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Mesenchymal Stem Cells Trigger Epithelial to Mesenchymal Transition in the HT-29 Colorectal Cancer Cell Line

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ABSTRACT

Background & Objective: Mesenchymal stem cells (MSCs) promote metastasis in colorectal cancer; however, the mechanism underlying this process is not fully understood. Epithelial to mesenchymal transition (EMT) is a key step in tumor acquisition of metastatic phenotype. We aimed to investigate the effect of MSCs on the expression of EMT markers, as well as cancer stem cell markers in HT-29 colorectal cancer cells.

Materials & Methods: MSCs were isolated from bone marrow tissue, and their multi potency was confirmed. The HT-29 cell line was prepared and co-cultured with MSCs for 3 days using 6-well transwell co-culture plates (membrane pore size: 0.4 µm). Cell morphology was observed by inverted microscopy. The expression levels of EMT-related genes, namely E-cadherin, Vimentin, and β-catenin, were investigated by the RT-qPCR method. Also, the surface expression levels of CD44 and CD133 cancer stem cell markers were analyzed by flow cytometry.

Results: The co-culture of HT-29 cells with bone marrow-derived MSCs resulted in changes in cell morphology from epithelial to mesenchymal forms. The expression of mesenchymal stem cell markers, namely Vimentin and β-catenin, were significantly increased (2.25 and 1.83 folds, respectively), while the expression of the epithelial marker, E-cadherin, was reduced (0.3 folds). The expression of CD133 was also increased (51.5%).

Conclusion: Tumor-resident mesenchymal stem cells can promote colorectal cancer metastasis inducing EMT as well as increasing cancer stem cell frequency in the tumor microenvironment. It seems that direct contact between MSCs and colorectal cancer cells is not required for the interaction. Our findings may help scientists to find effective strategies against cancer metastasis by targeting tumor-resident MSCs.

Keywords: Stem cells, Metastasis, Colorectal cancer, Epithelial Mesenchymal Transition, Biomarker

Introduction

Colorectal cancer (CRC) is the second most common cancer in the United States and also is the second cause of cancer-related deaths in the world, partially due to the high resistance of CRC to conventional treatments and its capacity to spread beyond its original location (metastasis) (1). CRC development is usually initiated in the form of adenoma in the intestinal epithelium and progresses very slowly into well-differentiated carcinoma. The intestinal epithelium is composed of a variety of specialized cell types that originated from rapidly proliferating intestinal stem cells (ISCs) at the bottom of the crypts (2). Under normal conditions, crypt morphogenesis and the population of intestinal stem cells are tightly regulated; however, any changes in the lumen content, such as hormonal or cytokine milieu, can affect the morphogenesis and ISCs (2). Genetic and other risk factors, including race, ethnicity, gender, age, hereditary mutations, inflammatory bowel disease, abdominal radiation, cystic fibrosis, cholecystectomy, and androgen deprivation therapy, are able to influence crypt morphogenesis and cause CRC development (3). Under normal conditions, crypt morphogenesis (ISC differentiation into well-differentiated epithelial cells) is unidirectional; however, chronic injury or infection

triggers the de-differentiation of specialized cells into partially differentiated cells, leading to a prominent change in the stem cell pool of the crypts (2). Under sustained stressful conditions, intestinal resident stem cells undergo transformation into neoplasia and cancer. Transformation is associated with the overexpression of oncogenes, along with the inactivation of tumor suppressors genes, both of which cause uncontrolled cell proliferation. Barker et al. showed that stem-cell-specific loss of the tumor suppressor gene, adenomatous polyposis coli (APC), has been associated with rapidly growing neoplasia in a mouse model (4). APC mutation is frequently detected in CRC cells.

Cancer stem cells (CSCs), a subpopulation of tumor cells, play an important role in the initiation, progression, and metastasis of cancer. CSCs are capable of generating other subpopulations of tumors, thus generating new tumor tissue. These types of cells show high resistance to irradiation and chemotherapeutic agents and, in most cases, are known as the etiology of cancer recurrence after recovery. CSCs possess specific surface molecular characteristics in comparison with other tumor cells that are directly involved in CRC development (5). CD133 is a commonly reported CSC marker in CRC, and this surface molecule has been frequently used for selective isolation of CSCs (6). In addition, CD44, CD166, CD24, CD29, Lgr5, Bmi1, and EpCAM have also been reported as CSC markers in CRC (5, 7).

