Article

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Impact of Adipose-Derived Stem Cell Microenvironment on Colon Cancer Progression

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Background: Malignant tumor cells can directly affect the biological behavior of the cells through the interaction with the surrounding microenvironment. This study aimed to investigate the effects of adipose-derived stem cell microenvironment (ADSCM) on the growth of colon cancer (CC) and the expression of malignant surface markers and abnormal pathways in the cells.

Method: Adipose-derived stem cells (ADSCs) were extracted using collagenase digestion, followed by culture and identification. A three-dimensional stem cell microenvironment was established and co-cultured with CC cell lines. Furthermore, clonogenic assays were conducted to assess cell proliferation. Annexin V staining was employed to detect cell apoptosis and an invasion assay was performed to study cell migration capabilities. Moreover, immunohistochemistry and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were employed to assess the expression levels of surface markers in CC cells, and WB analysis was used to determine the levels of signaling pathway proteins. A CC model was established by subcutaneous injection of ADSCs and CC cells into Balb/c mice. Additionally, western blot (WB) was performed to investigate changes in inflammatory factors within tumor tissues.

Result: After introducing CC cell lines into the ADSCM, a significant decrease in cell proliferation and invasion capability was observed, accompanied by a substantial increase in apoptosis rate. Furthermore, CC cells grown within the microenvironment exhibited reduced malignant phenotypic features, and the expression levels of common cancer signaling pathways were also diminished. Additionally, there was a decrease in the content of inflammatory factors within CC tissues.

Conclusion: ADSCM can inhibit the growth of colon cancer, reduce the malignant markers and abnormal pathways in cancer cells and hinder the progression of CC.

Keywords: adipose-derived stem cell microenvironment; growth transfer; colon cancer

Introduction

Colon cancer (CC), a malignant tumor arising from the epithelial cells of the colon or rectum, ranks as the third most common cancer type worldwide. Its incidence rates vary across different regions, primarily affected by various factors such as diet, lifestyle, and genetic predisposition, with occurrence increasing with age [1]. CC is often characterized by rapid cell growth and metastasis, posing a significant threat to the patient's survival and quality of life. Although conventional treatment approaches, including surgical intervention, radiation therapy, and chemotherapy offer certain effectiveness, they still pose challenges in treating advanced and recurrent CC patients [2]. The malignant phenotype of CC cells is crucial in driving disease progression, directly impacting tumor growth, spread, invasion, and treatment response. Common protein markers such as Carcinoembryonic antigen (CEA), cytokeratin-7 (CK-7), cytokeratin 20 (CK20), and an anti-epithelial cells monoclonal antibody (BerEP4) are highly expressed, promoting the growth and spread of CC. In contrast, the reduced caudal-related homeobox gene 2 (CDX2) expression in CC, is associated with disease severity. Malignant CC cells disrupt the normal cell growth process, leading to unregulated cell division and infiltration into adjacent tissues and organs. This increase in tumor volume facilitates the migration of the primary tumor to other sites, forming metastatic lesions, such as liver metastases. Therefore, there is an urgent need to explore new treatment approaches.

CC, being a highly prevalent malignant tumor, involves intricate and complex interactions among various cell types, signaling pathways, and microenvironmental factors, contributing to its onset and progression. Within CC, the overactivation of the protein kinase B/phosphatidylinositol-4,5-bisphosphate 3-kinase/mechanistic target of rapamycin complex 1 (Akt/PI3K/mTORC1) and Wnt/β-catenin pathways can stimulate the proliferation of cancer cells and enhance their resistance to apoptosis, thereby promoting disease progression [3,4]. While traditional cancer research has primarily

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focused on cancer cells, recent research has demonstrated the significant involvement of the tumor microenvironment in the progression, expansion, and metastasis [5,6]. The tumor microenvironment comprises various components, including different cell types interacting with cancer cells, extracellular matrix, immune cells, blood vessels, and growth factors [7,8]. Therefore, investigating how the tumor microenvironment influences CC behavior and interventions to mitigate these effects has become a crucial focus in cancer research.

Stem cells are characterized by unique features of self-renewal and differentiation potential [9]. These cells hold a crucial position in biology by virtue of their ability to differentiate into diverse cell types, thereby contributing to tissue regeneration and the sustenance of vital life processes [10]. Stem cells can originate from various origins, ranging from embryonic sources to those derived from mature tissues. Embryonic stem cells, derived from early-stage embryos, exhibit the highest degree of versatility regarding their differentiation potential. On the other hand, adult stem cells are primarily located in mature tissues, including bone marrow, adipose tissue, and skin, and under certain conditions, they can differentiate into various functional cell types.

Furthermore, due to their diverse potential functions, including organ and tissue regeneration, as well as their capabilities of treatment or amelioration of severe diseases, they are often referred to as "multipotent cells" in the medicine [11,12]. Adipose-derived stem cells (ADSCs) are a crucial and versatile subset of stem cells, initially identified within the adipose tissues of both humans and animals in the 1960s. These stem cells reside within the body's adipose tissue, playing a significant role not only in energy storage but also in crucial functions such as tissue repair, immune regulation, and hormone secretion [13,14]. This translation maintains coherence while minimizing similarity with other academic papers. Recent research suggests that ADSCs can potentially impact the tumor microenvironment [15]. Furthermore, they can secrete various cytokines, growth factors, and hormones, thereby influencing tumor growth and immune responses [16,17]. Moreover, recent research has suggested their potential role in impacting malignant tumors such as colon cancer [18].

The adipose-derived stem cell microenvironment (ADSCM) encompasses the interplay between adipose stem cells and CC cells, along with the environmental conditions generated by these interactions in the proximity of the tumor. Three-dimensional cell culture technology offers an experimental model resembling the *in vivo* biological environment. By constructing a three-dimensional microenvironment primarily featuring adipose-derived stem cells and co-culturing them with CC cells within this 3D framework, researchers can investigate the impact of the ADSCM on tumor growth, invasion, and metastasis. This approach aims to identify potential therapeutic strategies for modulating the tumor microenvironment, ultimately enhancing the treatment outcomes for CC patients.

Introducing adipose stem cells as a treatment approach for CC is an emerging therapeutic strategy that is still in the early research stage. This research holds the promise of providing a theoretical foundation for developing novel treatment strategies, thereby paving new avenues to increase cancer treatment outcomes, enhance patient survival rates, and improve their quality of life. Nevertheless, further research is required to delve into the mechanisms by which the three-dimensional ADSCM influences CC, which is crucial for advancing the development of improved therapeutic approaches.

Materials and Methods

Isolation, Culture, and Identification of Adipose Stem Cells

Under aseptic conditions, 5 grams of subcutaneous fat tissue was extracted from the abdomen of C57BL/6 male mice aged 6–8 weeks (Slake Jingda Laboratory Animal Company, Changsha, China) using vacuum liposuction. The adipose tissue was thoroughly washed with PBS 3 times and was cut into small sections. Subsequently, the fat tissue was immersed in a solution of 0.2% Type I collagenase (17100017, Life Technologies Corporation, Waltham, MA, USA) at a volume twice that of the tissue and digested at 37 °C in a water bath for 60 minutes. After digestion, the cell filtrate was centrifuged at 1800 r/min for 10 min, the upper-middle layer was discarded, and the concentration of cells was adjusted to 1×10^8/L using a 70 μm cell strainer. The isolated adipose-derived stem cells (ADSCs) were cultured with fresh, complete dulbecco's modified Eagle medium (DMEM) medium containing 10% fetal bovine serum (FBS). The cell cultures were maintained in a 5% CO_2 cell incubator at 37 °C, with fluid replacement every 2 days and removal of non-adherent cells. When the confluence of primary ADSCs reached 80%–90%, the cells were passaged, and cells from 3–5 generations were selected for subsequent experiments. All operations were aseptically performed to prevent cell contamination. This study was approved by the ethics committee of the Third Ward of Affiliated Hengyang Hospital of Hunan Normal University & Hengyang Central Hospital, China (NU20237799).

