The in vitro Treatment of Mesenchymal Stem Cells for Colorectal Cancer Cells

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Abstract

Colorectal cancer is the most common tumor of the gastrointestinal system. The conventional treatment options of colorectal cancer are troublesome for both patients and clinicians. Recently, mesenchymal stem cells (MSCs) have been the novel focus for cell therapy due to its migration to tumor sites. In this study, the apoptotic effect of MSCs on colorectal cancer cell lines has been aimed. HCT-116 and HT-29 were selected as the colorectal cancer cell lines. Human umbilical cord blood and Wharton's jelly were used as mesenchymal stem cell sources. To discriminate against the apoptotic effect of MSC on cancer, we also used peripheral blood mononuclear cells (PBMC) as a healthy control group. Cord blood-MSC and PBMC were obtained by ficoll-paque density gradient, and Wharton's jelly-MSC by explant method. Transwell co-culture systems were used as cancer cells or PBMC/MSCs at ratios of 1/5 and 1/10, incubation times of 24 hours and 72 hours. The Annexin V/PI-FITC based apoptosis assay was performed by flow cytometry. Caspase-3 and HTRA2/Omi proteins were measured by ELISA. For both ratios in both cancer cells, it was found that the apoptotic effect of Wharton's jelly-MSC was significantly higher in 72-hour incubations ($p<0.006$), whereas the effect of cord blood mesenchymal stem cell in 24-hour incubations were higher ($p<0.007$). In this study, we showed that human cord blood and tissue derived MSCs treatment led colorectal cancers to apoptosis. We anticipate that further in vivo studies may shed light on the apoptotic effect of MSC.

Introduction

Stem cells are known as a unique cell group that have the capacity to self-renewal and differentiate into many cell types [1–3]. Due to these special potentials stem cells have, they are used as a cell therapy method in regenerative medicine, recently [4–6]. A special type of stem cells known as mesenchymal stem cells (MSCs) have brought a new approach to regenerative medicine due to the ease of obtaining and isolating from various tissues [7]. MSCs can be obtained from many tissues such as bone marrow, adipose, dental pulp, cord blood, Wharton's jelly and also, they can differentiate into the tissues originating from all three germ layers, differ from other stem cells with their plastic adherent and multipotent properties [7–9]. Cord blood and Wharton's jelly are abundant, non-invasive, and easy to obtain stem cell sources in terms of MSC source. In addition to these features, MSC can express "CD73, CD90 and CD105" (> 95%) on cell surfaces, while "CD11b, CD14, CD19, CD34, CD45, CD79α and HLA-DR" markers at a low rate (< 2%) [10] (Dominici et al. 2006). Recently, they have been used in many studies of regenerative medicine and have gained importance especially in cancer studies because they lead the cancer cells to apoptosis [11–13].

Colorectal cancer is the third most common type of cancer in both male and female worldwide and is the most common tumor of the gastrointestinal tract [14, 15]. It is considered to be an aggressive type of cancer because it can metastasize through surrounding blood vessels and lymph nodes. Cancers can be treated by standardized methods such as chemotherapy, radiotherapy, surgical procedures, and immunotherapy. Each of these traditionally accepted treatments has various limitations [16–19]. Recent advances in cancer treatments are in the direction of investigating the anti-cancer properties of human

stem cells. The interaction between stem cells with tumor cells and the apoptotic pathways used by anti-inflammatory molecules secreted by stem cells for anti-cancer mechanisms need to be studied in more detail.

In the current experiment, we aim to investigate the apoptotic effect of human derived MSC in vitro HT-29 and HCT-116 colorectal cancer cells. Mesenchymal stem cells were isolated from human cord blood and human Wharton's Jelly. MSCs and colorectal cancer cells were cultured with a 24-transwell system, two incubation times of 24h and 72 h and two ratios 1:5 and 1:10 (Colorectal cancer cells: Mesenchymal stem cells). We added colorectal cancer cells without WJ-MSC (WJ-MSC(-)) or CB-MSC (CB-MSC(-)) into the study as a control group. We also used peripheral blood mononuclear cells (PBMCs) as another control group to discriminate the apoptotic effect of MSC on healthy cell groups. The effects of MSC were determined with Annexin V/PI-FITC Flow Cytometry, Caspase-3 ELISA, and HTR2A/Omi ELISA methods.

Materials And Methods

Material Collection

Our current study was approved by the Ethics Committee of Istanbul University Istanbul Faculty of Medicine with the date 27/05/2020, and no: 62261**.** Human umbilical cord blood and tissues used in the study were obtained from individuals who had healthy pregnancy and volunteered to participate to the study with the informed consent in the department of the Obstetrics and Gynecology Polyclinic at Istanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital. The peripheral blood mononuclear cells used in the study as the control group and were obtained from a healthy individual who volunteered to participate to the study with the informed consent in the department of Medical Biology at Istanbul Faculty of Medicine. The material storage and assays were performed in the department of Medical Biology at Istanbul University, Istanbul Faculty of Medicine.

Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells were obtained from whole blood samples. PBMC of 20-30 cc taken from healthy individuals into heparinized tubes was processed in a sterile cabinet and were isolated using Ficoll-paque gradient method [20, 21]. The cells were grown at 37°C in a humidified atmosphere with 5% CO_2 with CB-MSC or WJ-MSC at 1:5 or 1:10 ratios during 24 h and 72 h incubation periods. At the end of the incubation period, PBMC were collected, and the cells were used immediately for the apoptosis assays.

Derivation of Mesenchymal Stem Cells from Human Cord Blood

MSCs from cord blood were obtained by centrifugation using the Ficoll-paque density gradient method from blood taken into heparin tubes after cesarean section [20, 21]. The cells obtained after centrifugation were put in 75 cm^2 cell culture flasks by adding 10% Fetal Bovine Serum (FBS), 1%

penicillin/streptomycin-containing L-glutamine-rich Dulbecco's Modified Eagle Medium (DMEM) (Biosera, Nuaille, France). The cells were maintained and expanded at 37°C in a humidified atmosphere with 5% CO_2, and the medium was replenished when required. Only cells from the 3rd passage were used for the experiments.

Derivation of Mesenchymal Stem Cells from Human Wharton's Jelly

After cesarean section, the human umbilical cords that were cut up to 15-20 cm were carried in a sterile storage box. MSCs from Wharton's jelly were obtained by the explant method [22, 23]. Tissues were taken in a solution consisting of previously prepared PBS+penicillin+streptomycin and amphotericin B medium for 1-2 hour/s and were incubated at 37°C in a humidified atmosphere with 5% CO_2 in the incubator. After incubation, the arterial and vein vessels were carefully removed from the tissue using with scissors, scalpels, and pliers in petri dishes. After this procedure, Wharton's jelly tissues, which were separated into smaller pieces by <1 cm, followed by scalding in more smaller pieces were put in 75 cm^2 cell culture flasks by adding 10% FBS, 1% penicillin/streptomycin-containing L-glutamine-rich DMEM (Biosera, Nuaille, France). The cells were expanded at 37°C in a humidified atmosphere with 5% CO_2, and the medium was replenished when required. Only cells from the 3rd passage were used for the experiments.

Colorectal Cancer Cell Lines

In the study, HT-29 (RRID:CVCL_0320) and HCT-116 (RRID:CVCL_AS10) were selected as the CRC cell lines obtained from the American Type Culture Collection (Manassas, VA, USA). The dissolved cells were washed with DMEM and were centrifuged at 2000 rpm. After washing, the cell pellets at the bottom of the tube were carefully removed by pipetting and were added into to 75 cm^2 cell culture flasks with previously prepared 10% FBS, 1% penicillin/streptomycin-containing L-glutamine-rich DMEM (Biosera, Nuaille, France) medium content [24]. The cells were grown at 37°C in a humidified atmosphere with 5% CO_2, and the medium was replenished when required.

Characterization of Mesenchymal Stem Cells

The proliferation of from both cord blood- and Wharton's jelly-derived MSCs after obtaining confluent appearance via inverted microscopy (Motic China Group Co. Ltd., AE21, Xiamen, P.R.C.), the cell viability and counts were evaluated using the Trypan blue exclusion method. MSC characterization was performed by using the BD Stemflow Human MSC Analysis Kit (BD Biosciences, San Jose, CA, USA) and was analyzed using NaviosEX flow cytometry (Beckman Coulter Navios, in, USA) [25]. CD73, 90 and 105 positivity and CD34, 11b, 19, 45 and HLA-DR negativity were evaluated with NaviosEX software program (Beckman Coulter, Navios, IN, USA).

In Vitro Co-culture of Mesenchymal Stem Cells with Colorectal Cancer Cells

Before co-culture procedure, both cancer cells and MSCs were removed from the flasks by the trypsinization method [26]. HT-29 cells were seeded in 24-well plates ($5x10^4$ cells/well) and added the

transwells (Sarstedt AG, Nümbrecht, Germany) with a 0.4 µm filter was placed on. MSCs were seeded in transwells (for 1:5 ratio $25x10^4$ cells/transwell, 1:10 ratio $5x10^5$ cells/transwell) and for control groups, only HT-29 cells ($5x10^4$ cells/well) were seeded into the 24-wells without MSCs. Both experiment and control groups were incubated in medium at at 37°C in a humidified atmosphere with 5% CO_2 for 72 h. At the end of the incubation period, HT-29 cells were collected by trypsinization and recorded by counting the number of cells with a trypan blue dye of 0.1%.

Annexin V/PI-FITC Apoptosis Assay

The apoptosis assay of CRC cells was evaluated by flow cytometry using the Annexin V/PI-FITC method [27]. ApoFlowEx FITC kit (ExBio Praha, Vestec, Czech Rep.) was used for the determination apoptosis by flow cytometry. HT-29 cells were washed with cold PBS at 2000 rpm and were diluted to obtain 3-5 x 10^4 at 100 µL. Then, 5 µL of Annexin V, 5 µL of Propidium iodide were carefully mixed with pipetting on 100 µL cells and incubated in the dark for 15 minutes at room temperature. After incubation, 100 µL of 1X Annexin Binding Buffer was added. Then, after centrifugation and supernatant removal, the apoptosis analysis was performed by using the NaviosEX software program (Beckman Coulter, Navios, IN, USA).