Mesenchymal stem cells (MSCs) are multipotent stromal cells that are mainly present in the bone marrow but also found in other tissues, such as the brain, lung, kidney, liver, pancreas, and skin (8). MSCs contribute to wound healing and tissue repair processes, and their tumor-specific tropism and accumulation have been demonstrated in tumor tissues following systemic injection (9). MSCs express various chemokine receptors and respond to recalling signals released from tumor cells and accumulate in tumor tissues. In the tumor environment, MSCs secrete cytokines, chemokines, and growth factors to support cancer cell growth. They also suppress innate and adaptive immune responses in favor of tumor cells (9). CRC cells highly express CC chemokine receptors, while MSCs are strong producers of CCl3/CCL4 and CCL5; therefore, a strong correlation was observed between the expression of CCR5 by MSCs and the development of CRC. MSCs can also induce epithelial-mesenchymal transition (EMT) in tumor cells; in this way, MSCs have the main role in the emergence of cancer cells with metastatic phenotypes (10).

Metastasis is a multi-stage process in which tumor cells lose cell junction adhesion and gain a migratory phenotype to spread to other tissues and organs (11). Transformation of differentiated epithelial cells in the crypts to stem cell-like phenotype after chronic exposure to environmental stimuli has been explained above. EMT is a complex process that helps locally adherent tumor cells acquire migratory and invasive properties and spread (12). In addition, these types of cells also show high resistance to chemotherapy and radiation. They also exhibit decreased expression of E-cadherin and increased expression of β-catenin and Vimentin (12). β-catenin is an effector molecule in the Wnt pathway and is the main oncoprotein in colorectal cancer whose expression is prominently increased due to APC mutation, which is a tumor suppressor proteinm(13). Nuclear accumulation and activation of β-catenin can directly induce loss of epithelial differentiation and EMT. E-cadherin is an epithelial cell marker whose expression is downregulated during EMT. Vimentin is a mesenchymal cell marker whose expression is upregulated during EMT.

The in-vitro effects of MSCs on CRC cells have not been reported in the literature. We used transwell plates with 0.4 µm pore size to perform the co-culture of HT-29 cells with bone marrow-derived MSCs. The mRNA expression levels of EMT-related markers, including E-cadherin, β-catenin, and Vimentin, were analyzed by real-time PCR. Also,the expression levels of cancer stem cell markers, namely CD44 and CD133, were assessed by flow cytometry in HT-29 cells.

Materials and Methods

The primary antibodies against CD105, CD90, and CD73 were purchased from Becton-Dickinson Biosciences. The primary antibodies against CD45, CD14, and CD34 were procured from Chemicon Co., USA. The RevertAid cDNA synthesis kit and SYBR Green PCR Master Mix were purchased from ThermoFisher Scientific Inc. Phosphate-buffered saline (PBS) was used as washing buffer for the MSC isolation process supplemented with penicillin (300 U/ml), streptomycin (100 µg/ml) and amphotericin-B (5 µg/ml). A 6-well transwell cell culture system was procured from Costar Corp., USA. The RNA isolation kit was purchased from FavorPrep (FavorPrep™ Blood/Cultured Cell Total RNA Mini Kit: FABRK 001, Taiwan).

