carlsbad, CA) according to the corresponding manufacturer’s introductions. A complete medium change was performed twice per week. Twenty-one days after induction, the osteoblast differentiation and adipocyte differentiation were verified by oil red O staining (both from Sigma-Aldrich, St. Louis, MO), respectively. ADSCs were fixed in 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO) for 20 min at room temperature, followed by staining with 0.5% oil red O solution for 60 min or 0.5% alizarin red S for 20 min both at room temperature. The staining results were evaluated under a light microscope.

In addition, the expression of stromal markers on cellular membrane of expanded ADSCs (passage 5) was analyzed by flow cytometry. The presence of CD105, CD73, and CD90 and the absence of hematopoietic and endothelial marker CD34 were used for ADSC identification (CD105-PECy7, CD73-APC, CD90-FITC and CD34-PE antibodies were from eBioscience, San Diego, CA).

2.3. Cell lines and culture conditions

Human EOC cell lines SKOV3, CAOV3, A2780 and ES2 from China Center for Type Culture Collection were used in this study. SKOV3, CAOV3 and ES2 were cultured in DMEM/F12 cultural medium containing 10% FBS. A2780 was cultured in RPMI-1640 with 10% FBS. Primary carcinoma-associated fibroblasts (CAF) in ovarian cancer tissues were isolated and identified as described in our previous study [20]. All of these cell lines were grown in a humidified 37 °C, 5% CO2 incubator.

2.4. Direct and indirect co-culture

For direct co-culture, ADSCs mixed with CFDA-labeling ovarian cancer cells (CFDA, V12883; Invitrogen, Carlsbad, CA) in a 1:5 ratio (ADSCs 0.2 x 10^5 cells/well, cancer cells 1 x 10^5 cells/well) were seeded in six-well plates and cultured in cultural medium (DMEM/ F12 with 10% FBS for SKOV3, CAOV3 and ES2; RPMI-1640 with 10% FBS for A2780) for at least 4 days. Transwell plates (Corning, Life Sciences, Amsterdam, the Netherlands) with 0.4 μm pore polycarbonate membrane insert were used for indirect co-cultures. ADSCs and CAFs were seeded in the lower compartment (0.2 x 10^5 cells per well) and EOC cells in the upper compartment (1 x 10^5 cells per well). Cells were cultured for 4–6 days together. EOC cells cultured alone were used as negative control, and those co-cultured with CAFs were used as positive control.

2.5. Multiplex analysis

Cell culture supernatant of EOC cells indirectly co-cultured with ADSCs for 4 days or cultured alone were collected and assayed on a Bioplex (Bio-Rad) cytometer. In this study, we used Bio-Plex Pro Hu MMPs 9–plex Assay, which contains beads for detection of MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12 and MMP-13. Assays were performed following the manufacturer’s instructions. Bio-Plex Manager Software was used to analyze the results.

2.6. Western blotting

Cells were lysed with NP40 buffer (Beyotime, Shanghai, China) for 10 min on ice, and then centrifuged at 10,000 g at 4 °C to remove cell debris. Equal amount (30 μg) cell extracts were resolved by SDS-PAGE and transferred to a PVDF membrane (Bio-Rad, Hercules, CA) followed by incubating with primary rabbit monoclonal antibody against human MMP2, MMP9 and α-tubulin (1:1000 dilution; ProteinTech groups inc, Chicago, IL). Proteins were detected using peroxidase-conjugated Affiniture secondary antibodies IgG(H+L) (1:2000; ProteinTech groups inc, Chicago, IL), which was visualized by a chemiluminescence detection system. Bands were quantified by Image Lab™ Software, Version 5.1 (both from Bio-Rad, Hercules, CA).

2.7. Flow cytometry

The phenotype modification of the ADSCs indirectly co-cultured with EOC cells were analyzed by flow cytometry as previously described [21]. Briefly, 1 x 10^6 cells were incubated with CD105-PECy7, CD73-APC, CD90-FITC and CD34-PE antibodies in 100 μL PBS for 40 min in the dark at room temperature. After washing with PBS, cells were analyzed on a BD LSR II flow cytometer (BD Biosciences). FACSDiva software (BD Biosciences) was used for data acquisition and analysis.

Carboxyfluorescein diacetate succinimidyl ester (CFSE) is a cellular dye of high chemical stability and low toxicity. By halving the fluorescence value for each successive generation, the number of divisions can be measured by flow cytometry. In this study, CFSE (Invitrogen, Eugene, Oregon, USA) was added to the EOC cells (5 x 10^6) at a final concentration of 10 mM. After incubation at 37 °C for 30 min, cells were washed with PBS, pelleted by centrifugation and resuspended in a fresh medium. The CFSE-labeled cells were divided into three groups: parental group, control group, and co-culture group. The cells in the parental group were fixed in 4% paraformaldehyde for 60 min, resuspended in PBS and...