Caspase-3 Protein Level Change

The cell culture supernatants collected and stored after the incubation period were used in the ELISA method [28]. The collected samples were centrifuged at 1000 rpm for 20 minutes before being tested. All reagents to be used in the assay were brought to room temperature before the experiment. The Human Caspase-3 (Casp3) ELISA kit was used for Caspase-3 protein level change (Abbkine Inc., Wuhan, China). Appropriate dilutions of all solutions and reagents were prepared before starting the assay. All standard, assay and control wells were determined in the 96-well plate, and the samples were processed according to the manufacturer's instructions. Absorbance at 450 nm was measured using an ELISA reader (Allsheng, FlexA-200, Hangzhou, China).

HTRA2/Omi Protein Level Change

The cell culture supernatants collected and stored after the incubation period were used in the ELISA method [29]. The collected samples were centrifuged at 1000 rpm for 20 minutes before being tested. All reagents to be used in the assay were brought to room temperature before the experiment. The Human HTRA2 (Omi) ELISA kit was used for HTRA2/Omi protein level change (ThermoFisher Scientific Life Tech Corp., Carlsbad, CA, USA). Appropriate dilutions of all solutions and reagents were prepared before starting the assay. All standard, assay and control wells were determined in the 96-well plate, and the samples were processed according to the manufacturer's instructions. Absorbance at 450 nm was measured using an ELISA reader (Allsheng, FlexA-200, Hangzhou, China).

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 17.0 package statistical program. Multidimensional statistical evaluations were made on the obtained data. All values are expressed as mean ± standard errors of the mean. The continuous variables were compared by the Mann-Whitney U-test for two independent group comparisons and the Wilcoxon rank test for the differences of the dependent groups. The significance value was determined as $p<0.05$ for each test. All experiments and control groups were performed in nonuplicate (n=9).

Results

1 Characterization of the Mesenchymal Stem Cell

The cord blood- and Wharton's jelly-derived mesenchymal stem cells were identified under microscopy (**Fig. 1**). After microscopic observations, the cultures of the adherent MSC population were labeled by the antibodies targeting the cell-surface antigens such as CD 73+, CD 90+ and CD 105+ and CD 34-, CD 11b-, CD 19-, CD 45- and HLA-DR negativity and identified by flow cytometry(**Fig. 2**).

2 Annexin V/PI-FITC-based Apoptosis Assay

2.a. Wharton's Jelly-derived Mesenchymal Stem Cells Affect in vitro HCT-116 and HT-29 Cells

In order to comparing the apoptotic effects of WJ-MSCs in vitro colorectal cancer cells by flow cytometry results, WJ-MSC(+) treated HCT-116 /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 and total group were significantly ($p<0.05$) higher than WJ-MSC(-) control group measurements.

When the flow cytometric results of its apoptotic effects on HT-29 were analyzed, WJ-MSC(+) HT-29 /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 and total measurements were significantly ($p<0.05$) higher than WJ-MSC(-) measurements (**Fig. 3**).

2.b. Cord Blood-derived Mesenchymal Stem Cells Affect in vitro HCT-116 and HT-29 Cells

HCT-116 CB-MSC (+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 was significantly ($p<0.05$) higher than CB-MSC (-) control groups. It was also found that HT-29 CB-MSC (+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 groups were significantly ($p<0.05$) higher than in CB-MSC (-) control group measurements (**Fig. 4**). The apoptosis results of two MSC in colorectal cancer cells are shown in **Fig. 5**.

2.c. The Effect of Cord Blood-derived Mesenchymal Stem Cells and Wharton's Jelly-derived Mesenchymal Stem Cells on Healthy Peripheral Blood Mononuclear Cells

To evaluate the apoptotic effect of MSCs on healthy cell groups, we cultured CB-MSCs and WJ-MSCs with PBMC under the same conditions of the experimental groups. PBMC CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10, and the total groups were higher than CB-MSC(-) measurements ($p<0.05$). On the other hand, PBMC CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10, and total measurements were higher significantly ($p<0.05$) than CB-MSC(-) group (**Fig. 6**).

2.d. The Comparison of Cord Blood-derived Mesenchymal Stem Cells and Wharton's Jelly-derived Mesenchymal Stem Cells for Apoptotic Effect

In order to compare the apoptotic effects between WJ-MSC and CB-MSC in vitro human colorectal cancer cell lines, Annexin V/PI-FITC based flow cytometry assay was conducted. The data demonstrated that 24 h incubation of CB-MSC for both two ratios was higher than 24 h incubation of WJ-MSC for both HCT-116 and HT-29. Similarly, 72 h incubation of WJ-MSC for two ratios was higher than 72 h CB-MSC for the human colorectal cancer cells (**Fig. 7**).

3 Caspase-3 ELISA Assay

3.a. Caspase-3 Protein Change of Wharton's Jelly-derived Mesenchymal Stem Cells in vitro Colorectal Cancer Cells

Caspase-3 ELISA analysis was performed to check the potential role of apoptosis of WJ-MSC or CB-MSC on the human colorectal cancer cells. For HCT-116 WJ-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 and total experimental groups, the caspase-3 change was significantly ($p<0.05$) higher than the WJ-MSC(-) measurements. On the other hand, the caspase-3 change in the measurements of HT-29 WJ-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 and total experimental groups were significantly ($p<0.05$) higher than WJ-MSC(-) group measurements (**Fig. 8**).

3.b. Caspase-3 Protein Change of Cord Blood-derived Mesenchymal Stem Cells in vitro Colorectal Cancer Cells

When we evaluated the CB-MSC culture results we found that caspase-3 change for HCT-116 CB-MSC(+) /24h-1:10, /72h-1:5, /72h-1:10, and total group measurements was significantly higher than CB-MSC(-) measurements ($p<0.05$). Interestingly for HCT-116 CB-MSC(+) /24h-1:5 measurements were significantly ($p<0.05$) lower than CB-MSC(-) measurements. On the other hand, for HT-29 CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10, and total group measurements were significantly ($p<0.05$) higher than CB-MSC(-) measurements (**Fig. 9**).

3.c. The Comparison of Cord Blood- and Wharton's Jelly-derived Mesenchymal Stem Cells for Caspase-3 Change

According to the data from Caspase-3 ELISA analysis, the comparisons of caspase-3 change related to WJ-MSC and CB-MSC were evaluated. HCT-116 CB-MSC(+) /24h-1:5 and /24h-1:10 caspase-3 measurements were significantly ($p<0.05$) lower than the WJ-MSC(+) group. HCT-116 CB-MSC(+) /72h-1:10 caspase-3 change was significantly ($p<0.05$) higher than the WJ-MSC(+) group. Caspase-3 measurement for HCT-116 CB-MSC(+) /72h-1:5, and total group no significance was found compared to WJ-MSC(+) groups ($p>0.05$). Caspase-3 measurements were significantly ($p<0.05$) lower in the HT-29 CB-MSC(+) /24h-1:5 measurements than in WJ-MSC(+) group. Change of caspase-3 in the measurements of HT-29 CC-MSC(+) /24h-1:10, /72h-1:5, /72h-1:10, and total groups were not significant compared to WJ-MSC(+) groups ($p>0.05$). **Fig. 10** demonstrated the comparison of CB-MSC and WJ-MSC for Caspase-3.

3.d. The Comparison of Caspase-3 Change for Cord Blood- and Wharton's Jelly-derived Mesenchymal Stem Cells on Healthy Peripheral Blood Mononuclear Cells

When the protein change analyzes of caspase-3 of PBMC group were evaluated, PBMC WJ-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10 caspase-3 change was significantly higher ($p<0.05$) than WJ-MSC(-) measurements. Caspase-3 changes for PBMC CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10, and total group were higher than CB-MSC(-) groups measurements. The comparison of PBMC groups demonstrated in **Table 1**.

4 HTRA2/Omi ELISA Assay

4.a. HTRA2/Omi Protein Change of Wharton's Jelly-derived Mesenchymal Stem Cells in vitro Colorectal Cancer Cells

In order to determine the level of mitochondrial serine protease -HTRA2/Omi protein- which is contributed to the caspase-independent apoptosis pathway, we found that the significant HTRA2/Omi protein level change in human colorectal cancer cell lines with WJ-MSC incubation compared to the control groups. In the HTRA2/Omi measurements of HCT-116 WJ-MSC(+) /24h-1:5, /24h-1:10 , /72h-1:10 and total groups were significant ($p<0.05$) were higher than HCT-116 WJ-MSC(-) control group. We found no significant differences for HCT-116 WJ-MSC(+) /72h-1:5 HTRA2/Omi change compared to WJ-MSC(-) measurements. HT-29 WJ-MSC(+) /72h-1:5, /72h-1:10 and total group HTRA2/Omi changes were significantly ($p<0.05$) higher than WJ-MSC(-) measurements. We found no significant differences for HTRA2/Omi change for HT-29 WJ-MSC(+) /24h-1:5 and /24h-1:10 compared to WJ-MSC(-) groups ($p>0.05$; **Fig. 11**).

4.b. HTRA2/Omi Protein Change of Cord Blood-derived Mesenchymal Stem Cells in vitro Colorectal Cancer Cells

Similar to WJ-MSC measurements, we found that significant CB-MSC(+) related HTRA2/Omi protein change. HCT-116 CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:10 and total HTRA2/Omi groups were more significant than CB-MSC(-) groups ($p<0.05$). We found no significance for HCT-116 CB-MSC /72h-1:5 groups. HT-29 CB-MSC(+) /24h-1:10, /72h-1:10 and total groups were significantly ($p<0.05$) higher than CB-MSC(-) measurements. Interestingly, HTRA2/Omi change for HT-29 CB-MSC(+) /72h-1:5 group was significantly ($p<0.05$) lower than CB-MSC(-) measurements. We found no significance for HT-29 CB-MSC(+) /24h-1:5 ($p>0.05$) compared to CB-MSC(-) measurements (**Fig. 12**).