Isolation and characterization of mesenchymal stem cells

Mesenchymal stem cells (MSC) were isolated from iliac crest bone marrow (BM) samples obtained from patients who were candidates for transplantation. After obtaining informed written consent, bone marrow samples (3-5 ml) were taken by a specialist and then transferred to tubes containing an equal volume of anti-coagulated DMEM (EDTA, %0.01 w/v) and then mixed (14). The cells were passed through a 150-µm mesh and then layered on an equal volume of Ficoll-Paque solution (1.077 g/cm^3, GE health care, USA). After centrifugation (500g for 25 min at 25 °C), the cells at the interface were collected and washed twice in PBS+EDTA and maintained in DMEM (Dulbecco's Modified Eagle's medium, Gibco) supplemented with 15% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin (Invitrogen) for 24 hours. Free-floating cells were removed through several washing steps using PBS, and adherent cells were incubated in the enriched medium at 37ºC in a 5% CO2 in a humidified incubator. The medium was changed to a fresh one every three days, and confluent

cells were detached and aliquoted into new containers and propagated. After passage 4, the cells were detached and stained using fluorescent-labeled antibodies against MSC and hematopoietic lineage markers using flow-cytometry in terms of positivity for CD90, CD73, CD105, and negativity for CD14, CD34, and CD45 markers. The multipotency of cells was also confirmed by differentiation of them into osteoblast and adipocytes using osteogenic and adipogenic media, respectively.

Propagation of colorectal cancer cell line, HT-29

HT-29 cells, a colorectal cancer cell line, was purchased from the Pasteur Institute of Tehran, Iran, and maintained in RPMI-1640 medium (Sigma) supplemented with 10% FBS, 100 U/ml penicillin/streptomycin. The medium was changed with the fresh one every three days, and the cells were passaged to new containers upon reaching proper confluence. All incubations were carried out at 37 °C in a 5% CO2 incubator with %95 humidity.

Co-culture of HT-29 cells with MSCs

A 6-well transwell cell culture system (Pore size 0.4 µm; Costar Corp. USA) was used to perform the co-culture of colorectal cancer cells with MSCs. DMEM supplemented with 10% FBS and 1% penicillin/streptomycin was used to support the cells. HT-29 cells at a density of $2x10^5$ cells were transferred onto the lower chamber, and MSCs ($1.5x10^6$ cells) were layered on the upper surface of the membrane of the transwell insert. The plates were incubated under proper conditions for 3 days, and subsequently, HT-29 cells were detached and analyzed for the expression of EMT markers by flow cytometry and RT-qPCR assays. The control group includes HT-29 cells incubated in the absence of MSCs in the upper chamber.

Assessment of cancer stem cell markers by flow cytometry

The expression levels of cancer stem cell (CSC) markers, namely CD133 and CD44, on the surface of HT-29 cells co-cultured with MSCs and those cultured in the absence of MSCs were analyzed using flow cytometry. At the end of the incubation period, HT-29 cells were detached and resuspended in PBS containing 1% bovine serum albumin (BSA) and then counted. Totally, $1x10^5$ cells were treated with PE-conjugated CD133 and FITC-conjugated CD44 antibodies for 1 hour at 4 ℃ in a dark place. Following two washing steps, the cells were analyzed by flow cytometry apparatus, and data related to $4x10^4$ cells were acquired by the software. Isotype antibodies were used as controls and drawing quadrants.

Expression levels of EMT-related genes

RNA extraction and cDNA synthesis

Total RNA was isolated from the HT-29 cell line using a standard RNA isolation kit, obeying the instructions. The RNA concentration and purity were examined by a Nanodrop spectrophotometer (NanoDrop Techniques INC, USA), and RNA quality was analyzed using agarose gel electrophoresis (%2). The extracted RNA was reverse-transcribed into cDNA by RevertAid First Strand cDNA Synthesis Kit (cat no K1622, Thermo Scientific) using the oligo$(dT)_{18}$ primer.

Real-time quantitative PCR for mRNA detection

The expression of E-cadherin, Vimentin, and β-catenin genes were evaluated (in triplicate experiments) by real-time quantitative PCR. The GAPDH gene was used for data normalization. The PCR reactions were carried out using SYBR Green Master Mix (ThermoFisher Scientific Inc.) on the Rotor-Gene 5plex HRM instrument (QIAGEN, Hilden, Germany). Primers were designed by the Primer3 software, and their sequences are presented in Table 1.