Establishment of Mouse ADSCM Model

After digesting adipose stem cells with 0.1% collagenase IV enzyme (17104019, Life Technologies Corporation, Waltham, MA, USA) and washing them with PBS, approximately 5×10^4 cells were seeded into a three-dimensional extracellular matrix gel with reduced growth factors. The cells were then cultured in a stem cell medium for 3 days. Subsequently, 20 mM NH_4OH (PC015, Shanghai Zhoufu Science and Trade Co., Ltd., Shanghai, China) was added to the matrix gel to remove embryonic stem cells. After thorough washing with double distilled water and PBS was performed, a three-dimensional microenvi-

ronment was acquired, having undergone embryonic stem cell treatment but without any cells. Subsequently, CC cell lines CT26 and MC38 in the logarithmic growth phase were seeded at a concentration of 2.5×10^5/mL into the matrix gel. After 4 days of incubation, cells were extracted for further experiments. The control group included CC cells directly seeded into the matrix gel without undergoing stem cell treatment.

Cell Culture

The cell lines CT26 (YS3022C, YaJi Biological, Shanghai, China) and MC38 (YS997C, YaJi Biological, Shanghai, China) were cultivated using a growth medium containing 10% fetal bovine serum (FBS; 16140071, Gibco, Grand Island, NY, USA) and 5% antibiotics (15240062, Gibco, Grand Island, NY, USA). These cells were maintained in a constant-temperature incubator at around 37 °C with a controlled humidity level and 5% CO_2. This culture condition is essential to support optimal cell growth and viability for subsequent experiments. The cells underwent STR identification and mycoplasma detection, and there was no mycoplasma infection.

Clone Formation Experiment

CT26 and MC38 cells in the logarithmic growth phase were seeded into six-well plates at a density of 1500 cells per well and cultured in DMEM medium containing 10% FBS. The cells were incubated at 37 °C with 5% CO_2, and the culture medium was refreshed every 3 days. This culture process was sustained for a duration of 2–3 weeks. Upon visible cell growth in the culture dish, cultivation was stopped. The supernatant was removed, the cells were rinsed twice with PBS, and fixed with 4% paraformaldehyde for 15 minutes. Subsequently, the cells were stained with 0.1% crystal violet dye for 20 min. Finally, the staining solution was gently rinsed with tap water, and the cells were air-dried, followed by cell counting.

Transwell

The CC cells were seeded at a density of 5×10^5 cells onto the upper chamber of the Transwell's inserts (BD Biosciences, San Diego, CA, USA), pre-coated with 50 µL of Matrigel at a concentration of 200 mg/mL. Simultaneously, the lower chamber was filled with 600 µL of DMEM nutrient medium containing 10% FBS and incubated at 37 °C in a 5% CO_2 environment for 48 hours. Subsequently, the cells in the lower chamber were fixed by immersing them in a solution containing methanol and glacial acetic acid mixed in a 3:1 ratio for 30 minutes. After washing with PBS, cells were stained using 0.1% crystal violet dye. Finally, the cells were counted in five randomly selected fields utilizing a microscope (CKX53, Olympus Corp, Tokyo, Japan).

Flow Cytometry

The surface markers of ADSCs were assessed using flow cytometry. The cells were washed with PBS and then incubated each group of 10^5 cells with the primary antibodies at 4 °C for 30 minutes in the dark. The primary antibodies used in this process were as follows: anti-CD31 (ab306495, Abcam, Cambridge, UK) and anti-CD45 (ab210224, Abcam, Cambridge, UK) conjugated with APC, anti-CD34 (ab131589, Abcam, Cambridge, UK) and anti-CD105 (ab184667, Abcam, Cambridge, UK) conjugated with FITC, anti-CD73 (ab282789, Abcam, Cambridge, UK), anti-CD90 (ab24904, Abcam, Cambridge, UK), and anti-CD235a (MA5-17004, Thermo Fisher Scientific, Waltham, MA, USA) conjugated with PE. Subsequently, the cells were washed with PBS and analyzed using flow cytometry (660585, BD Technologies, Franklin Lake, NJ, USA).

We determined the apoptosis rate in the CC cell lines CT26 and MC38. For this purpose, the cells were rinsed with cold PBS and resuspended using fluorescein isothiocyanate (FITC) binding buffer at a concentration of 1×10^6 cells/mL. Next, 100 µL of cell suspension was added to an EP tube, followed by the sequential addition of the appropriate amounts of Annexin V-FITC and PI (abs50001a, absin, Shanghai, China). The mixture was then incubated for 20 minutes at room temperature in the dark. Finally, the cell apoptosis rate was determined using a flow cytometer (BD Technologies, Franklin Lake, NJ, USA).

Immunohistochemistry

The CT26 and MC38 cells were cultured on glass slides and then placed on PBS (Thermo Fisher Scientific, Waltham, MA, USA) containing 1 mL of 4% PFA (BL-G002, Nanjing Senbeijia Biological Technology Co., Ltd., Nanjing, China), followed by incubation at room temperature for 1 hour for fixation. Subsequently, they underwent three PBS washes, with each wash lasting for 10 minutes. Following this, the cells were treated with 10% normal goat serum (SBJ-SE-GO012, SenBeiJia Biological Technology Co., Ltd., Nanjing, China) for 1 hour, followed by another PBS wash. Subsequently, the slides containing the cells were incubated overnight at 4 °C with anti-CEA (abs110605, absin, Shanghai, China), anti-CK-20 (abs149777, absin, Shanghai, China), anti-CK-7 (abs149967, absin, Shanghai, China), anti-CDX2 (abs137086, absin, Shanghai, China), and anti-Ber-EP4 (abs149892, absin, Shanghai, China) antibodies. The next day, a goat anti-rabbit secondary antibody (abs998, absin, Shanghai, China) was applied to the cells and incubated at room temperature for 60 minutes. After this, a DAB reagent (PH0728, Yunke Biotechnology Co., Ltd., Taizhou, China) was used for the chromogenic reaction. Finally, counterstaining was performed with hematoxylin, and stained sections were captured using a microscope (Hitachi, Japan, Tokyo).

