Suppression of tumor cell proliferation and migration by human umbilical cord mesenchymal stem cells: A possible role for apoptosis and Wnt signaling

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Abstract. Human umbilical cord-derived mesenchymal stem cells (hUCMSCs) represent potential therapeutic tools for solid tumors. However, there are numerous inconsistent results regarding the effects of hUCMSCs on tumors, and the mechanisms underlying this remain poorly understood. The present study further examined this controversial issue by analyzing the molecular mechanisms of the inhibitory effects of hUCMSCs on the proliferation and migration of the human lung cancer A549 cell line and the human hepatocellular carcinoma (HCC) BEL7402 cell line *in vitro*. Flow cytometric analysis demonstrated that hUCMSCs arrested tumor cells in specific phases of the cell cycle and induced the apoptosis of tumor cells by using the hUCMSC-conditioned medium (hUCMSC-CM). The hUCMSC-CM also attenuated the migratory abilities of the two tumor cell types. Furthermore, the expression of B-cell lymphoma 2 (Bcl-2), the pro-form of caspase-7 (pro-caspase-7), β-catenin and c-Myc was downregulated, while that of ephrin receptor (EphA5), a biomarker of cancer cell dormancy, was slightly increased in these two tumor cell lines treated with hUCMSC-CM. Specifically, when co-cultured via direct cell-to-cell contact, hUCMSCs were able to spontaneously fuse with any of the two types of solid tumor cells. These observations suggested that hUCMSCs may be a promising candidate for the biological therapy of lung cancer and HCC. Future studies should focus on detailed evidence for cell fusion, as well as other mechanisms proposed in the present study, by introducing additional experimental approaches and models.

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Introduction

Solid tumors are often malignant and are a serious threat to human health. At present, lung carcinoma is the most common type of malignancy and hepatocellular carcinoma (HCC) is the second most common cause of cancer-associated mortality (1,2). Surgical resection followed by radiotherapy and/or chemotherapy remains a common treatment for malignant tumors (3,4). However, these treatments are rarely curative. Therefore, the development of specific and highly efficient therapies is important. Human umbilical cord mesenchymal stem cells (hUCMSCs), derived from the umbilical cord matrix, have been proposed as a promising tool for the attenuation of tumor growth and metastasis (5).

hUCMSCs are a type of adult stem cell exhibiting primitive stem cell characteristics, including self-renewal and multi-potency. Following specific induction, hUCMSCs may differentiate into cardiomyocytes, neuron-like cells, skeletal muscle, endothelial cells and pancreatic islet-like cell clusters (6). Therefore, the initial research on hUCMSCs primarily focused on their multi-directional differentiation ability, which is applicable for repairing damaged tissues. At present, human MSCs used in experiments are primarily acquired from adult bone marrow. Compared with bone marrow-derived MSCs, hUCMSCs have several advantages for use in cell-based therapy, including improved expansion capability, painless collection procedures and lower risk of viral contamination. In addition, these MSCs also share important characteristics, including low immunogenicity, cytokine secretion and trans-differentiation, which favor their potent application in the development of novel tumor therapies (7-9).

Along with the frequent research into the use of hUCMSCs as a tool for cancer treatment, the interactions between hUCMSCs and malignant cells have been increasingly reported. However, there are numerous inconsistent results regarding the effects of hUCMSCs on tumors. In breast carcinoma models, the interferon-β-transfected hUCMSCs were capable of migrating to tumor sites and attenuating the proliferation

of human triple-negative breast carcinoma MDA-MB-231 and Hs578T cell lines (10). For glioma, hUCMSCs exerted antitumor effects by inhibiting proliferation, modulating the cell cycle in G0/G1 phase, and downregulating the expression of β-catenin and c-Myc in C6 glioma cells (11); hUCMSCs may also induce apoptosis in glioma U251 cells, resulting in the significant upregulation of apoptotic genes, including caspase-3 and caspase-9, and the significant downregulation of anti-apoptotic genes, including survivin and X-linked inhibitor of apoptosis (12). By contrast, other studies (13,14) have indicated that hUCMSCs may promote tumor proliferation in the tumor microenvironment. For example, hUCMSCs activated by macrophages promote the proliferation and migration of gastric epithelial cells and gastric cancer cells (13); in addition, esophageal carcinoma (EC) cells recruit hUCMSCs, and hUCMSCs promote EC cell migration and invasion illustrated by the upregulation of metastasis-related proteins, including matrix metalloproteinase (MMP)-2 and MMP-9 in EC cells co-cultured with hUCMSCs (14).

In order to better understand the interactions between hUCMSCs and specific tumor types, the present study further examined this controversial issue by analyzing the underlying mechanisms of the influence of hUCMSCs on the lung cancer A549 cell line and the hepatocellular cancer BEL7402 cell line via indirect and direct co-culture. The results indicated that hUCMSCs inhibit lung cancer and HCC cell progression by inducing apoptosis and targeting Wnt signaling, and possibly via direct cell-to-cell contact.