Table 1. Primer sequences

Genes	Primer sequences
E-cadherin	F: 5′-TGCTCTTGCTGTTTCTTCGG-3′
	R: 5′-CTTCTCCGCCTCCTTCTTC-3′
Vimentin	F: 5′-CCAGGCAAAGCAGGAGTC-3′
	R: 5′-CGAAGGTGACGAGCCATT-3′
β-Catenin	F: 5′-GGGTAGGGTAAATCAGTAAGAGGT-3′
	R: 5′-GCATCGTATCACAGCAGGTT-3′
GAPDH	F: 5'-GCTCTCTGCTCCTCCTGTTC-3'
	R: 5'-ACGACCAAATCCGTTGACTC-3'

Statistical analysis

Data were analyzed by the Graphpad Prism software. Each experiment was performed on three consecutive days and in duplicate. The results were expressed as the means and standard error of the mean (mean ± SEM). The Student's T-test was used for the comparison of two sets of independent variables, and the level of the statistical significance was set at $p<0.05$.

Ethical consideration

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.CRC.REC.1400.003).

Results

Morphological changes in HT29 cells

Morphological characteristics of HT29 cells co-cultured with MSCs were observed under an inverted microscope and compared with control cells (HT-29 cells alone). As depicted in Figure 1, HT29 control cells show a normal morphology, while the appearance of HT-29 cells co-cultured with MSCs was elongated and is very similar to fibroblast cells. It seems that the co-culture of HT29 cells with MSCs resulted in the conversion of epithelial into fibroblastic-like shapes.

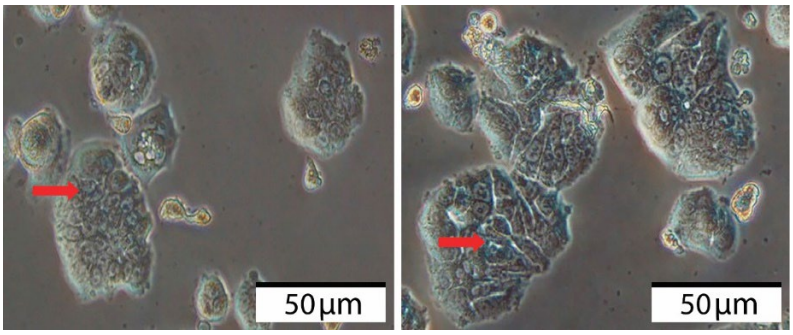

Figure 1. Morphological alterations in HT29 cells after 3 days of co-culture with MSCs; the upper image: normal morphology related to epithelial nature of HT-29 cells; the lower image: Elongated fibroblast-like appearance related to the EMT process of HT-29 cells; magnifications: ×200

Expression of EMT markers

The expression levels for E-cadherin, Vimentin, and β-catenin genes were assessed in HT-29 cells co-cultured with MSCs or cultured alone. As shown in Figure 2, the mRNA expression levels of β-catenin and Vimentin genes were increased by 1.83 and 2.25 folds in HT-29 cells cocultured with MSCs, compared with control cells, respectively. In contrast, the mRNA expression level of the E-cadherin gene was significantly decreased (0.3) in HT-29 cells cocultured with MSCs compared to the control cells

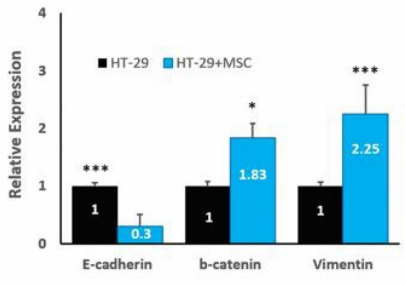

Figure 2. Changes in the mRNA expression levels of E-cadherin, β-catenin, and vimentin genes, as EMT markers, in HT-29 cells co-cultured with MSCs. The error bars represent standard errors of means (SEM). * p<0.05, *** p<0.001

Expression of CD44 and CD133

CD44 and CD133 are a group of proteins expressed on the surface of cancer stem cells, and their expression levels are increased following EMT induction by MSCs. The identification of these two markers ensures successful EMT induction in CRC cells. The analysis of CD44 and CD133 expression was performed by flow cytometry. A marked increase was found in the protein expression of CD133 in CRC cells following 72 hours of co-culture with MSCs. The expression levels of CD44 and CD133 markers were increased in CRC cells (0.46% and 51.5%, respectively) following indirect exposure to MSCs compared to the control cells. The significance of the differences was presented in Figure legends (Figure 3).