4.c. The Comparison of Cord Blood- and Wharton's Jelly-derived Mesenchymal Stem Cells for HTRA2/Omi Protein Change

We evaluated the comparison of HTRA2/Omi protein change of two different MSCs in both human colorectal cancer cells, also. HCT-116 CB-MSC(+) /24h-1:5 group was significantly ($p<0.05$) higher than the WJ-MSC(+) group. HCT-116 CB-MSC(+) /72h-1:5, /72h-1:10 groups were significantly ($p<0.05$) lower

than the WJ-MSC(+) group. No significant difference ($p>0.05$) was found for HCT-116 CB-MSC(+) /24h-1:10 and total groups compared to the WJ-MSC(+) group.

HT-29 CB-MSC(+) /24h-1:10 measurements were significantly ($p<0.05$) higher than the WJ-MSC(+) group. HT-29 CB-MSC(+) /72h-1:5, /72h-1:10 and total groups were significantly ($p<0.05$) lower than the WJ-MSC(+) groups. We found no significance for HT-29 CB-MSC(+) /24h-1:5 group ($p>0.05$) compared to the WJ-MSC(+) group (**Table 2**).

4.d. The Comparison of HTRA2/Omi for Cord Blood- and Wharton's Jelly-derived Mesenchymal Stem Cells on Healthy Peripheral Blood Mononuclear Cells

In order to analyze the apoptotic effect of MSC sources in healthy cell groups, we evaluated PBMC WJ-MSC(+) and PBMC CB-MSC(+) treatment groups. PBMC WJ-MSC(+) /24h-1:10, /72h-1:5, /72h-1:10 and total groups were significantly ($p<0.05$) higher than WJ-MSC(-) groups. We found no significance for PBMC WJ-MSC(+) /24h-1:5 ($p>0.05$) compared to the WJ-MSC(-) control group. PBMC CB-MSC(+) /24h-1:5, /24h-1:10, /72h-1:5, /72h-1:10, total groups ($p<0.05$)were more significant than CB-MSC(-) control groups (**Table 3**).

Discussion

Cancer has been a common interest for researchers for years. Among the types of cancer, colorectal cancer is the third most common cancer type worldwide [14, 15]. The researchers have been focused on experimental studies to solve the cancer problem. Recently, mesenchymal stem cells have generated interest for experimental and multidisciplinary cancer studies due to its apoptotic effect [30–32]. In the current experiment, we aimed to investigate the apoptotic effect of two different MSC sources in vitro human colorectal cancer cell lines, and also evaluate the apoptosis related proteins: Caspase-3 and HTRA2/Omi.

Based on the data from Annexin V/PI-FITC method, both WJ-MSC and CB-MSC have significant apoptotic effect on HCT-116 and HT-29 colorectal cancer cells. Consistent with our results, Rezaei-Tazangi et al. have demonstrated that WJ-MSC derived secretomes enhanced the apoptotic effect and also reported that caspase-3 and -9 proteins were increased in the WJ-MSC treated HT-29 cells [33]. Similar to this study, we found that Caspase-3 protein levels were significantly increased in both WJ-MSC and CB-MSC treated colorectal cancer cells compared to colorectal cancer cells without MSC(-) and healthy cell control groups at two ratios. To the best of our knowledge, human WJ-MSC and human CB-MSC treated in vitro colorectal cancer cell studies are limited and further studies are needed. On the other hand, many researchers have reported controversial results related to apoptotic effects of MSCs obtained from different sources. Numbers of studies have indicated that bone marrow derived-MSC promoted colorectal cancer progression [34, 35]. De Boeck et al. have shown that bone marrow derived-MSC migrated to the colorectal cancer cell proliferation and enhanced the tumor progression [36]. Chen et al. have demonstrated that the paracrine effect of adipose derived-MSC has an effect on colorectal cancer progression [37]. Conversely, there are studies indicating that MSCs can attenuate colon cancer growth

[33, 38]. We assume that while adult-type MSCs such as bone marrow-derived and adipose-derived MSCs induce cancer, also adult-type but ‘younger’ MSCs such as Wharton's jelly-derived and cord blood-derived MSCs may have a higher capacity to drive cancer cells to apoptosis [39–41]. In addition, we are agreed that the dual role in the effects of MSCs may be affected by the method used in the isolation of mesenchymal stem cells, the number of passages used, different level of secreted growth factors and the differences in the culture medium [42–45].

To compare the apoptotic effect of MSCs between cancer cells and healthy cells, we used PBMC as a healthy cell group due to being a suspension cell and obtained easily. Based on our data from the healthy cell control group, we found that human WJ-MSC and CB-MSC cannot affect PBMCs via apoptotic pathways. Therefore, in this study, we showed that human WJ-MSC and CB-MSC might not affect healthy cells but colorectal cancer cells. When we investigate the caspase-dependent and caspase-independent pathways, both Caspase-3 and HTRA2/Omi protein changes were significantly higher than MSC(-) groups. But we found that MSCs have a greater apoptotic effect on colorectal cancer cells compared to the effect on PBMC. This finding might be due to the culture duration of PBMC. It is recommended to culture PBMCs without mitogen using for up to 5-7 days [46–49].

We also compared the apoptotic effect of MSC for both colorectal cancer cells. We interestingly found that 24 h incubation of CB-MSC treatment was stronger than 72 h incubation, while 72 h incubation of WJ-MSC was stronger than 24 h incubation treatment for both ratios. We have presumed that these differences may be due to the MSC acquisition time and senescence capacities of MSC. We obtained MSCs from cord blood approximately in 7 days while Wharton’s jelly in 20. The CB-MSC senescence may be earlier than the WJ-MSC senescence. Similar to this finding, Block et al. have compared the human bone marrow derived MSCs from young and elderly donors and reported that MSCs from elderly donors expanded in the younger microenvironment increased 17,000 fold to 3 x 10^9 cells and achieved their youthful phenotype [50]. In addition to this finding, we also found that 1:10 ratio and 72 h incubation of WJ-MSC treatment was more effective cell therapy for human colorectal cancer cells.

In order to define whether caspase-3 pathway by MSCs was affected to colorectal cancer cells, we measured the caspase-3 protein level of colorectal cancer cells after 24 h and 72 h incubations. For both HCT-116 and HT-29 cells, caspase-3 protein levels were significantly higher than MSC(-) control groups, but HCT-116 CB-MSC(+)/ 24h-1:5 groups were significantly lower ($p<0.05$) than HCT-116 CB-MSC(-)/ 24h-1:5 control groups. In contrast, when we examined the HTRA2/Omi change, the same group was found to be higher than MSC(-) control groups. We also found interestingly that HTRA2/Omi protein change for HT-29 CB-MSC(+)/ 72h-1:5 group was significantly ($p<0.05$) lower than CB-MSC(-) control groups. Similarly, in this group, the caspase-3 protein change evaluation was found higher than MSC(-) groups. Although we cannot address these particular differences by apoptotic protein level changes, we demonstrated that the apoptosis occurred via alternative pathways according to the flow cytometry results.

Future Directions and Limitation of the Study

This study has some limitations. First, we used MSCs from the same passage. We only determined the 3rd passage of WJ-MSC and CB-MSC. We could have used earlier and later passages to compare the apoptotic effect of MSCs in vitro. Second, we could have considered differences in terms of donor features which influenced MSC functions such as age, weight, obstetric factors [51–53]. It should be considered that the choice of isolation, the medium used in the culture, and the number of passages may change the effect of MSC [43, 44]. Third, we performed only in vitro experiments. We predict that in vivo studies should be carried out in order to observe the immunosuppression effect of CB-MSC and WJ-MSC as well as the apoptotic effect. Fourth, we could have used conditional media to compare the results between conditional media and trans-well media culture to better understand the pro-apoptotic factors if it is thermostable or not. We only demonstrated that human CB-MSC and WJ-MSC are suitable for cell based-therapy in vitro. Now, we are planning to expand the in vitro studies and also in vivo studies related to the investigation of the behavior of WJ-MSC and CB-MSC.

Conclusion

We here demonstrated that the apoptotic effect of human MSCs in vitro human colorectal cancer cells depended on MSC sources with potential profile of 24 h and 72 h incubation and 1:5 and 1:10 ratios by CB-MSC and WJ-MSCs. Taken together, MSCs from human umbilical cord blood and Wharton's jelly may present a very promising cell therapy in regenerative medicine and these findings support the use of MSC administration for treatment of in vitro colorectal cancer cells.

Declarations

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Author Contributions

FAS: Original draft collection, cell culture, isolation and characterization of mesenchymal stem cells, flow cytometric apoptosis analysis, ELISA analysis, manuscript preparation and edition, AE: Cell culture, , isolation and characterization of mesenchymal stem cells, flow cytometric interpretation, manuscript edition, MS: Cell culture, isolation of mesenchymal stem cells, data correction, GS: Material and data collections, data correction, CKC: Material and data collections, DSK: Data collection, supervision of all

procedure, FSO: Data collection and correction, supervision of all procedure. All authors read and approved the final manuscript.

Data Availability

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

Compliance with Ethical Standards

Ethical Approval and Informed Consent

This study was performed in line with the principles of the Declaration of Helsinki. Our current study was approved by the Ethics Committee of Istanbul University Istanbul Faculty of Medicine with the date 27/05/2020, and no: 62261. Human umbilical cord blood and tissues used in the study were obtained from individuals who had healthy pregnancy and volunteered to participate to the study with the informed consent in the department of the Obstetrics and Gynecology Polyclinic at Istanbul Bakırköy Dr. Sadi Konuk Training and Research Hospital. The peripheral blood mononuclear cells used in the study as the control group and were obtained from a healthy individual who volunteered to participate to the study with the informed consent in the department of Medical Biology at Istanbul Faculty of Medicine.

Conflict of Interest

The authors declare that there are no competing interests associated with the manuscript.