Materials and methods

Cell culture. hUCMSCs were isolated from the umbilical cords of healthy neonates delivered in local hospitals with the written informed consent of their mothers. The research protocol was approved by the Institutional Review Board of the School of Life Science and Biopharmacology of Guangdong Pharmaceutical University (Guangzhou, China). Separation, expansion and identification of hUCMSCs were performed as previously described (15). Cells at passage 3 to 8, displaying a homogeneous MSC immunophenotype and multipotent differentiation potential into adipocytic, osteoblastic and chondrocytic lineages (data not shown), were selected for experiments.

The human lung cancer A549 cell line and the human HCC BEL7402 cell line were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Zhejiang Tianhang Biotechnology Co., Ltd., Hangzhou, China) at 37˚C in a humidified atmosphere containing 5% CO_2, according to the American Type Culture Collection protocols.

Treatment of tumor cells with conditioned medium from hUCMSCs. hUCMSCs were cultured in Dulbecco's modified Eagle's medium (DMEM)/F12 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS to 100% confluence. The culture medium was then harvested, filtered through 0.22-μm pore sterile filters (Pall Life Sciences, Port Washington, NY, USA), and stored at -80˚C in aliquots until use. A mixture of the aforementioned supernatant derived from hUCMSCs and RPMI-1640 medium containing 10% FBS at a volume ratio of 3:2, designated as hUCMSC-CM, was subsequently used to treat tumor cell lines at 37˚C for 72 h. During the incubation time, the hUCMSC-CM was replaced every 24 h (16) to prevent the rapid acidification of culture medium due to the pre-mixing of hUCMSC supernatant with fresh medium and to replenish the degradation of bioactive substances in hUCMSC-CM. In the control group, the same tumor cells were cultured with DMEM/F12 medium without FBS for 72 h.

Cell cycle analysis. A total of $1x10^6$ cells were harvested, washed twice with cold phosphate-buffered saline (PBS), fixed in 70% cold ethanol and stained with 5 μg/ml propidium iodide (PI; Invitrogen; Thermo Fisher Scientific, Inc.) in the dark for 15 min at 37˚C. The DNA content of the stained cells was detected using a flow cytometer (Gallios; Beckman Coulter, Inc., Brea, CA, USA) and analyzed using ModFit software (Verity Software House, Inc., Topsham, ME, USA).

Transwell migration assay. After culturing at 37˚C for 72 h with or without hUCMSC-CM, the A549 and BEL7402 cells ($1x10^5$ cells/well) were plated into the top chambers of Transwell plates (Corning Incorporated, Corning, NY, USA). RPMI-1640 medium containing 10% FBS was placed into the bottom chambers, followed by incubation at 37˚C in an atmosphere containing 5% CO_2 for 24 h. The cells that did not penetrate the polycarbonate membrane were removed with cotton stickers. The membrane was then fixed in 100% methanol at room temperature for 15 min. Subsequently, the morphological differences between the two tumor cell types became less clear. To distinguish them properly, two different staining agents, eosin and crystal violet, which are common in Transwell assays of tumor cells (17,18), were used to stain A549 cells and BEL7402 cells at room temperature for 5 min, respectively. The migratory ability of tumor cells was determined by counting the number of cells that had penetrated the membrane using a light microscope (Olympus Corporation, Tokyo, Japan), in at least 5 fields for each assay (magnification, x400).

Cell apoptosis assay. An Annexin V-fluorescein isothiocyanate (FITC)/PI apoptosis detection kit (MBL International Co., Woburn, MA, USA) was used to examine the percentage of apoptotic cells. In brief, $1x10^5$ cells were washed with cold PBS and re-suspended in binding buffer provided in the aforementioned kit. Cells were incubated at room temperature with 10 μl Annexin V-FITC and 5 μl PI for 15 min, and were then analyzed using a Gallios flow cytometer (Beckman Coulter, Inc.) and FlowJo software 7.6.1 (FlowJo LLC, Ashland, OR, USA).

Western blot analysis. Protein expression was measured via western blot analysis. Total protein was extracted from A549 and BEL7402 cells with ice-cold RIPA lysis buffer (Beyotime Institute of Biotechonology, Shanghai, China), and the protein concentration was determined using Bradford reagent (Beyotime Institute of Biotechnology). A quantity of 30 μg total protein per lane was separated by 10% SDS-PAGE

and transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were then blocked by 5% non-fat milk for 1 h at 4˚C under agitation, followed by incubation with primary antibodies at 4˚C overnight against EphA5 (cat. no. sc-1014; 1:1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), Bcl-2 (cat. no. 32124; 1:1,000; Abcam, Cambridge, MA, USA), pro caspase-7 (cat. no. 32067; 1:1,000; Abcam), β-catenin (cat. no. 8480; 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) and c-Myc (cat. no. 5605; 1:1,000; Cell Signaling Technology, Inc.). An antibody against GAPDH (cat. no. AB-P-R001; 1:1,000; Hangzhou Goodhere Biotechnology Co., Ltd., Hangzhou, China; http://www.goodhere.com/) was used as the loading control. Membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (cat. no. ZB-2301; OriGene Technologies, Inc., Beijing, China) at a dilution of 1:5,000 for 1 h at room temperature. Protein bands were visualized by incubating the membranes with enhanced chemiluminescence reagent (Bio-Rad Laboratories, Inc.) and recorded on X-film (Kodak, Rochester, NY, USA). Protein expression was quantified by densitometry using ImageJ software 1.51 (National Institutes of Health, Bethesda, MD, USA).

Cell co-culture and detection. Cell fusion experiments based on direct contact between tumor cell lines and hUCMSCs were performed as previously described (19,20) with slight modification. Tumor cells (A549 and BEL7402) and hUCMSCs were labeled with DIO and DID fluorescent dyes (Invitrogen; Thermo Fisher Scientific, Inc.), respectively, according to the manufacturer's protocols. Next, a total of $1x10^4$ DIO-labeled tumor cells were mixed with the DID-labeled hUCMSCs, at a ratio of 1:1, in 100-mm glass bottom dishes or 6-well cell culture plates containing DMEM/F12 supplemented with 10% FBS and 1% penicillin/streptomycin; these were incubated at 37˚C in a humidified atmosphere containing 5% CO_2. Monochrome fluorescence-labeled hUCMSCs or tumor cells cultured alone were set as controls. The co-cultured cells were observed under a confocal laser scanning microscope (FluoView FV1000, Olympus Corporation) at a magnification of x400 every 2-3 days and the images obtained were analyzed using FV10-ASW 1.6 software (Olympus Corporation) according to the manufacturer's protocol. On day 8 of co-culture, the hybrids (double-stained cells) were further confirmed using a Gallios flow cytometer (Beckman Coulter, Inc.) and the fusion efficiency was calculated using FlowJo software 7.6.1 (FlowJo LLC).

Statistical analysis. Statistical analysis was performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) software and GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA). Quantitative data are expressed as the mean ± standard deviation. The Student's t-test was used to test the probability of significant differences between groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

hUCMSC-CM influences the cell cycle of A549 and BEL7402 cells. We hypothesized that the influence of the supernatant from hUCMSCs on the growth of A549 and BEL7402 tumor cells may be associated with altered cell cycle progression. As expected, the analysis of cell cycle distribution indicated that A549 tumor cells were arrested in the G1 phase, as illustrated by an increased number of cells in the G1 phase (Fig. 1A). This was accompanied by a corresponding reduction in the number of cells in the S phase following incubation with hUCMSC-CM. The results for BEL7402 cells treated with hUCMSC-CM were different, with an increased cell number in the S phase and a corresponding reduction in the G1 phase population (Fig. 1A). Overall, analysis of the cell cycle distribution revealed that hUCMSC-CM inhibited the proliferation of tumor cells by arresting the cell cycle in specific phases.

hUCMSC-CM inhibits the migration of A549 and BEL7402 cells. The effect of hUCMSC-CM on the migration of A549 and BEL7402 tumor cells was investigated using 24-well polycarbonate Transwell inserts. The results revealed that fewer A549 and BEL7402 cells were able to cross the membrane following treatment with hUCMSC-CM, compared with the control group ($P<0.05$; Fig. 1B). Therefore, hUCMSC-CM markedly reduced the migratory abilities of A549 and BEL7402 tumor cells.

hUCMSC-CM induces the apoptosis of A549 and BEL7402 cells. In order to investigate whether apoptosis was involved in the hUCMSC-mediated inhibition of tumor cell growth, flow cytometric analysis was performed using Annexin V/PI staining. As demonstrated in Fig. 1C, incubation with hUCMSC-CM induced a significant increase in the percentage of apoptotic cells, with 46.27±9.96% of A549 tumor cells and 18.23±6.66% of BEL7402 tumor cells in late-stage apoptosis ($P<0.05$; Fig. 1C). Taken together, these results demonstrated that hUCMSC-CM induced the apoptosis of cancer cells.

hUCMSC-CM modulates molecular expression changes in A549 and BEL7402 cells. The results of western blot analysis revealed that hUCMSC-CM slightly increased the expression of EphA5, which has been identified as a biomarker of cancer cell dormancy. hUCMSC-CM also resulted in the downregulation of Bcl-2 and caspase-7 in these two tumor cell types (Fig. 2A and B). The primary antibody against caspase-7 (cat. no. 32067; Abcam) that was used in the present study only recognizes the pro-form but does not react with cleaved forms of caspase-7. Accordingly, the caspase-7 bands in Fig. 2A presented the pro-form, but not the cleaved form of caspase-7. Wnt signaling is known to serve critical roles in regulating the proliferation and progression of tumor cells. Therefore, the involvement of Wnt signaling was investigated in the present study. The expression levels of β-catenin and c-Myc, two key signaling molecules in the Wnt pathway, were downregulated in A549 and BEL7402 tumor cells treated with hUCMSC-CM. Taken together, these results indicated the involvement of cell apoptosis molecules, as well as that of the Wnt signaling pathway, in the inhibitory effects of hUCMSCs on A549 and BEL7402 tumor cells.