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Table 1. The list of primers used in quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Gene	F (5′-3′)	R (5′-3′)
CEA	ACTCAACAAGCTACCCCAGG	TGTCTCTCGACCGCTGTATG
CK20	GAACGACAGATTGGCAAACTACC	GATCAGCTTCCACAGCTATACGC
CK-7	ACCAGCGTGCCAAGTTAGAGTC	GATATTCACAGGTCCCATTCCGT
CDX2	AATACCACGCGCACCATCAC	TTCCGCATCCACTCGCACA
BerEP4	CGAAGAACCGACAAGGACACG	TGACTGCTAATGACACCACCACA
PI3K	GAGATGTTACAGAAAGTCACCA	CTGTGAAATAACTTGGGAACTG
Akt	TCACCCAGTGACAACTCAG	AAACTCGTTCATGGTCACAC
mTORC1	CGATGGTCGGATACATTTTAGGC	TGCTTTCTTATGGGCTGGTTCT
Notch-1	GGTGCTCTGATGGACGACAA	ATGTAACGGAGTACGGCCCA
Wnt	AACAGTAGTGGCCGATGGTG	CTCTGGAGCCCTCCACTCA
β-catenin	GGCCTCTGATAAAGGCTACTGTTG	ACGCAACGGTGCATGATTTG
Slit2	CAGTAACCCATGTAAACATGGAG	CCATCAGCACAAATACACCAG
Robo1	GCATCGCTGGAAGTAGCCATACT	CTAGAAATGGTGGGCTCAGGAT
IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
IL-8	GCATCTTCGTCCGTCCCTG	GCCAACAGTAGCCTTCACCCAT
IL-10	GACAACATACTGCTAACCGACTCCT	GCCTGGGGCATCACTTCTACC
GAPDH	TCAAGATCATCAGCAATGCC	CGATACCAAAGTTGTCATGGA

CEA, Carcinoembryonic antigen; *CK20*, cytokeratin-20; *CK-7*, cytokeratin-7; *CDX2*, caudal-related homeobox gene 2; *BerEP4*, an anti-epithelial cells monoclonal antibody; *PI3K*, phosphatidylinositol-4,5-bisphosphate 3-kinase; *Akt*, protein kinase B; *mTORC1*, mechanistic target of rapamycin complex 1; *Notch-1*, Notch receptor 1; *Wnt*, Wingless-Related Integration Site; *β-catenin*, Beta-Catenin; Slit2, Slit guidance ligand 2; *Robo1*, Roundabout guidance receptor 1; *IL-1β*, interleukin-1 beta; *IL-8*, interleukin-8; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted employing the Trizol reagent kit (abs9331, absin, Shanghai, China) and was reverse transcribed into cDNA using a cDNA reverse transcription kit (abs601510, absin, Shanghai, China). mRNA levels were assessed using SYBR Green PCR Master Mix (Takara, Kyoto, Japan). The relative expression levels of the target genes were calculated utilizing the $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH)* as the internal control. The list of primers used in qRT-PCR is given in Table 1.

Western Blot Analysis

Total protein was extracted using RIPA buffer (abs9229, absin, Shanghai, China) and quantified utilizing a BCA assay kit (abs9232, absin, Shanghai, China). Proteins were resolved through SDS-PAGE gel electrophoresis and subsequently transferred to a PVDF membrane (abs931, absin, Shanghai, China). The membrane underwent blocking using 5% skim milk at room temperature for two hours. Following this, the membrane was incubated overnight with antibodies targeting Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) (1:1000, ab302958, abcam, Cambridge, UK), protein kinase B (Akt) (1:1000, ab138504, Abcam, Cambridge, UK), mechanistic target of rapamycin complex 1 (mTORC1) (1:1000, ab120224, Abcam, Cambridge, UK), Notch receptor 1 (Notch-1) (1:1000, ab52627, Abcam, Cambridge, UK), Wnt (1:1000, ab219412, Abcam, Cambridge, UK), β-catenin (1:1000, ab305261, Abcam, Cambridge, UK), Slit guidance ligand 2 (Slit2) (1:1000, ab246503, Abcam, Cambridge, UK), and Roundabout guidance receptor 1 (Robo1) (1:1000, ab7279, Abcam, Cambridge, UK) at 4 °C. The following day, the unbound antibodies on the membrane were washed, followed by incubation with goat anti-rabbit IgG secondary antibody (1:1000, ab96899, Abcam, Cambridge, UK) at room temperature for 2 hours. The membrane was treated with an Enhanced Chemiluminescence (ECL) detection kit (abs9434, absin, Shanghai, China), and the protein bands were visualized employing a photosensitive luminescence analyzer system (Amersham Imager 600, Aylesbury, Buckinghamshire, UK) and analyzed using Image J software (V1.8.0.112, NIH, Madison, WI, USA).

Heterologous Transplantation of Tumors

Male BALB/c nude mice (n = 30), aged 6–8 weeks and weighing 18–22 g, were purchased from Hunan SJA Laboratory Animal Co., Ltd., Changsha, China and were raised under specific pathogen-free conditions. The mice were randomly divided into 6 groups (CT26 cells: the CT26 group, the co-culture group, the the gamma-secretase inhibitor N-[N-(3,5-diuorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester (DAPT) group, MC38 cells:

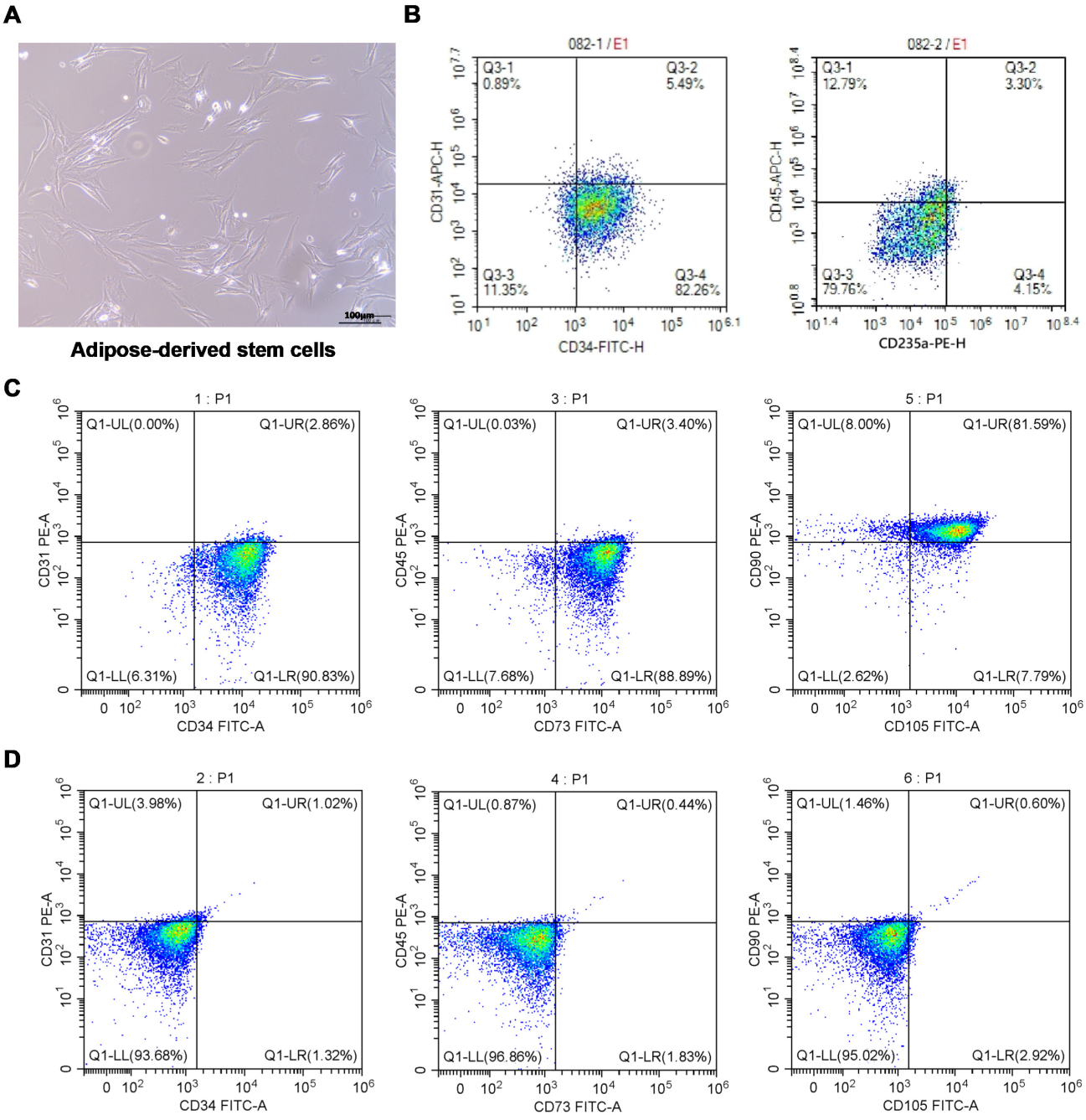

Fig. 1. Effect of ADSCM on colon cancer cell growth. (A) Adipose-derived stem cells (ADSCs). (B,C) Adipose-derived stem cell phenotype. (D) Adipocyte phenotype (negative control group). n = 3. ADSCM, adipose-derived stem cell microenvironment, CC, colon cancer.