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Tables

Table 1. Caspase-3 protein change for healthy PBMC group

		Caspase-3 Change				p*
		WJ-MSC(+)		WJ-MSC(-)		
		Ave±SD	Median	Ave±SD	Median	
PBMC	/24-1-5	0,24 ± 0,01	0,24	0,13 ± 0,00	0,13	***0,007***
	/24-1-10	0,18 ± 0,05	0,21	0,08 ± 0,05	0,11	***0,007***
	/72-1-5	0,30 ± 0,01	0,30	0,13 ± 0,00	0,13	***0,007***
	/72-1-10	0,31 ± 0,04	0,31	0,12 ± 0,00	0,12	***0,024***
	Total	0,26 ± 0,06	0,26	0,12 ± 0,03	0,12	***0,000***

		Caspase-3 Change				p*
		CB-MSC(+)		CB-MSC(-)		
		Ave±SD	Median	Ave±SD	Median	
PBMC	/24-1-5	0,22 ± 0,03	0,21	0,12 ± 0,00	0,12	***0,007***
	/24-1-10	0,22 ± 0,01	0,21	0,11 ± 0,00	0,11	***0,007***
	/72-1-5	0,31 ± 0,07	0,29	0,10 ± 0,05	0,13	***0,007***
	/72-1-10	0,29 ± 0,05	0,27	0,09 ± 0,05	0,12	***0,002***
	Total	0,26 ± 0,06	0,23	0,11 ± 0,04	0,12	***0,000***

*For PBMC and MSCs treatment comparison, Wilcoxon test were used. The significance value was determined as $p<0.05$.

Table 2 The comparison of HTRA2/Omi related to change of MSC sources

		HTRA2/Omi Change				p*
		WJ-MSC(+)		WJ-MSC(-)		
		Ave±SD	Median	Ave±SD	Median	
HCT-116	/24-1-5	0,054 ± 0,005	0,051	0,057 ± 0,003	0,055	***0,044***
	/24-1-10	0,055 ± 0,005	0,056	0,057 ± 0,012	0,058	0,687
	/72-1-5	0,114 ± 0,019	0,121	0,072 ± 0,010	0,075	***0,000***
	/72-1-10	0,124 ± 0,023	0,135	0,091 ± 0,003	0,092	***0,001***
	Total	0,087 ± 0,036	0,075	0,069 ± 0,016	0,066	0,171
		HTRA2/Omi Change				p*
		CB-MSC(+)		CB-MSC(-)		
		Ave±SD	Median	Ave±SD	Median	
HT-29	/24-1-5	0,050 ± 0,008	0,048	0,054 ± 0,001	0,054	0,227
	/24-1-10	0,054 ± 0,004	0,054	0,061 ± 0,002	0,061	***0,005***
	/72-1-5	0,093 ± 0,005	0,094	0,064 ± 0,003	0,064	***0,000***
	/72-1-10	0,100 ± 0,001	0,101	0,076 ± 0,008	0,077	***0,001***
	Total	0,074 ± 0,023	0,074	0,064 ± 0,009	0,062	***0,000***

*For PBMC and MSCs treatment comparison, Wilcoxon test were used. The significance value was determined as $p<0.05$.

Table 3. HTRA2/Omi protein change for healthy PBMC control group

		HTRA2/Omi				
		WJ-MSC(+)		WJ-MSC(-)		p*
		Ave±SD	Median	Ave±SD	Median	
PBMC	/24-1-5	0,059 ± 0,001	0,060	0,059 ± 0,002	0,060	0,335
	/24-1-10	0,203 ± 0,223	0,060	0,052 ± 0,002	0,052	***0,048***
	/72-1-5	0,073 ± 0,005	0,070	0,067 ± 0,002	0,067	***0,024***
	/72-1-10	0,064 ± 0,002	0,064	0,056 ± 0,005	0,058	***0,024***
	Total	0,100 ± 0,123	0,063	0,059 ± 0,006	0,059	***0,000***

		HTRA2/Omi				
		CB-MSC(+)		CB-MSC(-)		p*
		Ave±SD	Median	Ave±SD	Median	
PBMC	/24-1-5	0,086 ± 0,007	0,084	0,063 ± 0,005	0,060	***0,007***
	/24-1-10	0,081 ± 0,008	0,083	0,057 ± 0,006	0,057	***0,007***
	/72-1-5	0,083 ± 0,001	0,083	0,068 ± 0,004	0,068	***0,007***
	/72-1-10	0,314 ± 0,357	0,080	0,057 ± 0,005	0,059	***0,002***
	Total	0,141 ± 0,199	0,083	0,061 ± 0,007	0,061	***0,000***

*For PBMC and MSCs treatment comparison, Wilcoxon test were used. The significance value was determined as $p<0.05$.

Figures

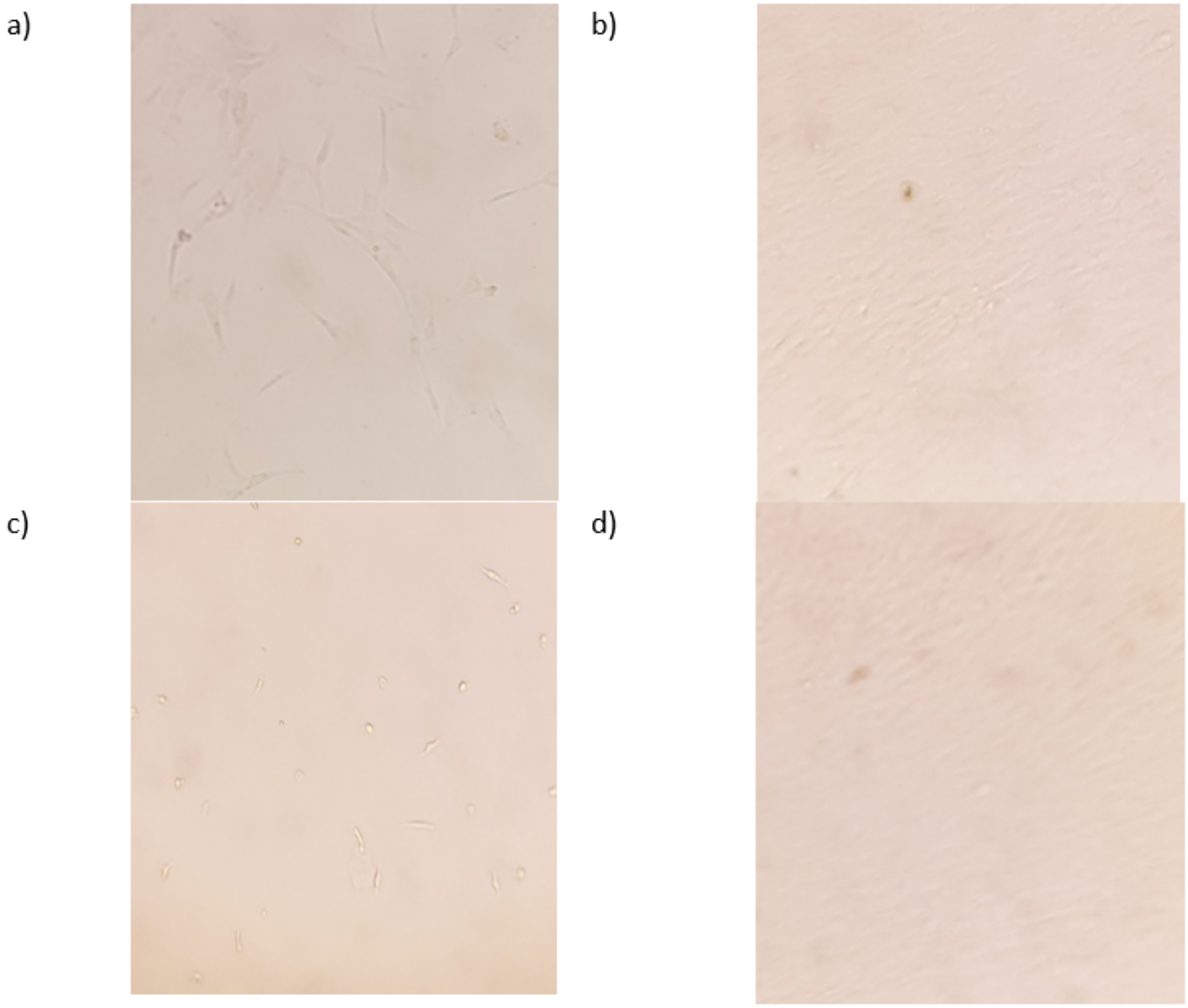

Figure 1

Images of Wharton's jelly- and cord blood-derived MSCs for adherent feature

(a) At day 20, image of Wharton's Jelly-derived MSCs. **(b)** At day 29, image of Wharton's Jelly-derived MSCs. **(c)** At day 7, the image of cord blood-derived MSCs**. (d)** At day 20, the image of cord blood-derived MSCs