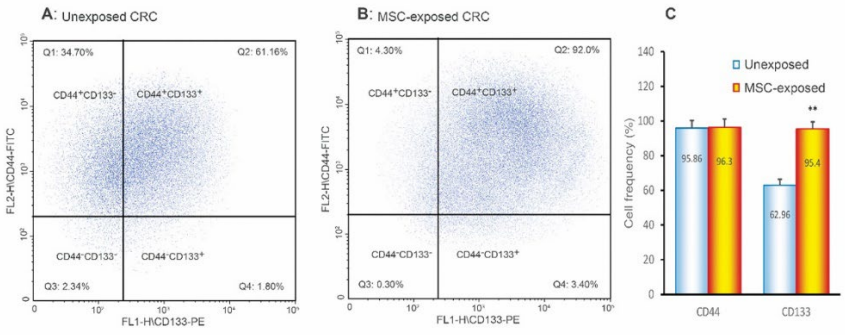

Figure 3. The frequency of CD44+ and CD133+ cells among HT-29 cells co-cultured with MSCs (B) and cultured alone (A). The flow cytometry analysis showed an increased frequency of CD133+ cells among HT-29 cells after being co-cultured with MSCs (62.96% was increased to 95.40%). The number of double-positive cells CD44+CD133+ was also increased among HT-29 cells co-cultured with MSCs from 61.16% to 92.0%. (C) The bar chart represented significant differences between HT-29 cells co-cultured with MSCs and those cultured alone in terms of the mean frequency of CD133-positive cells. ** p<0.01

Discussion

The interaction between tumor and stromal cells affects the growth and metastasis of colon cancer (15). Tumor-associated fibroblasts (TAFs), originating from bone marrow-derived MSCs, are an important part of the tumor stroma. MSCs show high tropism toward wounds and tumor tissues (16). Bone marrow-derived MSCs selectively migrate to tumor stroma and differentiate into TAFs and potently promote tumor growth through cell-cell contact and soluble interactions (17). Both promotion and inhibition of tumor growth and metastasis by MSCs have been reported. The conditioned medium of bone marrow-derived MSCs led to increased expression of aquaporin 1 in both osteosarcoma and hepatocellular carcinoma tumor cell lines. Aquaporin 1 is a water channel known to promote metastasis and neo-angiogenesis (17). Shinagawa et al. used a mouse model of colon cancer and showed that systemically injected MSCs led to the migration of these cells into tumor stroma and promoted tumor growth and metastasis (18). Co-implantation of CRC cells with MSCs into the cecal walls of mice produced larger tumors, and MSCs promoted tumor growth and lymph node metastasis through increased angiogenesis, migration, and invasion, as well as inhibiting apoptosis in tumor cells (19). Zhu et al. also reported in-vivo cancer-promoting effects of MSCs using F6 and SW480 (human colon cancer) cell lines in a mouse model (20). The effects of MSCs on tumor growth inhibition and proliferation were also investigated using chronic myelogenous leukemia cell line, K562, Kaposi's sarcoma cells, and two human hepatoma cell lines (21).

Mesenchymal stem cells are plastic-adherent cells with a high capacity to differentiate into various cellular lineages, such as adipocytes and chondrocytes. In addition, due to their self-renewal abilities, MSCs actively contribute to tissue regeneration processes (9).

Tumor tropism of MSCs has been reported in the literature. In-vitro migration of MSCs into spheroids of pancreatic cancer cells was observed in a number of studies. The injection of GFP-marked MSCs in nude mice showed migration of cells preferentially into tumor xenografts (22).

Proteomics and secretomics analyses of MSCs have revealed their high potential to secrete immune stimulators (cytokines and chemokines) and growth factors (23), thereby promoting cell proliferation (fibroblast, epithelial and endothelial cell division) and angiogenesis (24).