the MC38 group, the co-culture group, and the DAPT group), with 5 mice in each. The CT26 and MC38 cells (3×10^5 cells) were subcutaneously inoculated into mice. Once the tumor size reached 350 ± 50 mm^3, the Notch 1 pathway inhibitor DAPT (10 mg/kg, dissolved dimethyl sulfoxide (DMSO) was administered subcutaneously once every 4 days to investigate its effect on tumor growth. Tumors were measured with calipers, and the volumes were calculated using the formula as follows: (3.14 × length × width × depth)/6. After 28 days, mice were euthanized with sodium pentobarbital (150 mg/kg), and tumor tissue and serum samples were collected. The isolated tumors were weighed and stored at –30 °C for subsequent analysis. The experimental protocol adhered to the principles outlined in the "Guidelines for Experimental Animals" and was approved by the Ethics Committee of Third Ward of Affiliated Hengyang Hospital of Hunan Normal University & Hengyang Central Hospital, China (NU20237799).

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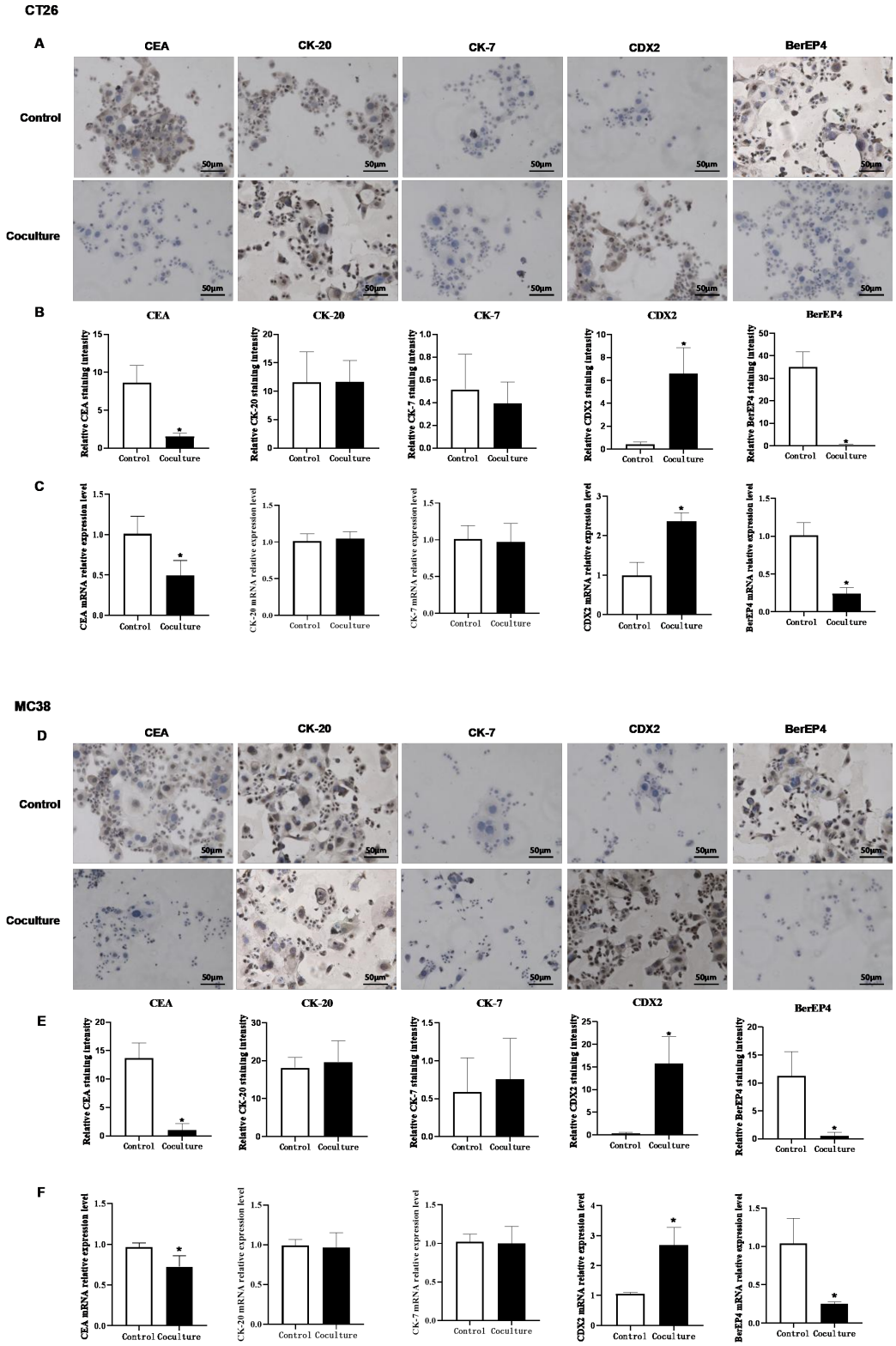

Fig. 2. Effect of ADSCM on the malignant phenotype of colon cancer cells. (A–C) Changes in the malignant characteristics of CT26 CC cells. (D–F) Changes of the malignant phenotype in MC38 CC cells. n = 3. $^{*}p < 0.05$ versus the control group. CEA, carcinoembryonic antigen; CK-7, cytokeratin-7; CDX2, caudal related homeobox gene 2.

Enzyme-Linked Immunosorbent Assay (ELISA)

The expression levels of interleukin-1 beta (IL-1β), interleukin-8 (IL-8), and interleukin-10 (IL-10) were assessed using their corresponding standard ELISA assay kits (Jiangsu Meimian Industrial Co., Ltd., IL-1β: MM-0132M1, IL-8: MM-0123M1 and IL-10: MM-0163M1),