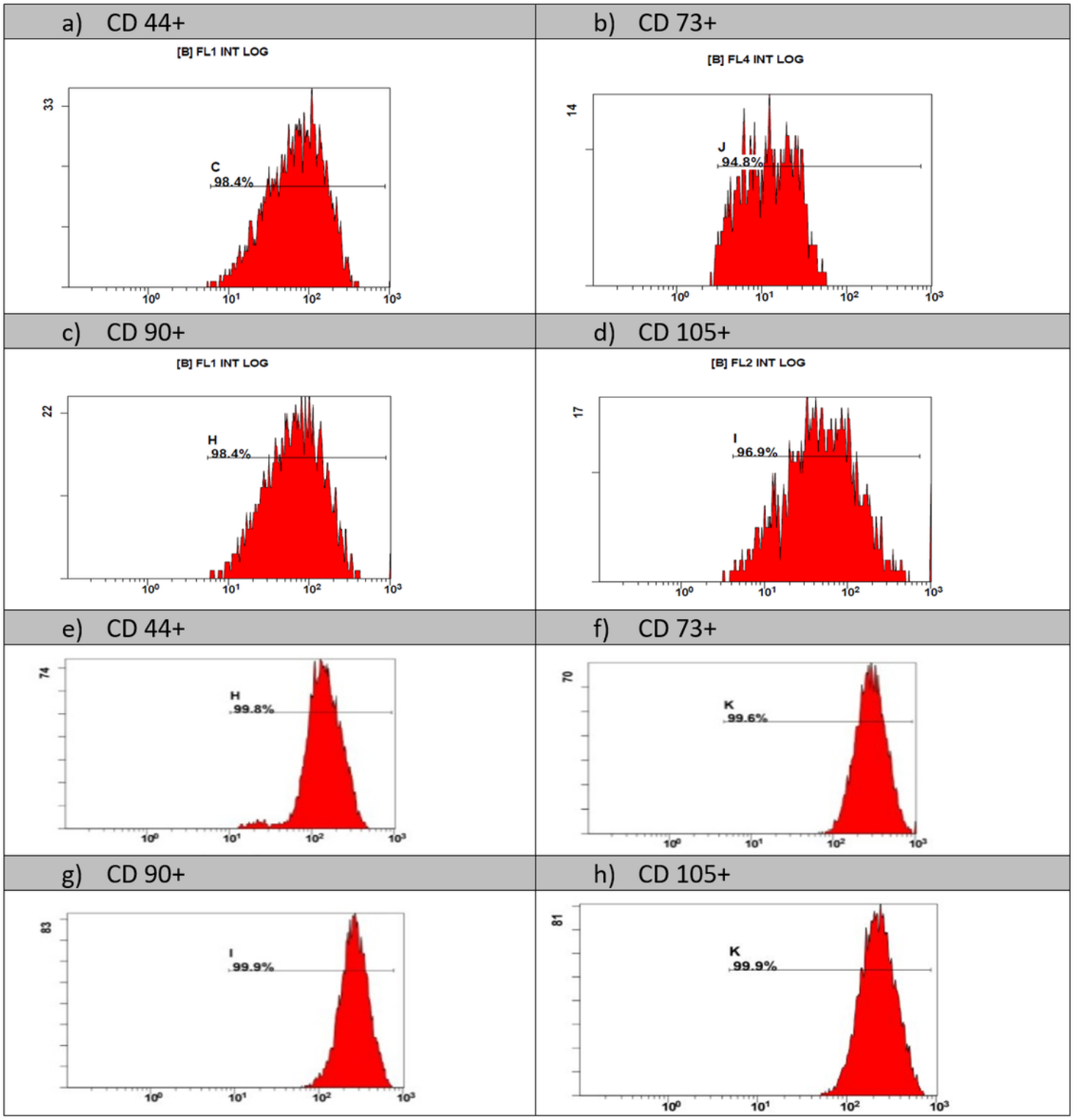

Figure 2

Characterization of mesenchymal stem cells by Flow Cytometry

(a-d) Human umbilical cord blood-derived mesenchymal stem cell specific surface markers.

(e-h) Human Wharton's jelly-derived mesenchymal stem cells specific surface markers.

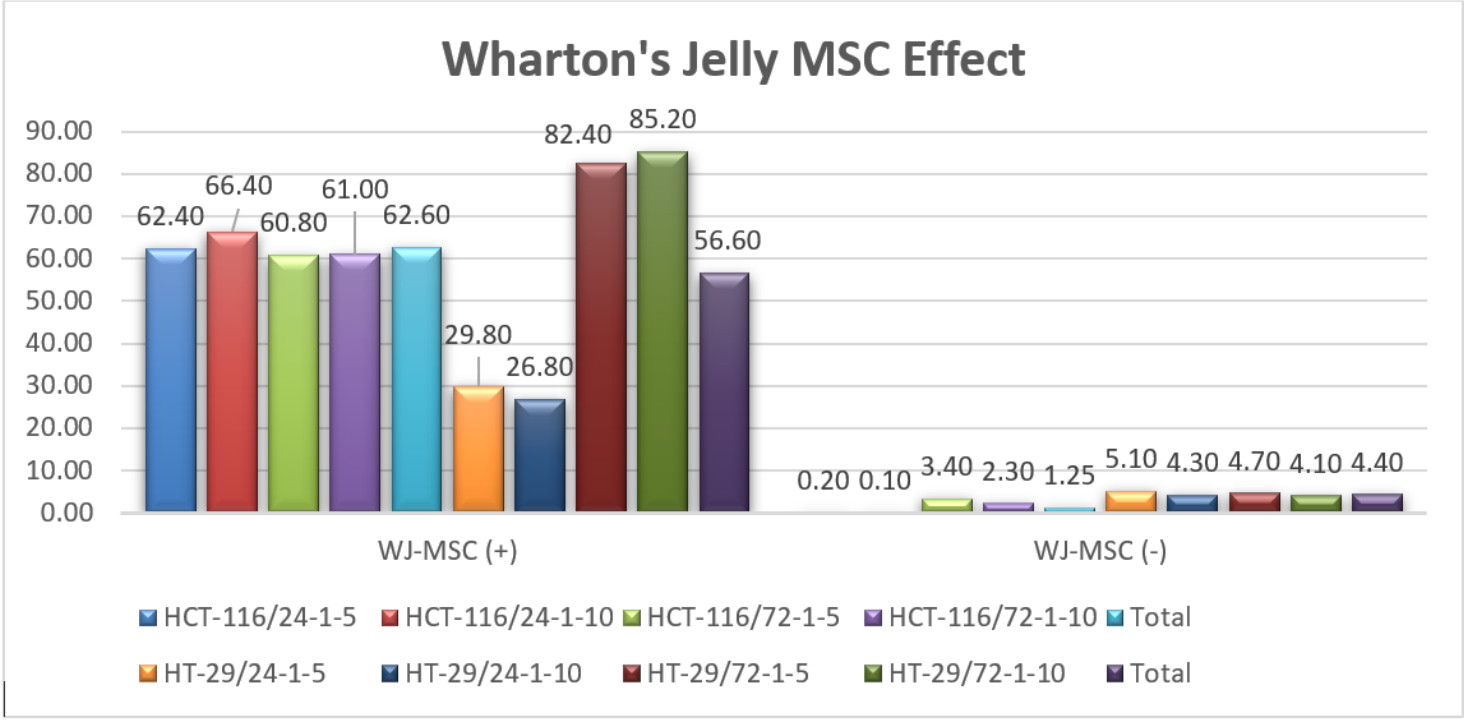

Figure 3

The Apoptotic Effect of WJ-MSC on Colorectal Cancer Cells

The figure shows flow cytometric results comparing WJ-MSC treated and non-treated cancer cells at 1:5 and 1:10 ratios, with the 24h and 72h incubation periods.

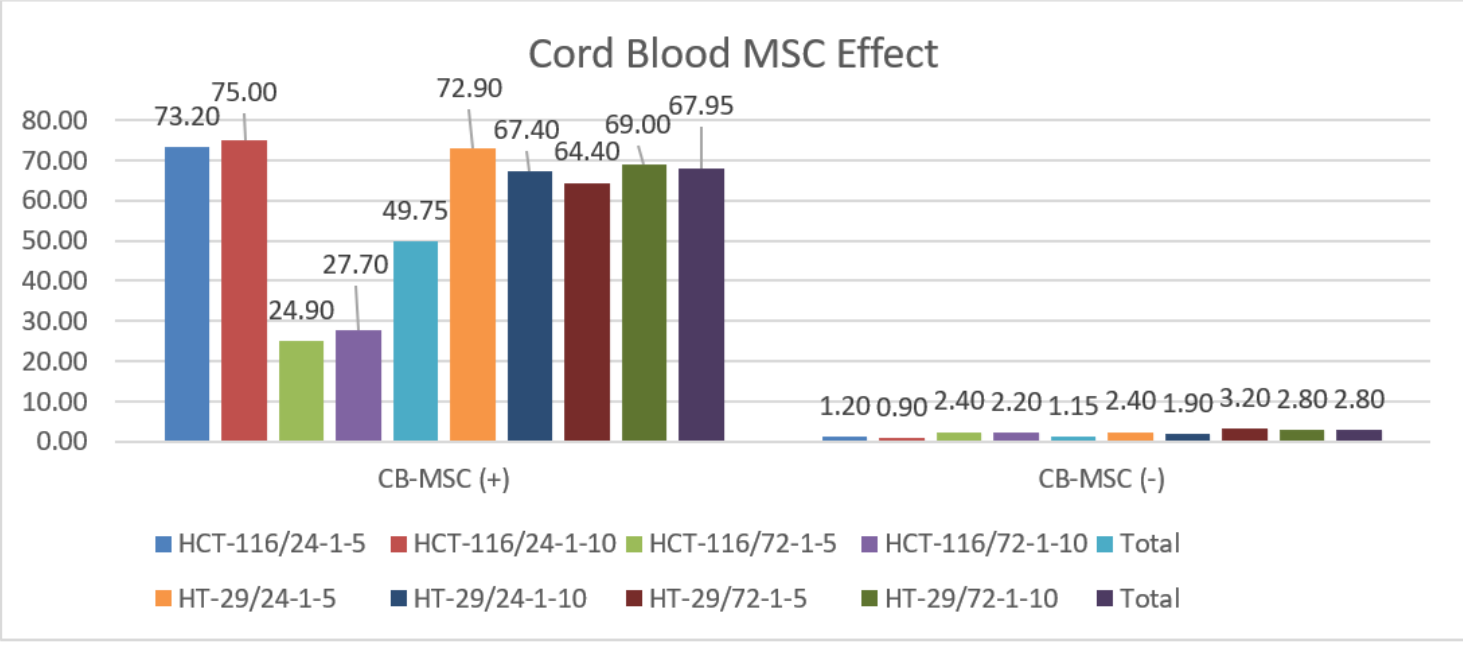

Figure 4

The Apoptotic Effect of CB-MSC on Colorectal Cancer Cells

The figure shows flow cytometric results comparing CB-MSC treated and non-treated cancer cells at 1:5 and 1:10 ratios, with the 24h and 72h incubation periods.