Bone marrow-derived MSCs migrate to tumor tissues and differentiate into TAFs and vascular pericytes (21). Tumor-educated MSCs promote cancer progression through producing pro-tumorigenic cytokines, changing metabolic pathways, and releasing exosomes. In addition, the hypoxic and acidic tumor microenvironment affects other tumor resident cells and induces a tumor-promoting phenotype in MSCs (25). On the other hand, tumor cells secrete soluble factors and extracellular vesicles containing different biomolecules for educating tumor-associated MSCs. MSCs that migrate to the tumor microenvironment show higher cellular plasticity with a more stem-like phenotype and acquire some biomarkers of CSCs upon communication with cancer cells through secretory factors (26). MSCs are capable of suppressing innate and adaptive immune cells in favor of tumor cells. MSCs can also induce EMT and colon cancer cell metastasis (10, 27). MSCs produce IL-6, IL-8, CCL5, VEGF, and TGF-β that promote tumor cell proliferation, survival, migration, invasion, and metastasis (28).

Above, we presented several lines of evidence about the promoting effects of MSCs on tumor growth, invasion, and metastasis. MSCs exert their promoting ability through the induction of epithelial to mesenchymal transition (EMT), a phenomenon that results in the generation of cancer stem-like cells (CSCs). CSCs show increased stemness properties and can produce all subpopulations of tumor tissues upon migrating into the target tissue. These “tumor-initiating cells” play a significant role in the drug resistance of CRCs and in postoperative cancer recurrence (29). CSCs show distinct surface molecular characteristics in different cancer types, known as CD markers. Many studies have emphasized the increased expression of CD133, as a significant marker, for distinguishing CSCs in malignant tumor tissues (30). In general, CD44 and CD133 are the most widely studied markers in identifying CSCs in colorectal cancer (7); however, other markers, such as CD166, CD24, CD29, Lgr5, and Bmi1, were also investigated in CRC. Clinically high CD133 levels are inversely correlated with the 5-year overall survival and disease-free survival rates in patients with some types of cancer, including colorectal cancer (31).

The present research evaluated indirect interactions (via soluble factors secreted by tumor cells and MSCs) between mesenchymal stem cells and colorectal cancer cells and determined the mRNA expression levels of EMT-related genes (E-cadherin, β-catenin, and Vimentin). We also analyzed the protein expression of CSC surface markers (CD44 and CD133). Transwell plates with a membrane pore size of 400 nm were used for indirect co-culture of the two cell types. Membrane pores are small enough to prevent MSC infiltration into the lower chamber; however, they are also large enough to allow free distribution of almost all soluble substances between the two chambers (between upper and lower chambers). The CRC cell line, HT-29, along with isolated bone marrow-derived MSCs, was co-cultured in the lower and upper parts of the chamber, respectively. They incubated in optimum conditions. The control cells include HT-29 cells that were cultured alone in the absence of MSCs. At the end of the third day, the morphology of cells was assessed (by

an inverted microscope), and then they were detached. The mRNA expression levels of E-cadherin, β-catenin, and Vimentin genes were analyzed. The frequency of CD44+ and CD133+ cells was also determined. After the co-culture process, the expression of CD133+ cells was increased in HT-29 colorectal cancer cells by 51.5% compared to the control cells, while the expression of CD44+ cells was increased only slightly (0.46%). Notably, the expression of double positive cells, CD44+CD133+, was elevated from 61.16% to 92% (30.84% increase after being co-cultured with MSCs). Altogether, the co-culture of HT-29 cells with MSCs resulted in the increased frequency of CSCs harboring CD44 and CD133 markers on their cell surface.