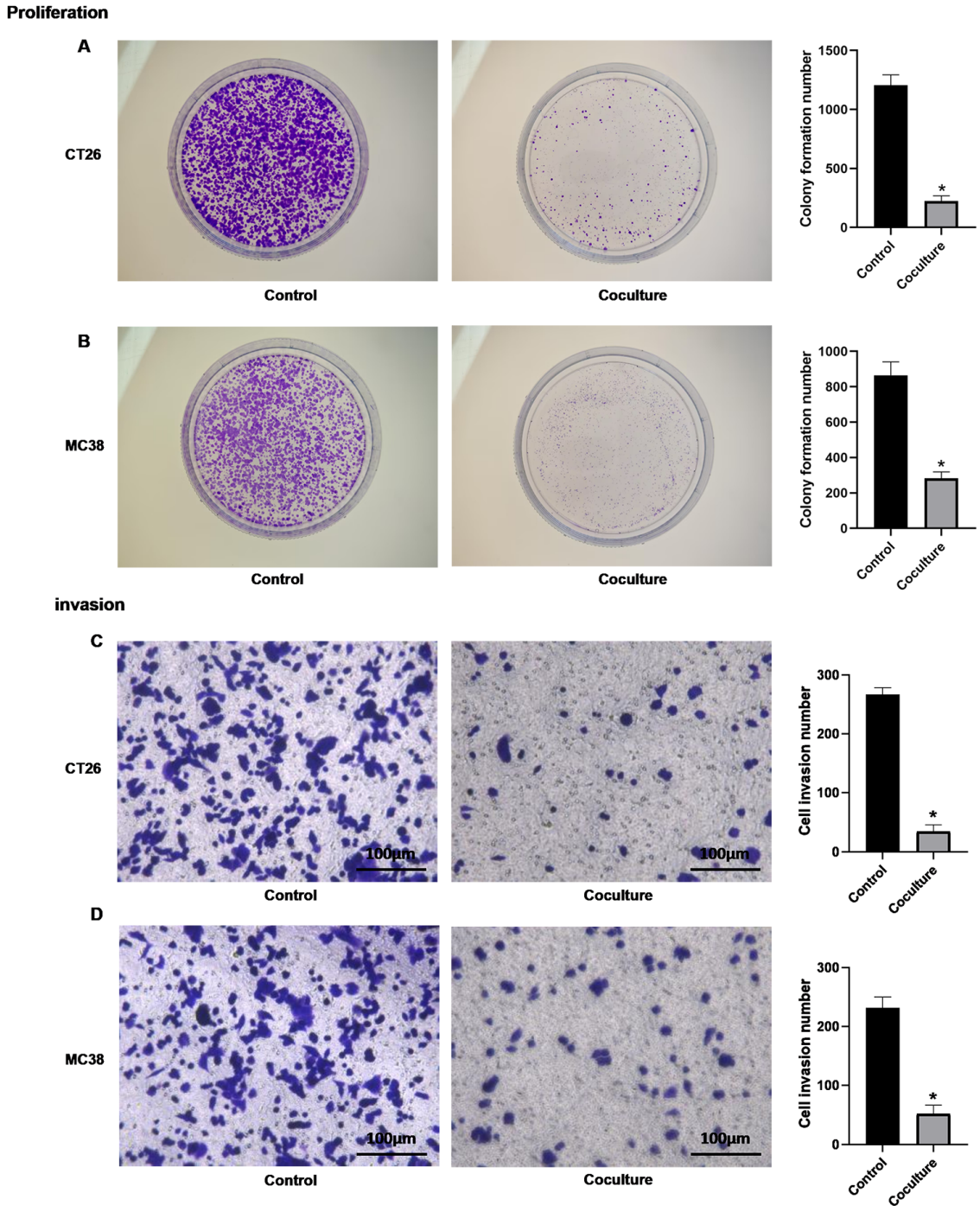

Fig. 3. Effects of ADSCM on proliferation and invasion of CC cell lines CT26 and MC38. (A,B) Proliferation and invasion of CT26 cells. (C,D) Proliferation and invasion of MC38 cells. n = 3. $^{*}p < 0.05$ versus the control group.

following the manufacturer's instructions. The serum samples were centrifuged to obtain serum supernatant. The absorbance (450 nm) of each sample was observed using a standard automatic microplate reader (BioTek, Winooski, VT, USA).

Statistical Analysis

The statistical analysis was conducted with GraphPad Prism 8.0 software (Dotmatics, Boston, MA, USA), and the data were expressed as mean ± standard deviation. To evaluate the differences between two or more groups, the student *T*-test, Mann-Whitney U test, or one-way analysis of variance (ANOVA) was used, followed by the Tukey method for comparative analysis. Statistical significance was determined at a p-value < 0.05.

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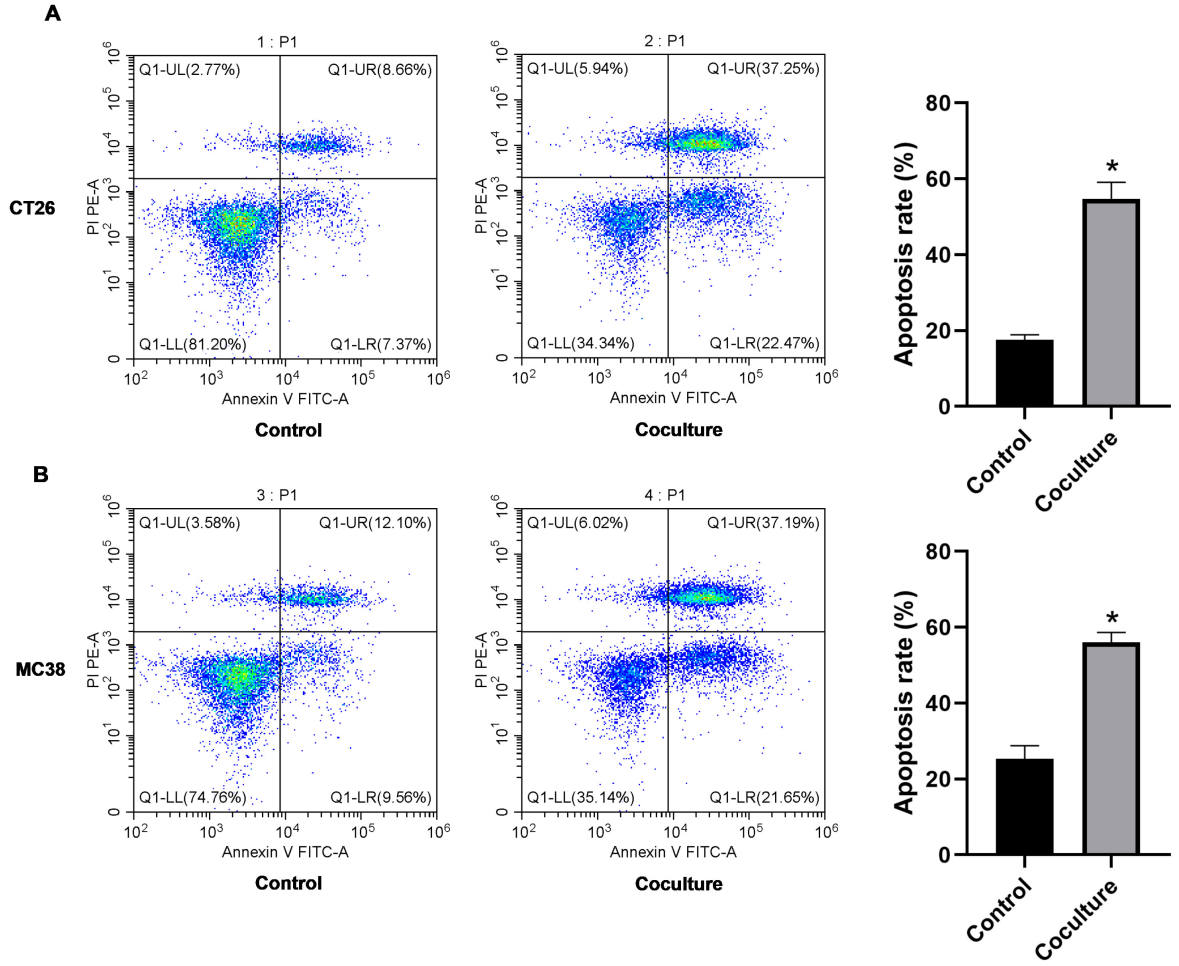

Fig. 4. Effect of ADSCM on apoptosis of colon cancer cells. (A,B) Apoptosis of CT26 and MC38 cells. n = 3. $^{*}p < 0.05$ versus the control group.