Co-culture of hUCMSCs with A549 and BEL7402 cells induces cell fusion. hUCMSCs were mixed with the two

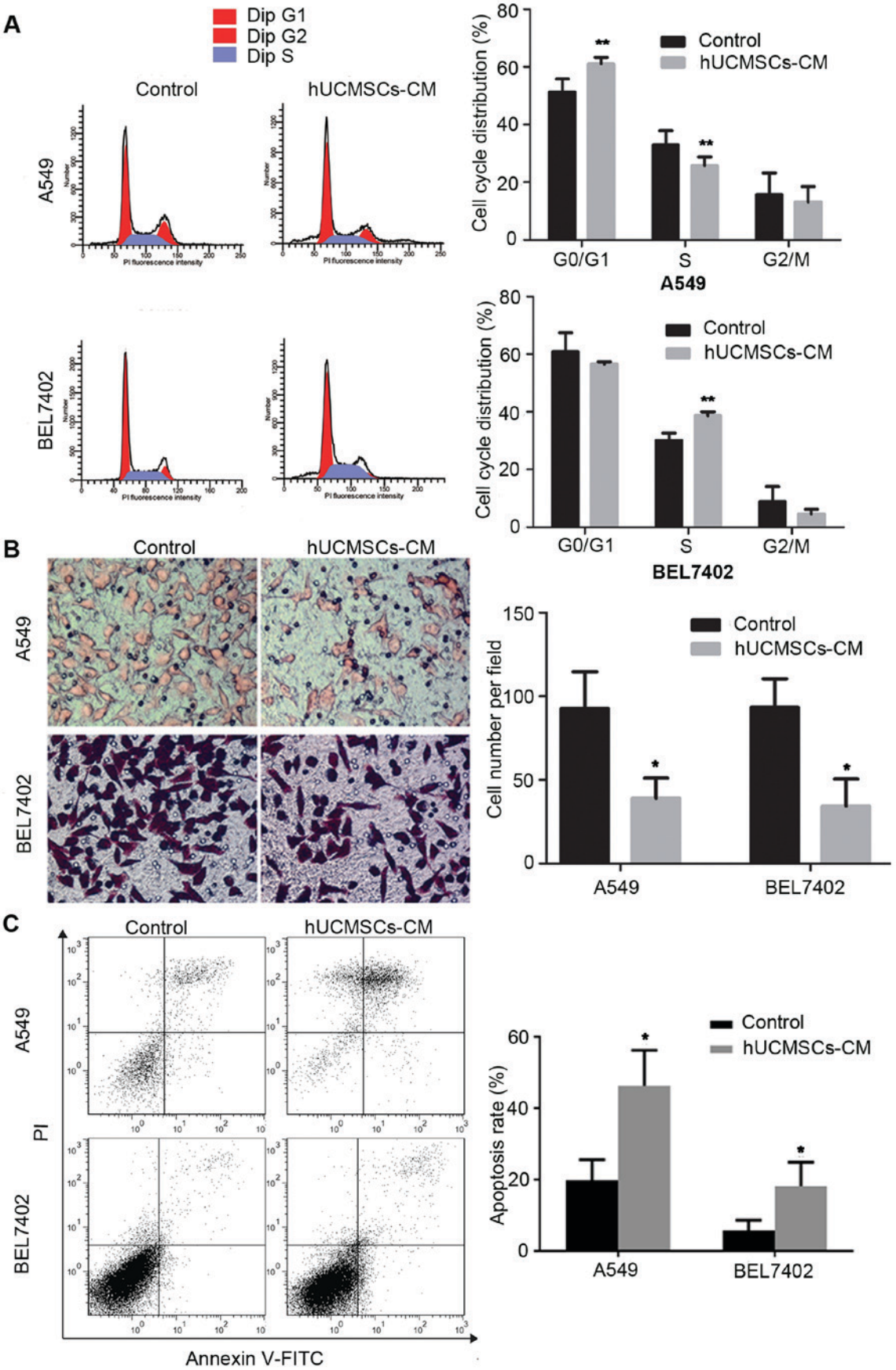

Figure 1. Effects of hUCMSC-CM on the proliferation, migration and survival of A549 and BEL7402 tumor cells. Serum-free culture medium was used instead of hUCMSC-CM in control groups. (A) Effect of hUCMSC-CM on the cell cycle of A549 and BEL7402 tumor cells. The percentage of cells in the G1, S and G2 phases is presented in the left panel and statistical analysis is presented in the right panel. **P<0.01 vs. control. (B) The migratory ability of tumor cells in the hUCMSC-CM group was analyzed by a Transwell assay. Representative images of migratory cells on the membrane (original magnification, x400; left). Average number of migrated cells per field from three independent experiments (right). *P<0.05 vs. control. (C) Flow cytometry was performed to examine cell apoptosis in each group. Representative flow cytometric analysis (left). Quantification of flow cytometric analysis (right). *P<0.05 vs. control.