Primary cancer cells have epithelial nature and morphology, in which the cells highly express E-cadherin, a membrane adhesion molecule, and bind to adjacent cells through cell-cell junctions. The cells also show very low migration and invasion abilities. However, colorectal cancers are usually highly metastatic compared to other tumors. In other words, CRCs possess a higher frequency of CSCs in tumor tissues. Our finding showed a 61.16% frequency of CD44+CD133+ cells even before the co-culture process, implying that the frequency of CSCs is initially high among HT-29 cells; however, the co-culture process increased the frequency of these cells to 92%. The phenomenon that causes primary cells to become more aggressive and metastatic is called epithelial-mesenchymal transition, EMT. EMT enables tumor cells to migrate from their original site into other parts of the body **(32)**. During EMT, primary cancer epithelial cells acquire fibroblast-like shapes, as confirmed by our observations. Such morphological changes in tumor cells have also been reported in the lung, colon, and colorectal cancer cells **(33)**. EMT results in reduced expression of Ep markers, E-cadherin, and cytokeratin, as well as a concomitant increase in mesenchymal markers, such as Vimentin, N-cadherin, Snail, ZEB1, ZEB2, Fibronectin, and β-catenin. In the present study, the mRNA expression levels of the three EMT markers were evaluated in HT-29 colorectal cancer cells, induced by being co-cultured with bone marrow-derived MSCs to elucidate the nature of possible interactions that may occur between CRCs and MSCs. After 3 days of co-culture of HT-29 cells with MSCs, and mesenchymal markers, the expression levels of Vimentin and β-catenin genes were prominently increased in CRC cells compared to the control cells. On the other hand, the expression of the epithelial marker, E-cadherin, was dramatically reduced in HT-29 cells co-cultured with MSCs compared to the control.

Our findings demonstrated that the co-culture of HT-29 colorectal cancer cells with MSCs resulted in a prominent increase in the mRNA expression of Vimentin, a mesenchymal marker. Other researchers have reported similar findings; for example, Zhang et al. co-cultured SW48 colorectal cancer cells with MSCs derived from colon cancers and observed reduced E-cadherin and increased Vimentin expression in cells, denoting the EMT induction by MSCs **(34)**. Martin et al. co-cultured breast cancer cells with bone marrow-derived MSCs and observed more than 3 folds increases in Vimentin expression, as well as a significant reduction in E-cadherin expression **(35)**. Azizi et al. co-cultured AGS cells with MSCs and reported several folds increase in Vimentin expression and a substantial reduction in E-cadherin expression **(36)**. Generally, bone marrow-derived mesenchymal stem cells are potent inducers of EMT in primary cancer cells and promote cancer invasion and metastasis partly through the increment in CSC frequency in tumor tissues.

Vimentin, a cytoskeletal filament, is involved in cell migration, motility, and adhesion. It also facilitates EMT when overexpressed in tumors; however, Vimentin is known as one of the typical biomarkers of mesenchymal cells. Increased expression of Vimentin usually leads to the disruption of cellular adhesion junctions, thus enhancing the motility and invasiveness of tumor cells **(32)**.

β-catenin is an intracellular signal transducer in the Wnt signaling pathway. β-catenin, in conjunction with E-cadherin, is considered a component of the cell adhesion complex; however, reduced expression of E-cadherin results in the accumulation and localization of the protein in the nucleus which has a promoting effect on EMT **(37)**.

E-cadherin is an adhesion glycoprotein and is categorized as one of the major factors involved in the EMT process. The downregulation of E-cadherin is regarded as a prognostic marker in colorectal cancer patients **(38)**. The expression of E-cadherin may be suppressed during the overexpression of some transitional genes, such as Slug, Snail, and Twist. Taken together, our findings show that MSCs can communicate with CRCs in a co-culture system and show a high capacity to induce EMT in cells.

Conclusion

In the current study, we found that the co-culture of HT29 cells with MSCs led to morphological alterations in cancer cells from epithelial to mesenchymal forms. The expression levels of EMT biomarkers, namely Vimentin and β-catenin, were increased, while the epithelial marker, E-cadherin, was reduced in the co-culture system compared with cancer cells cultured in the absence of any treatments. Also, the specific surface marker of CSC, CD133, was upregulated in the co-culture system compared with HT-29 cells cultured alone. The understanding of the precise mechanism underlying the EMT process could pave the way to finding effective therapeutic approaches to inhibit tumor invasion and metastasis.

Acknowledgments

None.

Conflict of Interest

The authors declared no conflict of interest.

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