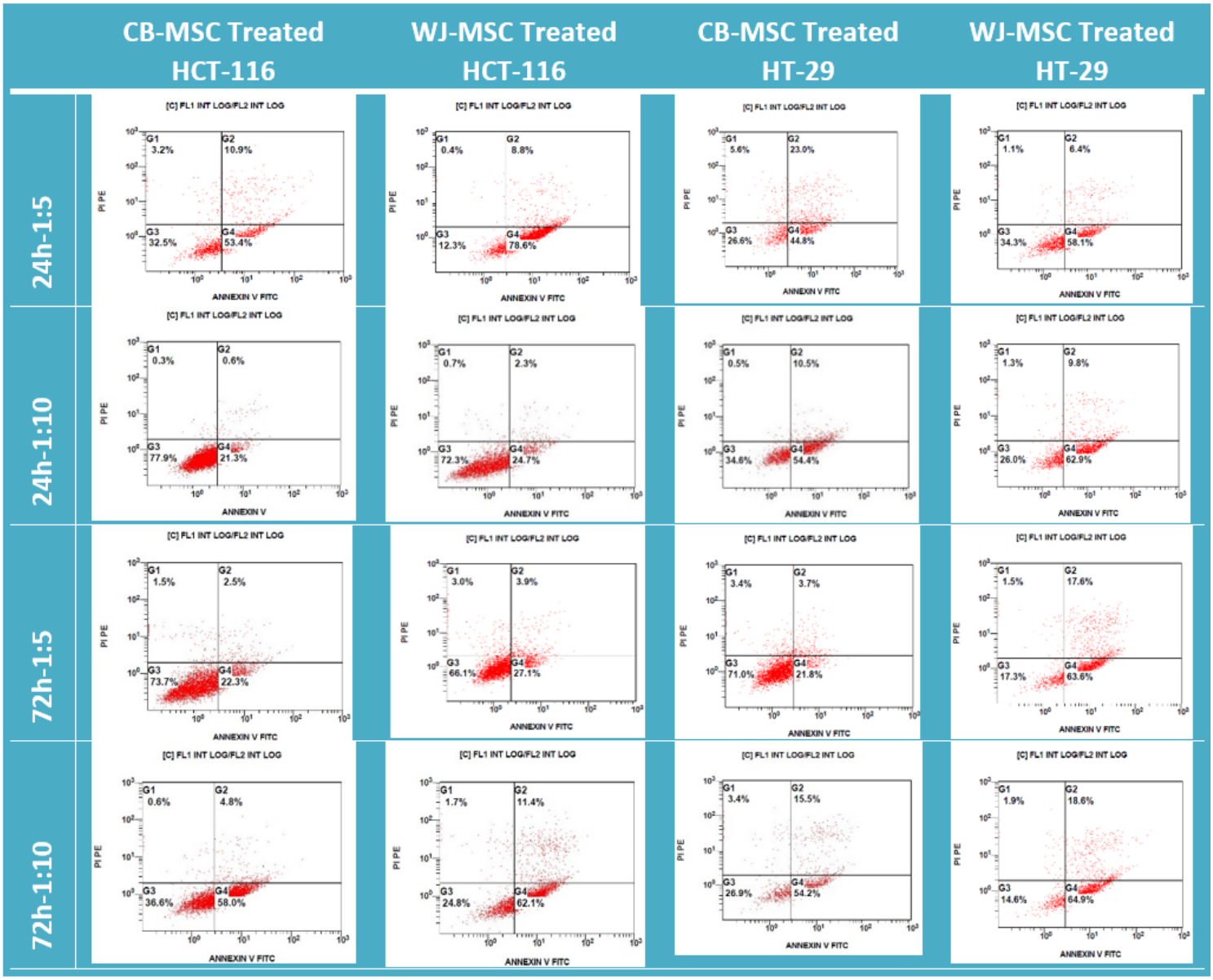

Figure 5

The Comparison of Flow Cytometric Result of both types of MSC in vitro Colorectal Cancer Cells

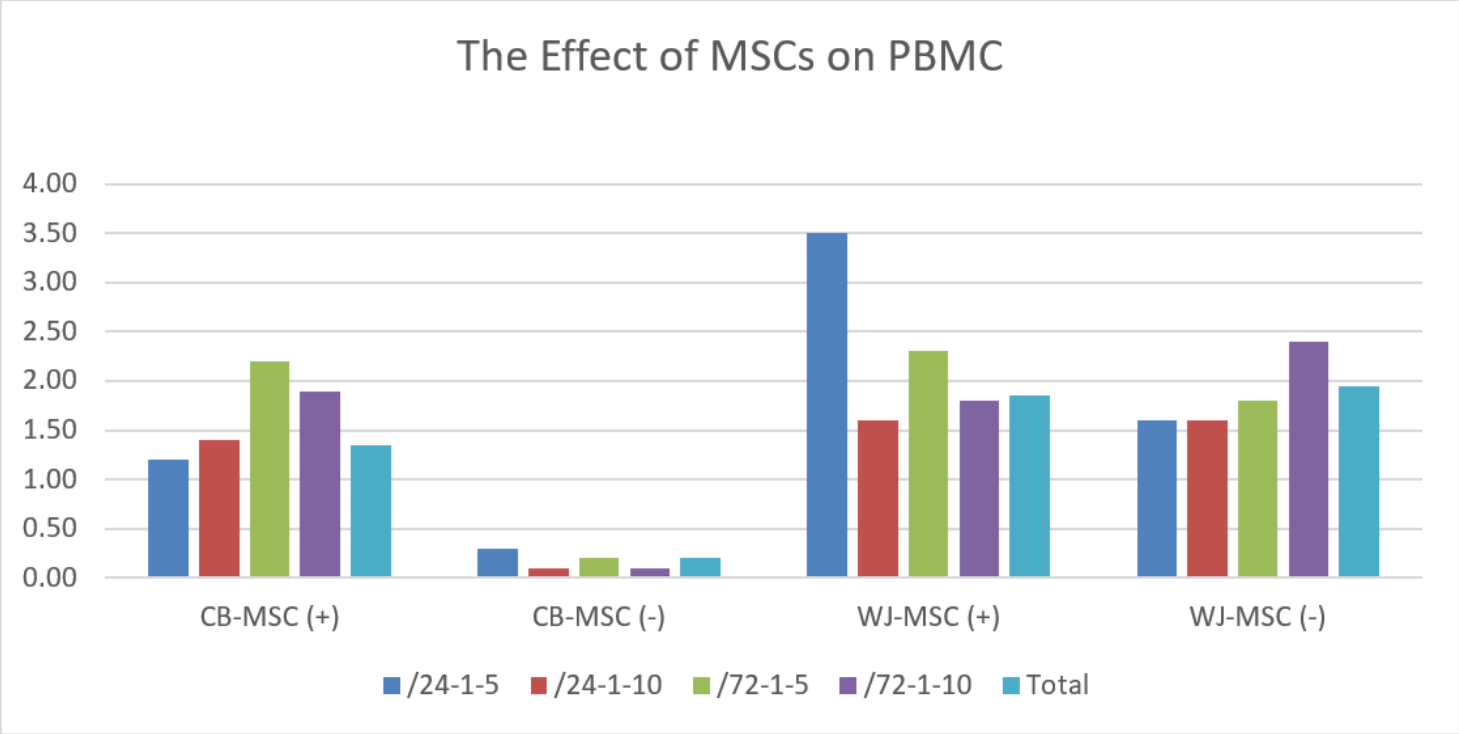

Figure 6

The apoptotic effect of WJ-MSC and CB-MSC on healthy PBMC control group

Median values were used for the graphic. According to the data, the apoptotic effects of both of MSCs remain weaker than experiment groups (MSC treated groups).

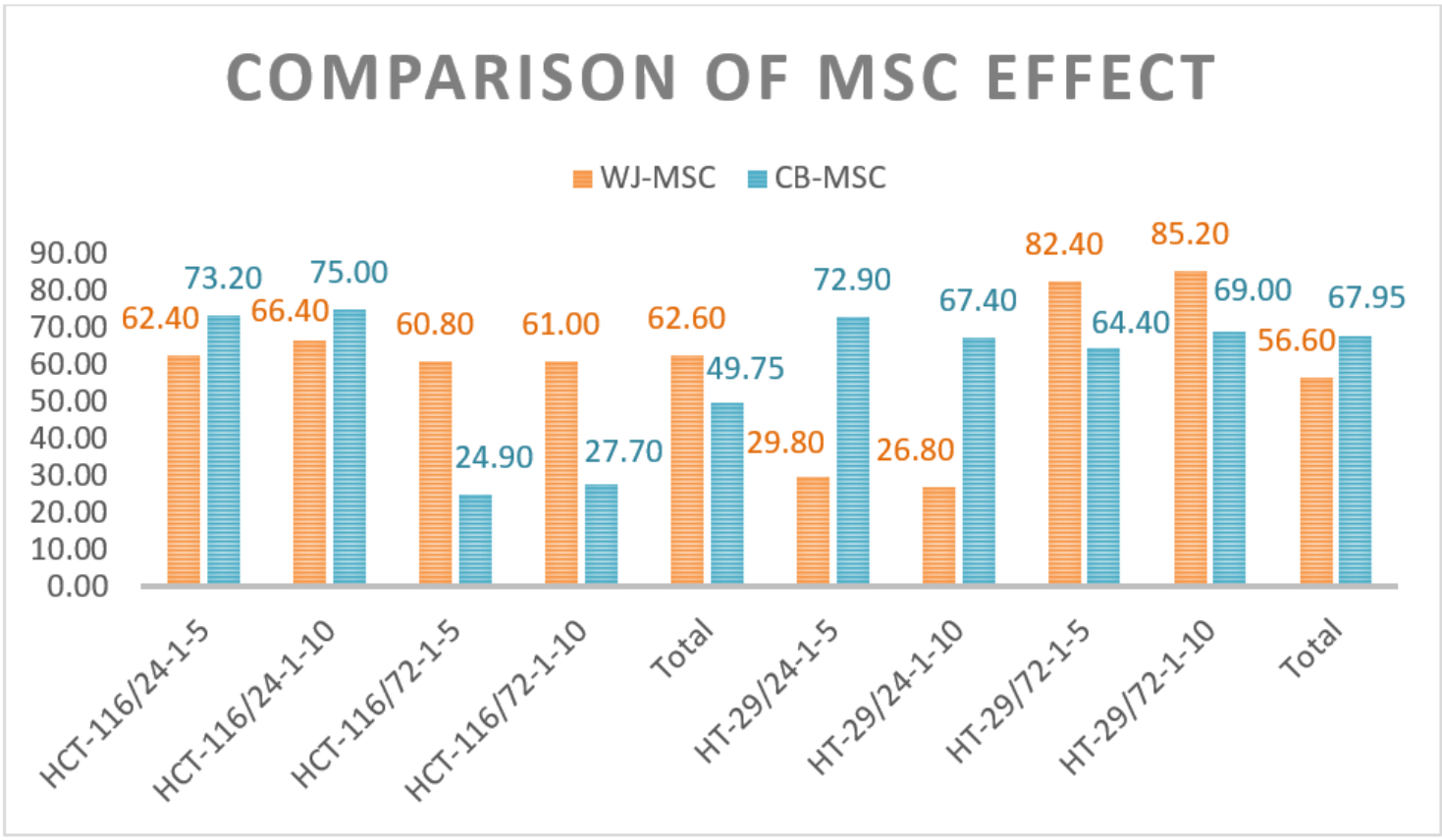

Figure 7

The comparison of WJ-MSC and CB-MSC on human colorectal cancer cell lines

Median values were used to compare the apoptotic effect of MSC sources.