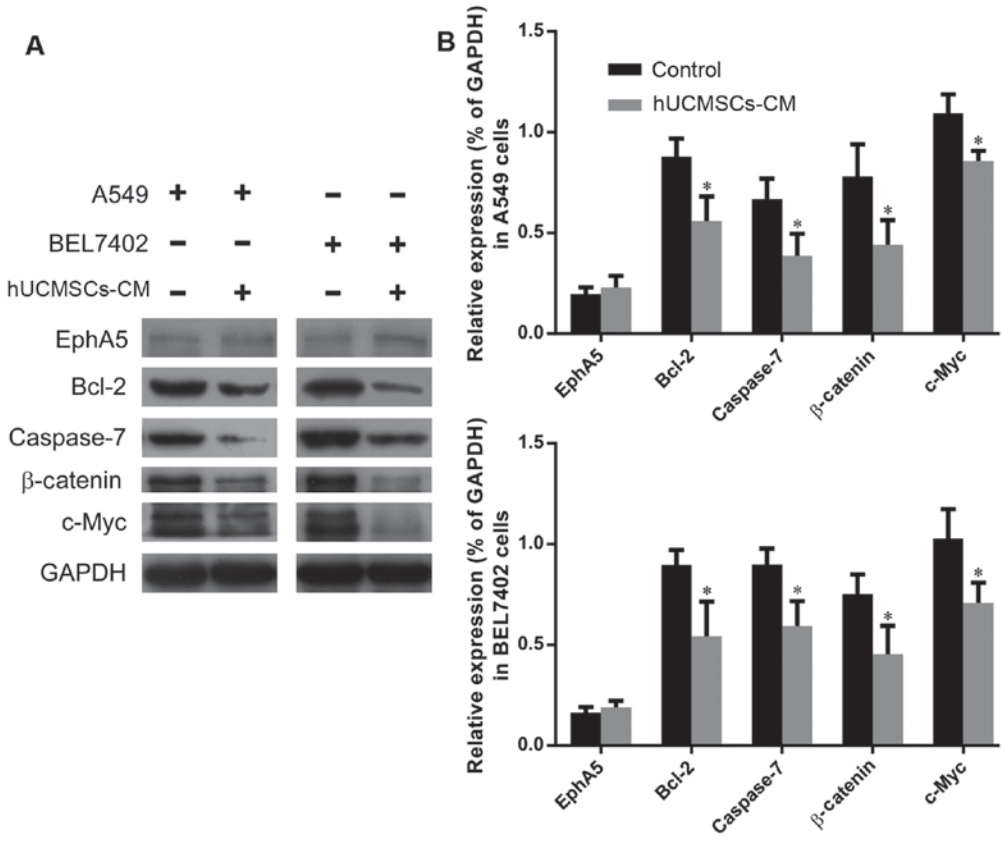

Figure 2. Molecular changes in A549 and BEL7402 tumor cells treated with hUCMSC-CM. The expression levels of EphA5, Bcl-2, pro caspase-7, β-catenin and c-Myc were detected by western blot analysis. Treatment with hUCMSC-CM decreased the protein expression of Bcl-2, pro caspase-7, β-catenin and c-Myc in A549 and BEL7402 tumor cells. Serum-free culture medium was used as control for hUCMSC-CM. (A) Representative western blot analysis images. (B) Quantitative data were acquired via densitometric analysis. Data are presented as the mean ± standard deviation of three independent experiments. *P<0.05 vs. control. EphA5, ephrin receptor A5; Bcl-2, B-cell lymphoma 2.

solid tumor cell types by direct cell-to-cell co-culture. The cell fusion event and cell fusion efficiency were demonstrated using confocal laser-scanning microscopy and flow cytometry, respectively. After co-culture for 72 h *in vitro*, the hUCMSCs were revealed to have merged into A549 or BEL7402 tumor cells, as illustrated by the existence of bi-nucleated hybrid cells of dual color (DiO and DiD double-stained cells), which were detected by confocal laser-scanning microscopy (Fig. 3). Following co-culture for 8 days, flow cytometry revealed that the fusion efficiencies were approximately 79.0±2.6% in A549 cells and 76.4±4.3% in BEL7402 cells (Fig. 4).