Results

Cultivation and Identification of Adipose Stem Cells and Establishment of a Three-Dimensional Stem Cell Microenvironment Model

Adipose-derived stem cells (ADSCs) were extracted from adipose tissue through collagenase digestion (Fig. 1A), and the surface markers of adipose-derived stem cells were assessed through anti-human antibodies using flow cytometry analysis. The initially harvested cells were found to express CD31-/CD34+/CD45-/CD235a- surface markers (Fig. 1B). After the specific incubation period, compared with the negative control group, the cell phenotypes of ADSCs were CD31-/CD34+/CD45-/CD73+/CD90+/CD105+ (Fig. 1C,D).

Impact of the ADSCM on the Malignant Phenotypes of CT26 and MC38 Cells

Immunohistochemical analysis of malignant features in CC cells revealed that in both CT26 and MC38 cells, compared to the control group, the co-culture group exhibited minimal changes in CK-20 and CK-7 positivity rates. However, there was a significant decrease in the positivity rates of CEA and BerEP4, along with an increase in CDX2 positivity (Fig. 2A–F) ($p > 0.05$).

The Impact of the ADSCM on the Proliferation and Invasive Capability of CT26 and MC38 Cells

We observed that co-culture of CC cell lines CT26 and MC38 with ADSCs led to a reduction in both cell proliferation and invasion capabilities compared to the control group (Fig. 3A–D) ($p > 0.05$). This observation suggests that the ADSCM exerts a specific inhibitory effect on the growth and metastasis of CC cells.

The Impact of ADSCM on Apoptosis Rate in CT26 and MC38 Cells

As depicted in Fig. 4A,B, co-culture of CC cells CT26 and MC38 resulted in an increased apoptosis rate compared to the control group ($p > 0.05$). This suggests that the ADSCM may promote apoptosis in CC cells, potentially improving the progression of CC.

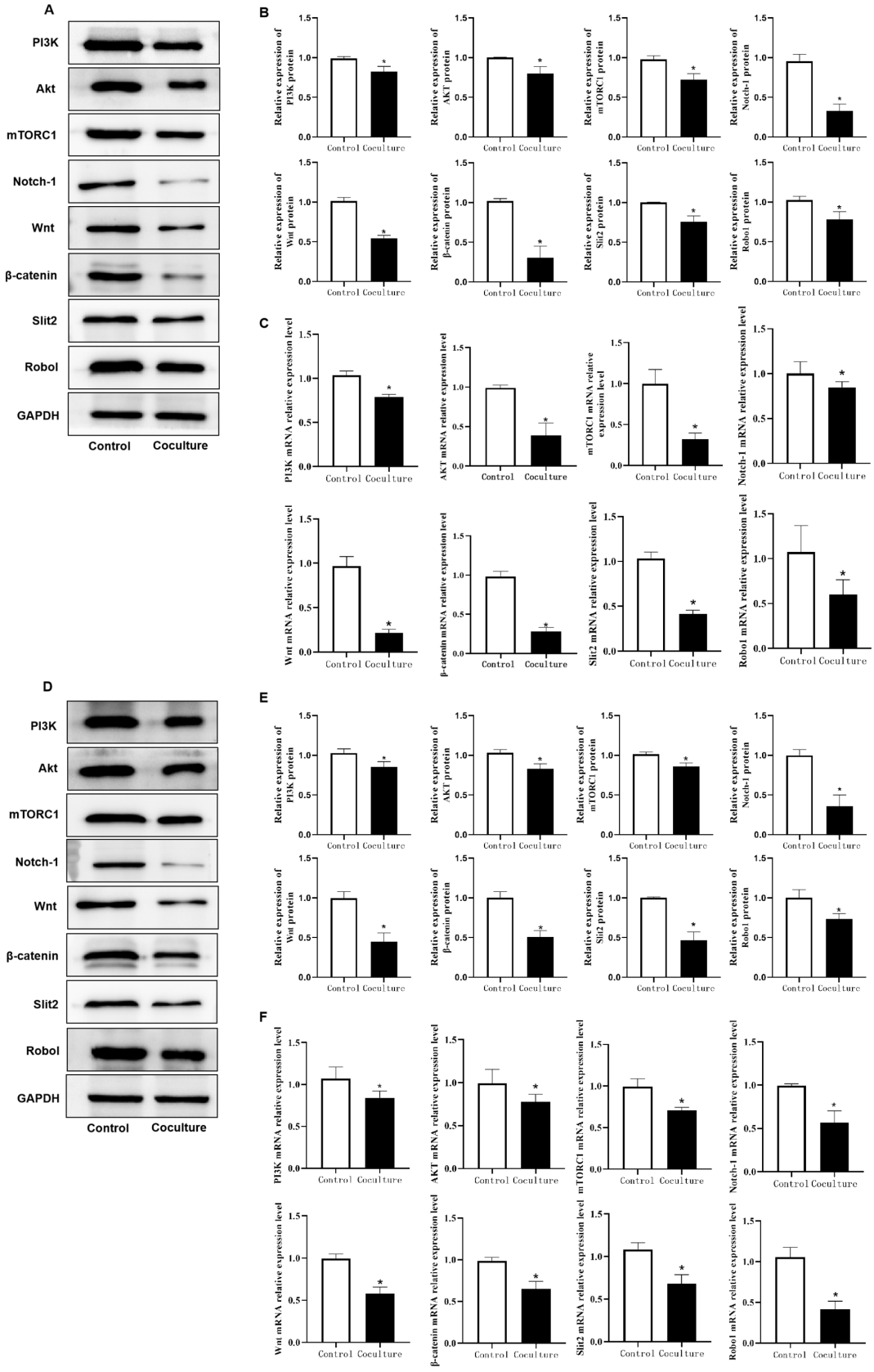

Fig. 5. Effects of ADSCM on common cancer pathways in colon cancer cell lines. (A–C) Western blot (WB) and qRT-PCR were used to determine the expression levels of cancer pathway-associated proteins in CT26 cells. (D–F) The expression levels of cancer pathway-associated proteins in MC38 cells were evaluated using WB and qRT-PCR. n = 3. $^{*}p < 0.05$ versus the control group.