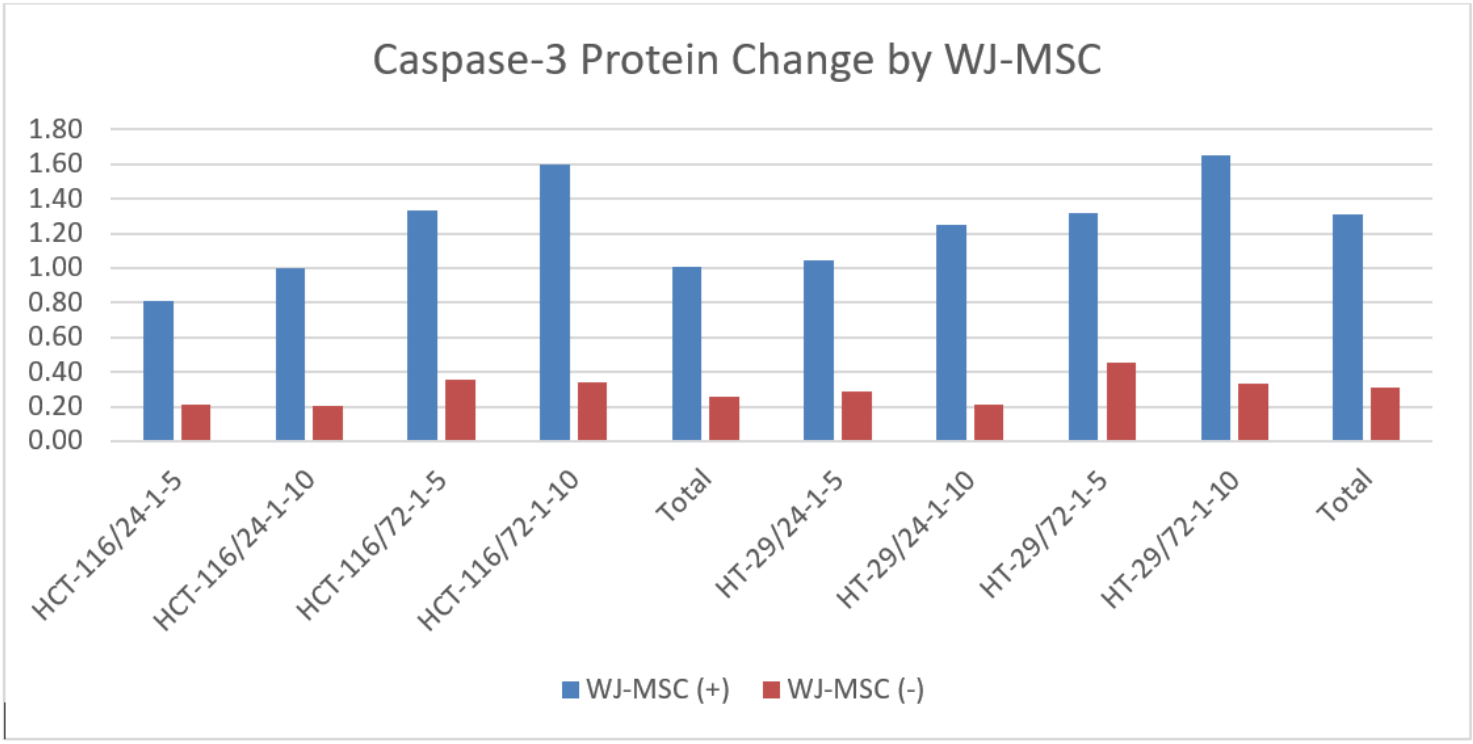

Figure 8

Caspase-3 protein change related to WJ-MSC by ELISA

The median values were used for comparison of WJ-MSC(+) and WJ-MSC(-) for both colorectal cancer cells.

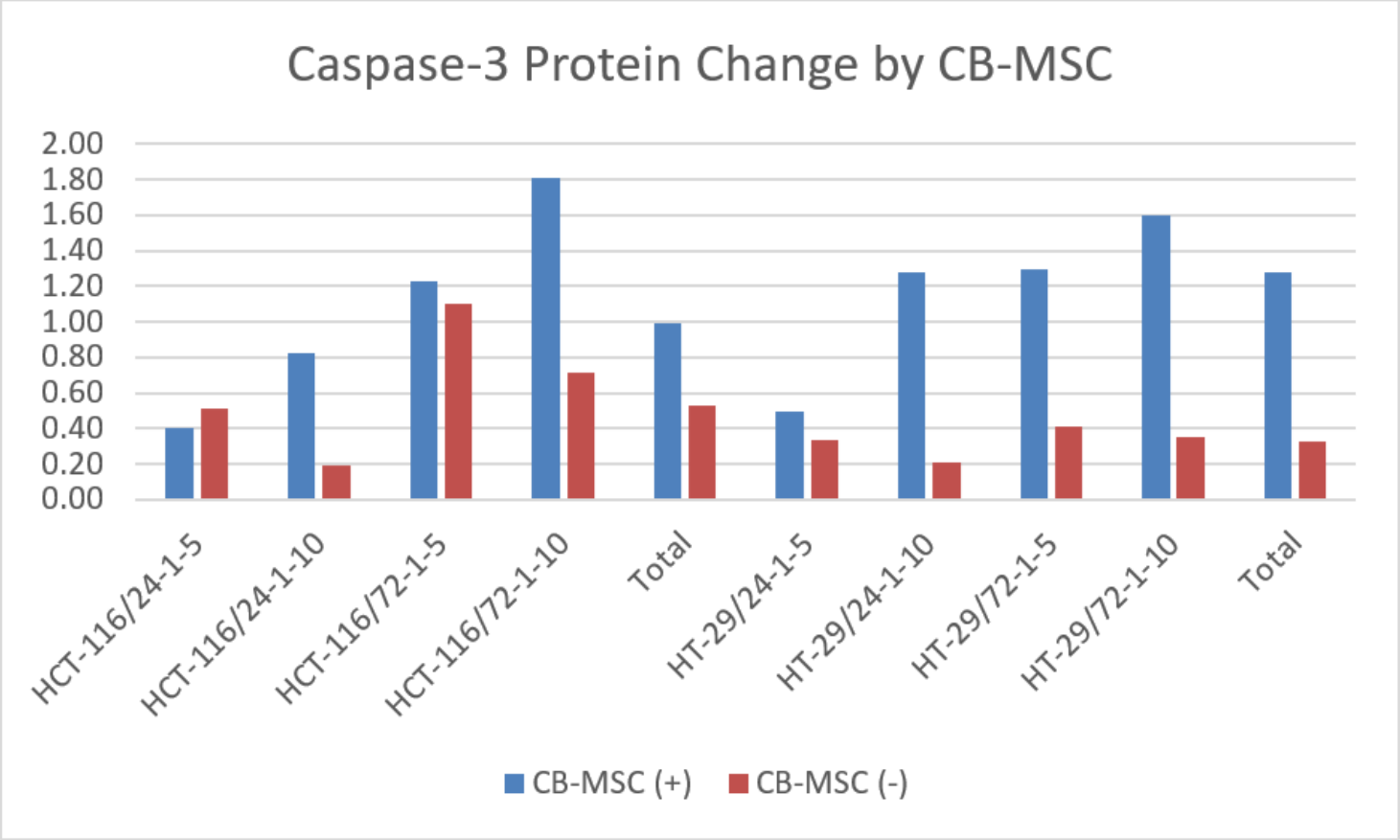

Figure 9

Caspase-3 protein change related to CB-MSC by ELISA

The median values were used for comparison of CB-MSC(+) and CB-MSC(-) for both colorectal cancer cells.