Discussion

Increasing evidence has indicated that adult stem cells may be effective therapeutic tools for cancer therapy (21,22). hUCMSCs are a group of adult stem cells that are easy to collect, may be expanded extensively *in vitro* and have a low immunogenicity. It is important to study the effects of hUCMSCs on tumor growth in order to develop novel therapies for the treatment of cancer. Recently, certain studies have reported that hUCMSCs have an intrinsic ability to attenuate the growth of several types of cancer cells. Ma *et al* (23) directly injected hUCMSCs into an immunodeficient xenograft mouse model transplanted with MDA-MB-231 breast cancer stem cells. The hUCMSCs reduced tumor volume and tumor weight in these mice. Zhang *et al* (24) reported that modified hUCMSCs that were transfected with the inerleukin-21 gene inhibited the proliferation of ovarian cancer cells *in vitro* and *in vivo*. Subramanian *et al* (25) confirmed that hUCMSCs did not transform into tumor-associated fibroblasts, making them safer than bone marrow MSCs.

In our previous study, hUCMSCs were successfully separated from the umbilical cords of healthy donors (15). hUCMSCs have the general characteristics of MSCs. The aim of the present study was to investigate the effects of hUCMSCs on the malignant behaviors, including proliferation, migration and survival capabilities, of the two types of solid tumor cells *in vitro*. Cell proliferation is specifically controlled in the G1 phase and the G1/S phase transition in the cell cycle. When cell cycle analysis was conducted, significantly more tumor cells were observed in the G1/S phase following treatment with hUCMSC-CM *in vitro*. However, the cell cycle arrest in these two types of tumor cells was different. Lung cancer A549 cells were arrested in the G0/G1 phase, while HCC BEL7402 cells were arrested in the S phase of the cell cycle. Therefore, we hypothesized that hUCMSCs may interfere with the growth of tumor cells through regulating cell cycle arrest in different phases. These outcomes also suggest that the anticancer effect of hUCMSCs may depend on the specific types of tumor cells. Metastasis is one of the most important biological behaviors of malignant tumors. The present study demonstrated that hUCMSCs also significantly inhibited the migratory behavior of the two types of tumor

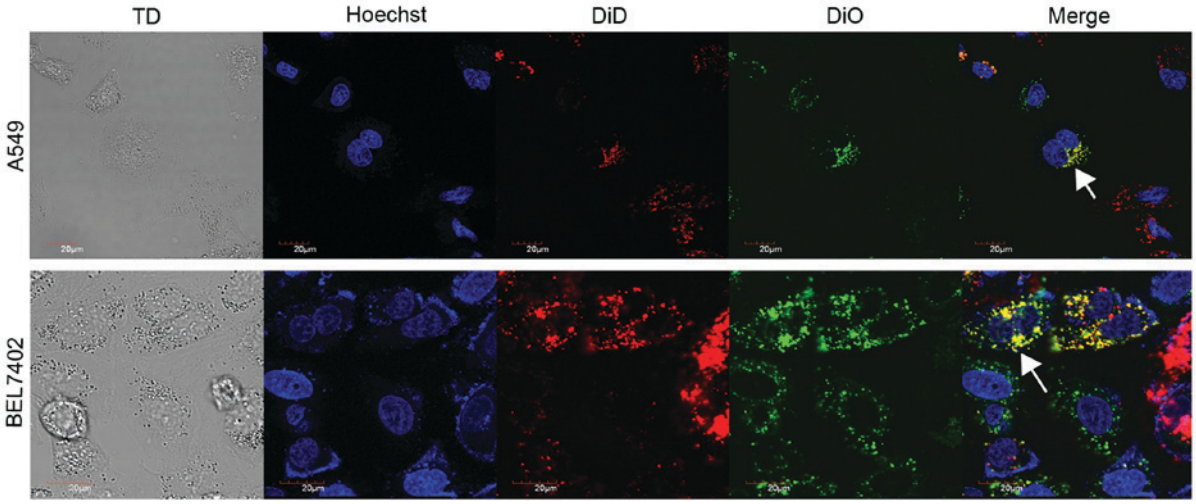

Figure 3. Confocal laser-scanning images of cell fusion between hUCMSCs and tumor cells. Tumor cells and hUCMSCs were stained with DiO (green) and DiD (red), respectively. All samples were counterstained with Hoechst 33342 (blue) to indicate the nucleus. After co-culture for 72 h, the hUCMSCs were revealed to have merged into A549 or BEL7402 tumor cells, as illustrated by the existence of hybrids with double nuclei and yellow cell membranes (DiO and DiD double-stained cells indicated by the white arrow) in the representative images. Scale bar, 20 μm; magnification, x400.