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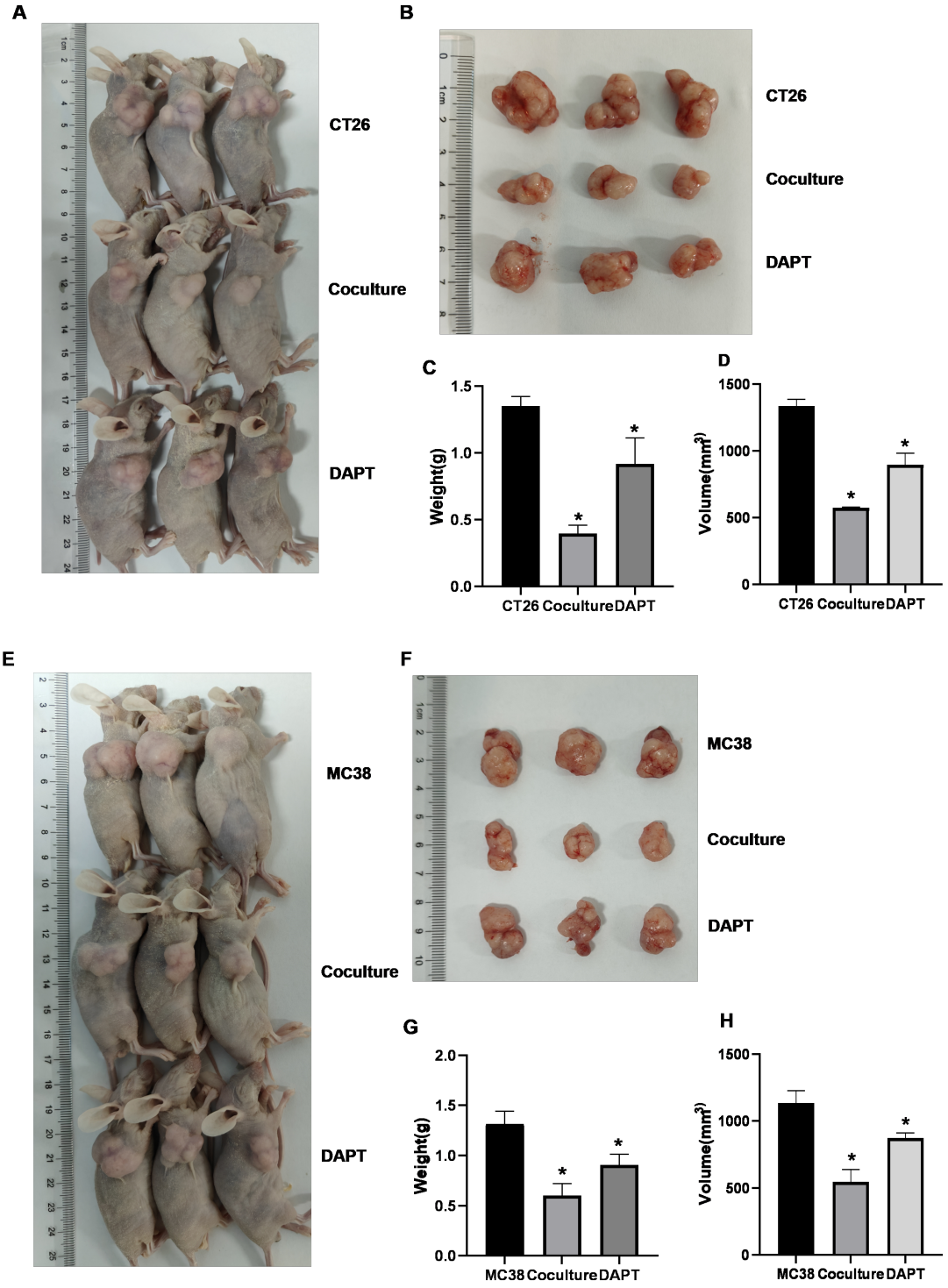

Fig. 6. Effect of adipose-derived stem cell microenvironment on the growth and metastasis of colon cancer. (A–D) The CT26 group of nude mice exhibited changes in tumor growth. (E–H) The MC38 group of nude mice exhibited changes in tumor growth. n = 3. $^{*}p < 0.05$ versus the control group. DAPT, the gamma-secretase inhibitor N-[N-(3,5-diuorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester.

The Impact of the ADSCM on Common Cancer Pathways in CT26 and MC38 Cells

The qRT-PCR and WB analysis revealed that the expression levels of PI3K, Akt, mTORC1, Notch-1, Wnt, β-catenin, Slit2, and Robol proteins were significantly decreased in the co-culture group compared to the control group (Fig. 5A–F) ($p > 0.05$). These findings suggest that ADSCM can reduce the expression levels of common cancer pathways in colon cancer cells.

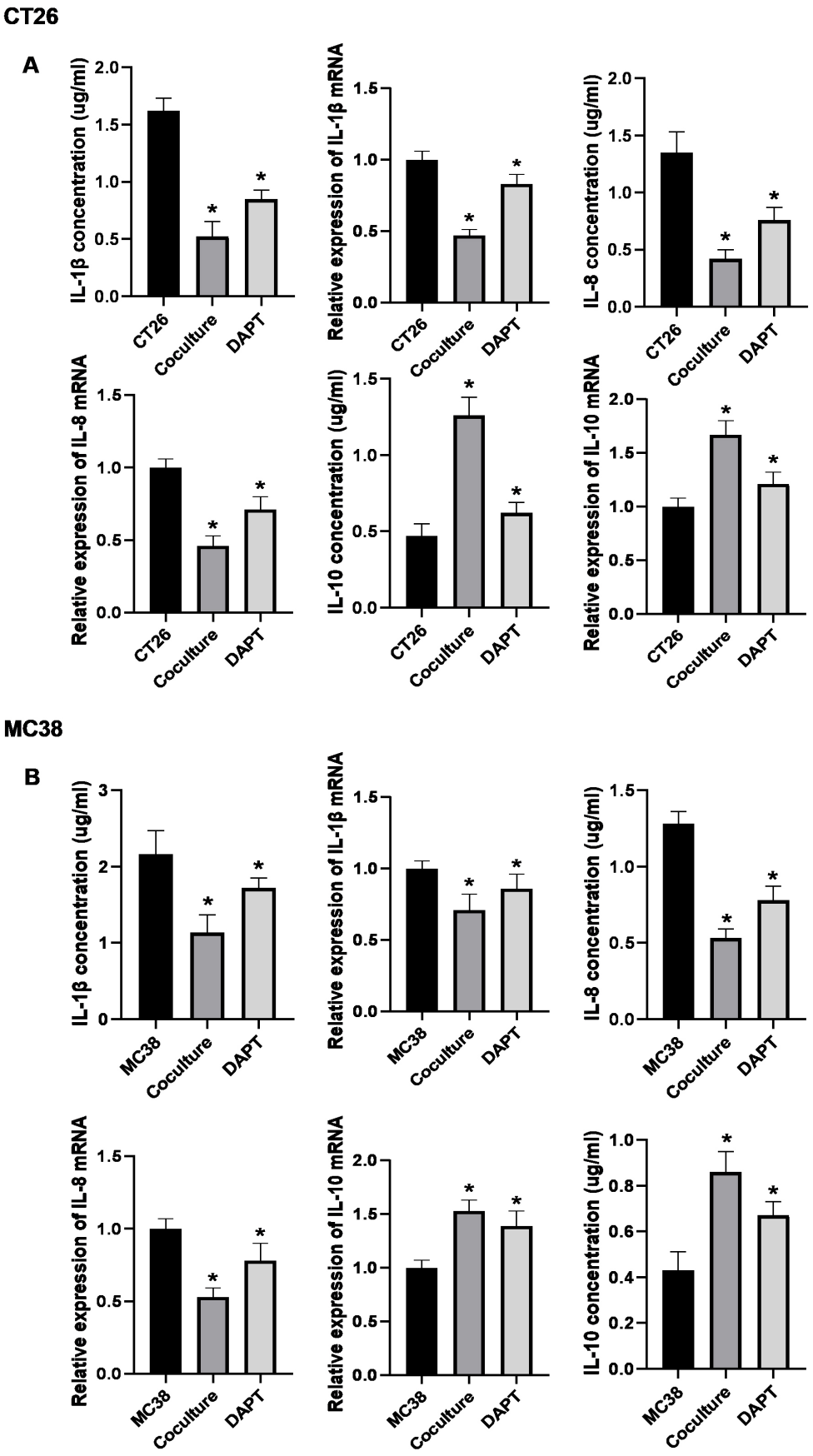

Fig. 7. Effect of adipose stem cell microenvironment on expression levels of inflammatory cytokines in mice. (A,B) The levels of interleukin-1 beta (IL-1β), interleukin-8 (IL-8), and interleukin-10 (IL-10) in mouse tissues, n = 3. $^{*}p < 0.05$.