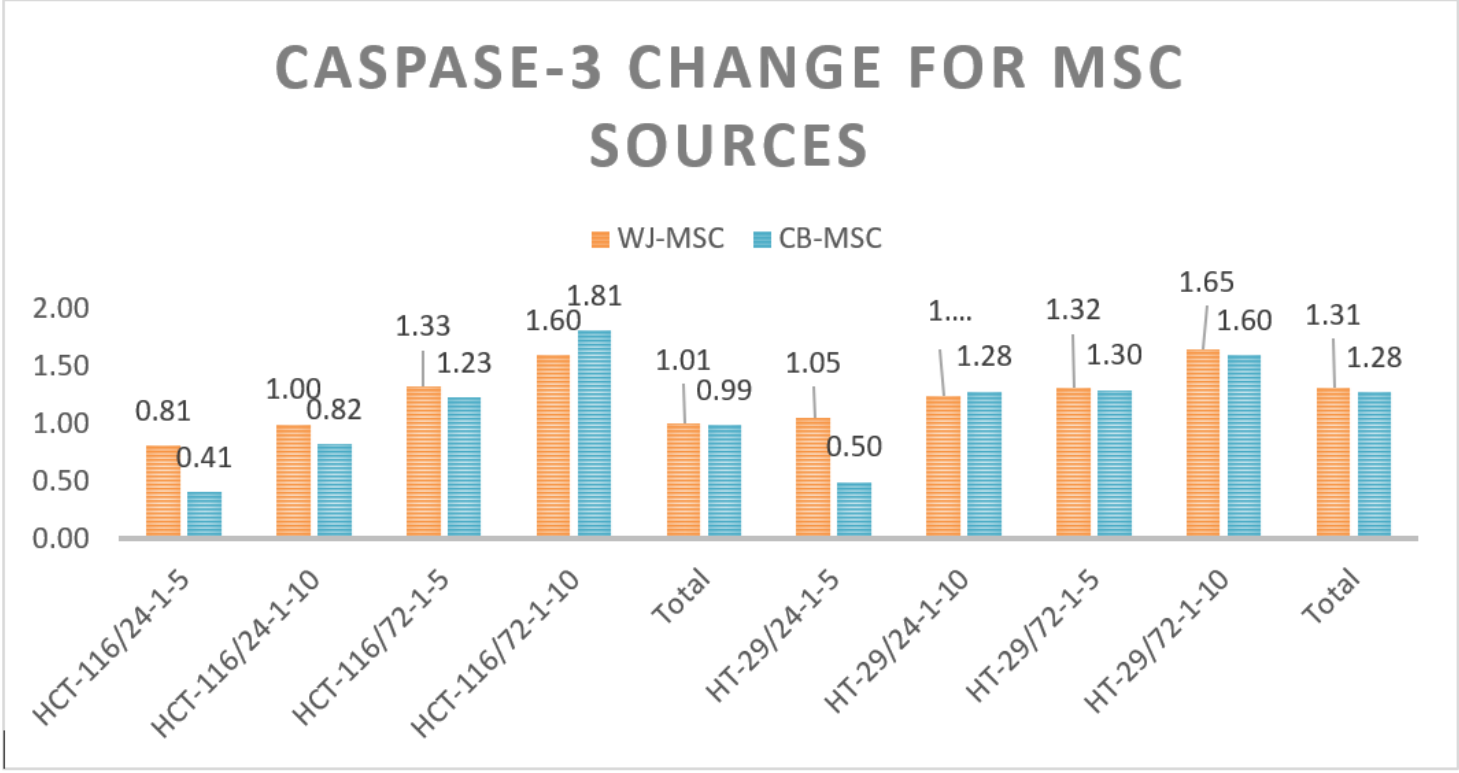

Figure 10

The comparison of Caspase-3 effect related to MSC sources on human colorectal cancer cell lines

Median values were used to compare the Caspase-3 protein change of MSC sources.

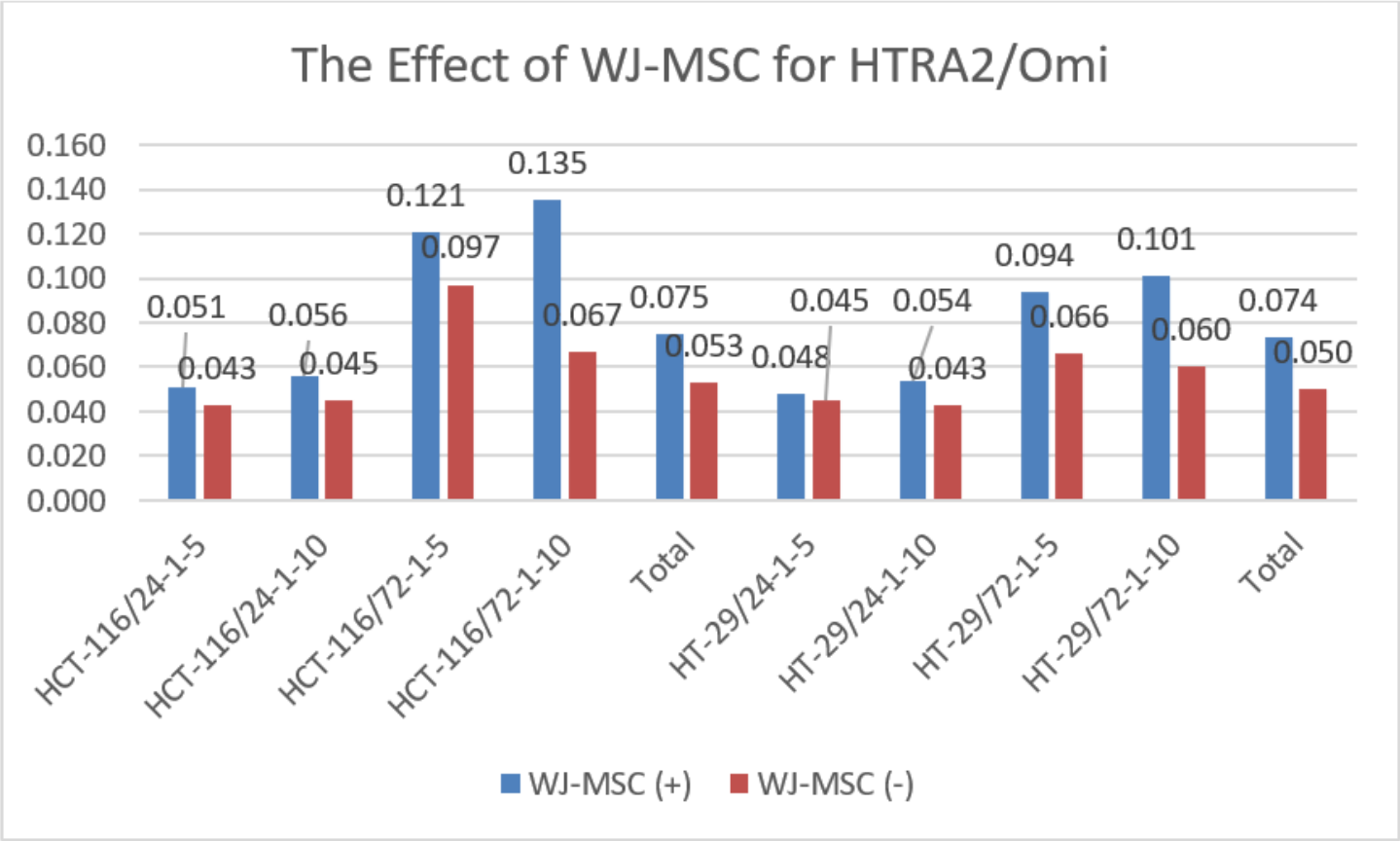

Figure 11

The effect of WJ-MSC on HTRA2/Omi protein level

For both colorectal cancer cells, it is found that the 72 h incubations were more significant than 24 h.

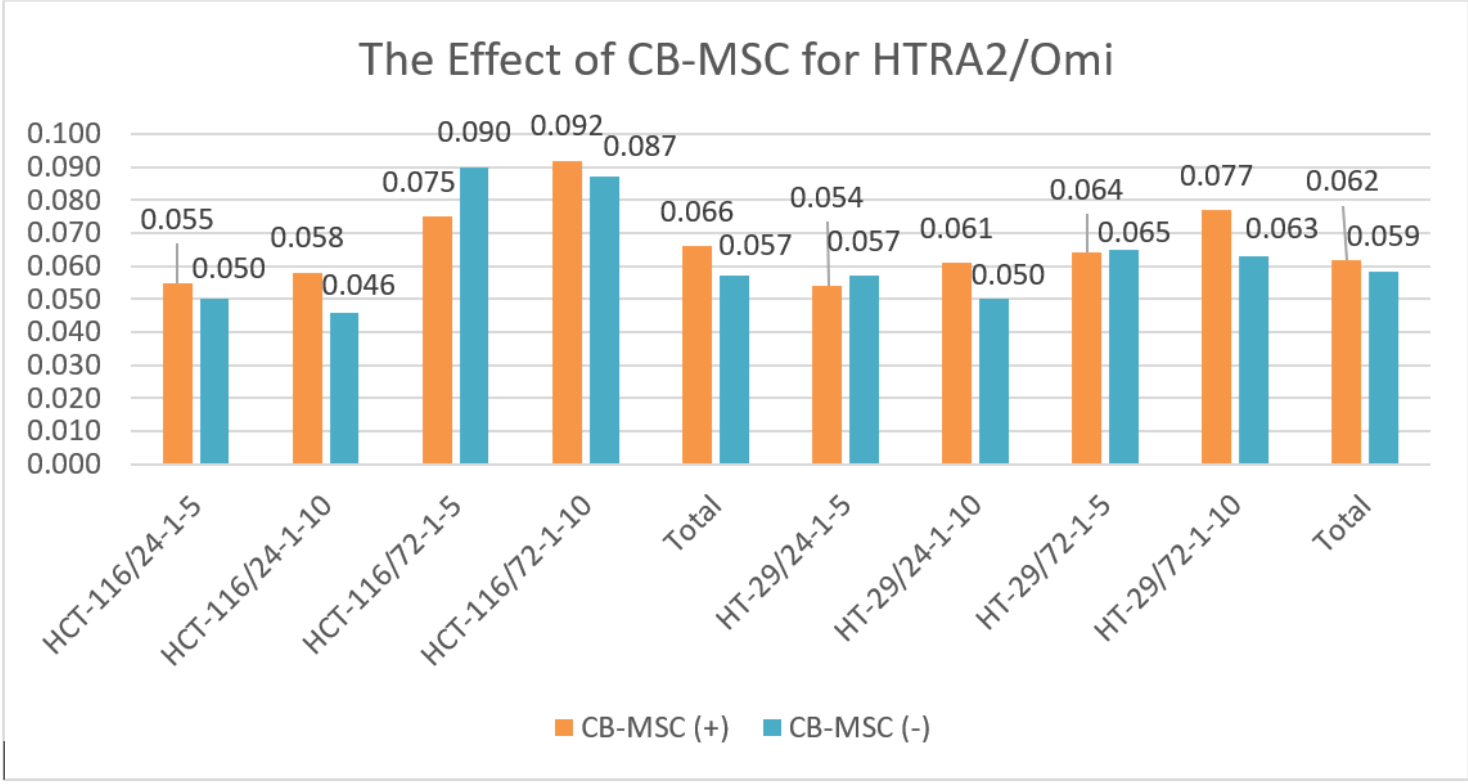

Figure 12

The effect of CB-MSC related HTRA2/Omi protein change on human colorectal cancer cells