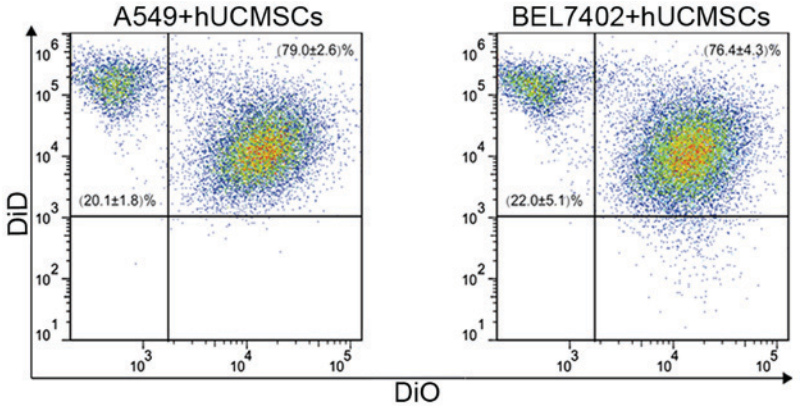

Figure 4. Flow cytometric analysis of spontaneous cell fusion between DiO-labeled tumor cells and DiD-labeled hUCMSCs.

cells in Transwell chambers. Furthermore, it was revealed that hUCMSC-CM significantly increased the proportion of Annexin V/PI-positive cells. Consistently, the western blot analysis revealed that hUCMSC-CM inhibited the expression of apoptosis-related proteins, including Bcl-2 and pro caspase-7, in A549 and BEL7402 cells. Caspase-7 has been identified as a major contributor to the execution of apoptosis. During apoptosis, caspase-7 is activated through proteolytic processing to produce the mature and active subunits, resulting in the decrease of pro-caspase-7 and emergence of cleaved caspase-7 (26). Therefore, the downregulation of pro-caspase-7, as well as that of Bcl-2, indicated the activation of intrinsic and extrinsic apoptosis pathways, and constituted the molecular mechanisms underlying the hUCMSC-induced apoptosis of tumor cells.

To further investigate other molecular mechanisms by which hUCMSCs inhibited tumor cells, the present study focused on the influence of hUCMSC-CM on Wnt signaling and tumor dormancy biomarkers in A549 and BEL7402 tumor cells.

Wnt/β-catenin signaling has been demonstrated to serve an important role in regulating tumor initiation and progression in various malignant tumor types, including lung cancer and HCC (27,28). Furthermore, Wnt signaling may regulate genes that are involved in cell-cycle regulation and cell apoptosis, including Cyclin and Bcl-2 (29). Therefore, we hypothesized that this signaling pathway may be involved in governing the inhibitory effects of hUCMSCs on lung cancer A549 cells and HCC BEL7402 cells. β-catenin is a key mediator in Wnt signaling, regulating multiple cellular functions. Stabilized β-catenin translocates into the nucleus, binds with T-cell factor/lymphoid-enhancing factor transcriptional factors and regulates the expression of downstream β-catenin-dependent genes, including cyclin D1 and c-Myc (30). The results of western blot analysis in the present study demonstrated that the treatment of A549 and BEL7402 cells with hUCMSC-CM resulted in the downregulation of β-catenin. To further assess whether known β-catenin targets were also reduced, the expression of c-Myc was analyzed. Consistent with the observed reduction in β-catenin, the expression levels of c-Myc

in these two tumor cell types receiving hUCMSC-CM were also downregulated. These molecular data revealed that the hUCMSC-mediated tumor inhibition was associated with the depression of Wnt signaling in the two tumor cell types. It has been demonstrated that MSCs may secret bioactive molecules, including signaling proteins, cytokines and chemokines, into culture medium (31). Some of these soluble factors may have helped the triggering of the downregulation of Wnt signaling in tumor cells.

Tumor dormancy describes a process through which malignant cells exit the cell cycle and survive in a quiescent state (32). Namely, the dormant cancer cells usually experience the static stage at which they do not proliferate or no longer enter the cell cycle. The maintenance or reversal of tumor dormancy involves the changes of a series of malignant biological behaviors, including the metastasis, proliferation and apoptosis of tumor cells in a specific environment. Accordingly, one possible explanation for tumor dormancy is proliferative arrest, and the other is the equilibrium between proliferation and apoptosis (32). At the beginning of the present study, it was observed that hUCMSCs induced cell cycle arrest, an increase in apoptosis and a decrease in the migration of A549 and BEL7402 tumor cells, which indicated the possible involvement of dormancy mechanisms. However, it has been demonstrated that the interactions between cancer cells and their normal neighbors act as an important microenvironmental control of the onset and maintenance of dormancy during cancer development (33). MSCs are one of the critical components in the tumor microenvironment. We hypothesized that MSCs may also serve a role in tumor dormancy. On the basis of the aforementioned speculations, the present study aimed to investigate the expression of dormancy-specific biomarkers (34). EphA5, one of the tumor dormancy markers that was detected, was upregulated at the mRNA and protein levels in the two tumor cell types following incubation with hUCMSC-CM. This may be a possible explanation for how hUCMSCs support tumor dormancy. Similarly, Glick and Yuspa (35) observed that the tumor microenvironment may inhibit or reverse the malignant characteristics of tumor cells that are in a dormant state, and tumors are able to develop only when the stability of the microenvironment is disrupted. Alt-Holland *et al* (36) also proposed that the signaling network interaction between tumor cells and adjacent normal cells may control tumor growth and maintain the dormancy of tumor cells.