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The Impact of ADSCM on Subcutaneous Tumor Formation in CC Mouse Model

We observed that, compared to the CT26 group of nude mice, the DAPT group exhibited a reduction in tumor volume and body weight. In the co-culture group, nude mice showed a more pronounced decrease in tumor volume and body weight (Fig. 6A–D, $p > 0.05$). Furthermore, when compared to the MC38 group, both the DAPT group and co-culture group displayed significantly smaller tumors, with the co-culture group indicating a more substantial reduction in tumor size (Fig. 6E–H, $p > 0.05$).

The Impact of ADSCM on Inflammatory Factors in CC Tissues

The levels of inflammatory factors in CC mice were evaluated utilizing ELISA and qRT-PCR analyses. It was observed that compared to the CT26 group, both the DAPT and co-culture groups demonstrated significantly reduced expression of pro-inflammatory cytokines IL-1β and IL-8 ($p > 0.05$). Conversely, the levels of the anti-inflammatory cytokine IL-10 were significantly increased. Furthermore, the changes were particularly pronounced in the co-culture group (Fig. 7A, $p > 0.05$). Additionally, similar outcomes were observed in the MC38 group of mice (Fig. 7B).

Discussion

With the growth of China's population, an aging demographic structure, and socioeconomic development, the number of new cases and deaths from colon cancer (CC) has been increasing annually, with a rising trend towards onset at younger age [19]. CC typically ranks among the leading cancers in terms of incidence, second only to lung and breast cancer. In particular, its incidence is relatively high in developed countries [20]. This situation is affected by various factors, including dietary habits and genetic predispositions. In developing countries, the incidence of CC has gradually increased with the widespread adoption of Western dietary habits and lifestyles. The occurrence of CC is closely associated with its malignant characteristic markers, which typically consist of various biomolecules, primarily proteins.

In CC, the abnormal expression, or elevated levels of specific malignant characteristic markers may be associated with the occurrence, diagnosis, and progression of cancer [21,22]. Carcinoembryonic antigen (CEA), a common malignant marker, is often elevated in many cases of CC, particularly in advanced stages [23]. Conversely, abnormal overexpression of CK-20 suggests tumor origin from colonic epithelial cells [24]. CK-7, on the other hand, typically exhibits reduced or negative expression levels in CC [25], although some cases may show limited CK-7 positivity. Decreased or no expression of CDX2 might be correlated with the malignant phenotype and poor prognosis of CC [26,27]. BerEP4, a widely used marker for diagnosing CC cells, indicates the presence of malignant tumors when expressed at high levels. It provides essential prognostic information and assists in diagnosing tumor, evaluating treatment efficacy, and monitoring postoperative recurrence [28]. Beyond their diagnostic and prognostic applications, the occurrence of CC typically involves abnormalities in multiple signaling pathways closely associated with the onset, progression, and metastasis of the disease. Aberrant activation of the Notch-1 pathway, the Wnt/β-catenin pathway, and the PI3K/Akt/mTORC1 signaling pathway may promote tumor cell proliferation and metastasis.

Conversely, the Slit2/Robo1 signaling pathway is believed to contribute to the migration and invasion of CC cells [29–32]. The high expression of these signaling pathways is closely associated with the malignant characteristics of CC. Further understanding their regulatory mechanisms can help elucidate the pathogenesis of CC and provide novel targets and directions for therapeutic strategies.

Adipose-derived stem cells (ADSCs) are a group of mesenchymal stem cells with multipotent differentiation potential originating from adipose tissue. Since their isolation from adipose tissue was initially reported by Zuk *et al*. in 2001 [33], ADSCs have provided new therapeutic avenues for numerous diseases due to their widespread availability and convenient sourcing [34]. Research has found that ADSCs have an extraction efficiency 1000 times higher than that of bone marrow stem cells and lack expression of major histocompatibility complex class II molecules and costimulatory molecules, rendering them minimally immunogenic and potentially applicable for allogeneic ADSC applications [35]. Additionally, ADSCs can exert immunomodulatory effects by regulating the phenotypic differentiation of immune cells [36]. Due to their easy accessibility and lack of ethical concerns, we constructed an ADSC microenvironment to mimic the embryonic microenvironment and observed its effects on tumor cell growth through both *in vitro* and *in vivo* experiments. The results revealed that compared to colon cancer cells cultured alone, colon cancer cells co-cultured with the ADSC microenvironment exhibited a reduced growth rate, inhibited proliferation and invasion capabilities, and enhanced apoptosis.

Furthermore, expression of malignant phenotype markers CEA and BerEP4 was downregulated, whereas CDX2 expression was upregulated in the co-culture group, along with decreased expression levels of several aberrant pathway proteins, including PI3K/Akt/mTORC1, Notch-1, Wnt/β-catenin, and Slit2/Robo1. Subsequently, co-cultured colon cancer cells were introduced into nude mice, and the Notch-1 pathway inhibitor DAPT was administered to explore their effects on subcutaneous tumors and inflammatory factors. The findings indicated that both the ADSC microenvironment and the Notch-1 pathway inhibitor could inhibit CC growth. Additionally, the expression levels of inflammatory factors IL-1β and IL-8 decreased, whereas IL-10 expression increased in the co-culture and DAPT groups, suggesting that the ADSC microenvironment and Notch-1 pathway inhibitor may alleviate tumor-induced inflammation.

These findings suggest that the ADSC microenvironment may have the potential to exert anti-tumor and anti-inflammatory effects, providing new insights and possibilities for CC treatment. However, further research is warranted to elucidate the molecular mechanisms underlying the interaction between the ADSC microenvironment and CC cells and to optimize treatment strategies to enhance therapeutic efficacy. Nonetheless, the study still has some limitations. Firstly, the mechanisms regulating the interaction between the ADSC microenvironment and CC cells remain incompletely understood and require further investigation. Secondly, the nude mouse model has limitations in simulating the human body environment and requires optimization to better mimic the human body's physiological conditions. Additionally, further in-depth research is needed to identify potential therapeutic targets to advance CC treatment.

Conclusion

Adipose-derived stem cells inhibit tumor growth and metastasis by establishing ADSCM, and reduce the phenotypic characteristics of malignant cells and the expression of abnormal pathways. Consequently, this reduces the progression of CC and holds promise for therapeutic potential.

Availability of Data and Materials

The datas used and/or analyzed during the current study are available from the corresponding author.

Author Contributions

QL and XML contributed to the conception and design of the research study, analyzed and interpreted the data, and drafted the manuscript. BL and YSD participated in the acquisition of data, contributed to the analysis and interpretation of the data, and critically revised the manuscript for important intellectual content. WDG and GH contributed to the conception and design of the research study, provided technical support in the acquisition of data, and critically revised the manuscript for important intellectual content. YCZ and HW contributed to the analysis and interpretation of the data, provided statistical expertise, and critically revised the manuscript for important intellectual content. BYL and JY provided guidance on the design of the research study, contributed to the interpretation of the data, and critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript, and have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Ethics Approval and Consent to Participate

The experimental protocol adhered to the principles outlined in the "Guidelines for Experimental Animals" and was approved by the Ethics Committee of Third Ward of Affiliated Hengyang Hospital of Hunan Normal University & Hengyang Central Hospital, China (NU20237799).

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Conflict of Interest

The authors declare no conflict of interest.

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