The majority of solid tumor cells and MSCs are adherent cells. Therefore, in order to avoid the interference of MSCs with the detection of tumor cells, the majority of experiments prefer to culture tumor cells with conditioned medium from MSCs. However, MSCs will inevitably come into contact with tumor cells after entering the body when they are used for tumor therapy. To better reflect this situation, in the present study, hUCMSCs were co-cultured with the two solid tumor cell types by direct cell-to-cell contact. With confocal scanning, bi-nucleated hybrid cells were observed due to the fusion of hUCMSCs with the co-cultured tumor cells, and it was re-affirmed by flow cytometry. Specifically, hybrid cells with two clear nuclei were observed until the end of 6 days of confocal tracking in the present study (data not shown), which may aid in distinguishing cell fusion from other mechanisms, including phagocytosis among cells as well as endocytosis of MSC-secreted exosomes to a certain extent. Phagocytosis refers to the process of specifically engulfing and destroying particulate targets via diverse mechanisms (37). Targets of phagocytosis include microorganisms, dead or dying cells, and environmental debris. By contrast, cell fusion is a nuclear reprogramming process that involves fusing two or more cell types to form a single identity and generally does not cause deadly damage to the two sides of the fusion (19). However, membranous vesicle transport, particularly the exosome-mediated endocytosis, is one of the important mechanisms by which mesenchymal stem cells exert their biological functions, possibly including the communication between MSCs and tumor cells (38). Exosomes and other extracellular vesicles belong to subcellular components without nuclear structures, although they usually contain cell-specific proteins, lipids and nucleic acids. However, in the present study, bi-nucleated cells were observed under confocal microscope, which indicated the direct fusion of hUCMSCs into tumor cells. Considering the limitations of the present study, including the absence of electron microscopy data, the aforementioned observation does not exclude the involvement of exosomes or other mechanisms, but emphasized the potential roles of cell fusion in the crosstalk between MSCs and tumor cells.

It has been widely demonstrated that numerous cell types in the tumor microenvironment are able to merge with malignant cells by cell fusion (39,40). As one of the critical components in the tumor microenvironment, MSCs are also a putative fusogenic candidate. Similarly, the study of Wei *et al* (19) co-cultured RFP-expressing MSCs with eGFP-expressing lung cancer H441 cells without any fusogenic agent and demonstrated that MSCs fuse spontaneously with lung cancer cells. Transcriptome profiles revealed that the lung cancer cells are reprogrammed to slow growth and a stem-like state upon MSC fusion, accomplished by the restoration of p21 function and the upregulation of forkhead box F1, a putative tumor suppressor (19). Wang *et al* (20) also generated fusion progeny by fusing DiD-labeled MSCs and DiO-labeled esophageal carcinoma cells with PEG1500, and confirmed that the fusion aids in controlling the malignant phenotype of esophageal cancer cells.

In summary, the results of the present study suggested that hUCMSCs may inhibit the malignant biological behaviors of human lung cancer and hepatocellular cancer cells *in vitro* by activating cell apoptosis and inhibiting Wnt signaling. hUCMSCs also have the potential ability to induce tumor dormancy, at least through the mechanism of cell cycle arrest. In addition, the present study provided evidence to support spontaneous cell fusion between hUCMSCs and tumor cells, which may contribute to the antitumor effects of hUCMSCs. Unlike individual molecules, including protein and DNA/RNA, each complete cell is functionally independent and the cell-cell interaction involves complex mechanisms. As a preliminary attempt to investigate such a complicated issue, the present study may only provide limited conclusions for the time being. Notably, the results of the present study provide valuable clues for in-depth research on the interactions between MSCs and tumors, including the identification of bioactive molecules in hUCMSC-CM as upstream modulators of Wnt signaling.

Therefore, the mechanisms emphasized in the present study warrant further efforts by using additional approaches and more suitable models in the future.

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Availability of data and materials

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

Authors' contributions

XL and YY designed the experiments. YY performed the hUCMSC isolation. CZ performed tumor cell culture. YY, CZ, and XC performed Transwell assay and western blot analysis. YY and XC performed flow cytometric analyses. CT and XL performed confocal laser scanning and image analysis. HC performed cell co-culture. YY, CZ, and XL wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Human umbilical cord collection for research was approved by the institutional review board of the School of Life Science and Biopharmacology of Guangdong Pharmaceutical University (Guangzhou, China). MSCs were isolated from umbilical cords with donor's informed consent.

Consent for publication

The patients consented to the publication of this data.

Competing interests

The authors declare that they have no competing interests.

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