An answer to colon cancer treatment by mesenchymal stem cell originated from adipose tissue

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OBJECTIVE(s): Colon cancer is risen up with its complex mechanism that directly impacts on its treatment as well as its common prevalence. Mesenchymal stem cells (MSCs) have been considered as a therapeutic candidate for conventional disease including cancer. In this research, we have focused on apoptotic effects of adipose tissue-derived MSCs in colon cancer.

Materials and Methods: MSCs were obtained from adipose tissue and characterized by flowcytometer using suitable antibodies. MSCs, HT-29, HCT-116, RKO and healthy cell line MRC5 were cultured by different seeding procedure. After cell viability assay, changes in caspase 3 enzyme activity and the level of phosphatidylserine were measured.

Results: For cell viability assay, a 48 hr incubation period was chosen to seed all cells together. There was a 1.36-fold decrease in caspase 3 enzyme activity by co-treatment of RKO and MSCs in addition to 2.02-fold decrease in HT-29 and MSCs co-treatment, and 1.103-fold increase in HCT-116 and MSCs. The results demonstrated that HCT-116 led to the highest rate of apoptotic cell death (7.5%) compared with other cells.

Conclusion: We suggest that MSCs might remain a new treatment option for cancer by its differentiation and repair capacity.
completed using anti-CD34 PE-Cy7 (BD Biosciences, San Diego, CA, USA), CD45 APC-Cy7 (BD Biosciences Pharmingen, San Diego, CA, USA), anti-CD73 PE (BD Biosciences Pharmingen), and anti-NG2 PE (Beckman Coulter, Marseille, France) antibodies by flowcytometry (BD FACSCanto-II, USA) and DiVA software (BD Biosciences Pharmingen) (10,11).

Cell culture for HT-29, HCT-116, RKO and healthy cell line MRC5

The colon cancer cell lines HT-29, HCT-116, and RKO and MRC5 as healthy cell line were purchased from American Type Culture Collection (ATCC, Manassas, VA). McCoy’s 5a modified medium was used for culturing for HT-29 and HCT-116 cells; Eagle’s Modified medium was used for culturing RKO and Dulbecco’s modified medium was used to culture MRC5 cells. All medium were prepared by adding supplemented materials including fetal bovine serum (FBS), and penicillin/streptomycin. Culture conditions were 37 °C and 5% CO₂.

MSCs and HT-29, HCT-116, RKO and healthy cell line MRC5 seeding procedure and cell viability assay

Three different groups were organized for seeding at a density of 1×10⁶ cells/well in 24-well plates for all cell lines including healthy cell line. In the first group, all cells (HT-29, HCT-116, RKO and MRC5) were seeded on different plates and incubated for 24 hr period. After attachment of the cells to the plate, MSCs were seeded as a treatment agent in concentration of 10⁴–10⁶ cells/well.

In the second group, MSCs were seeded first at a density of 10⁴–10⁶ cells/well and incubated for 24 hr period. After incubating, the cells (HT-29, HCT-116, RKO and MRC5) were seeded at a density of 1×10⁶ cells/well.

All cells including MSCs were seeded all together in the third group at a density of 1×10⁶ cells/well (HT-29, HCT-116, RKO and MRC5) and in concentration of 10⁴–10⁶ MSCs cells/well.

After seeding the all groups, WST-1 colorimetric cell proliferation assay was conducted after each incubation period; 24 hr, 48 hr and 72 hr. For this purpose, 25 ul of WST-1 solution was added to each well. According to the assay procedure, wells were incubated 4 hr at 37 °C. To measure color development, microplate reader (Thermo Fisher Scientific, Germany) was used at 450 nm. Percentage of proliferation was calculated compared to the untreated cells.

Changes in Caspase 3 enzyme activity

Change in Caspase 3 enzyme activity as an important sign for apoptosis process was examined by Caspase 3 colorimetric assay kit (BioVision Research Products,USA) and BCA protein assay kit. The absorbance values were normalized to protein concentrations and determined using Bradford assay. The main principles of the kit to measured of labeled substrate DEVD-pNA. The final reaction was measured spectrophotometrically after necessary incubation period at 400 nm wavelengths for Caspase 3 and 562 nm wavelengths for BCA on an ELISA reader (Thermo Electron Corporation Multiskan Spectrum, Finland).

Figure 1. Viability level of colon cancer cell lines HT-29, HCT-116, RKO and healthy cell line MRC5 after treatment with MSCs. (Chosen seeding group and the time of incubation is shown)

**Results**

**Viability level of colon cancer and healthy cell lines after treatment with MSCs in a time- and dose-dependent manner**

Cytotoxic effects of MSCs were determined by WST-1 colorimetric assay. The three different seeding group that mentioned earlier were incubated with increasing concentration of MSCs (10⁴–10⁶) for 24 hr, 48 hr and 72 hr. The results showed that the third group (the group in which all cells including MSCs seeded all together) was the statistically important effects in a time-dependent manner (*P*-value<0.05). The concentration of MSCs was 10⁵ cells/well and the concentrations of HT-29, HCT-116, RKO and MRC5 were 10⁶ cells/well during 48 hr incubation period (*P*-value<0.05) (Figure 1).

**Changes in caspase 3 enzyme activity in a time- and dose-dependent manner**

Caspase-3 enzyme activity was measured after 48 hr incubation period. The values are normalized to protein concentrations and determined using a Bradford assay. There were a 1.36-fold decrease in caspase-3 activity in RKO and MSCs and 2.02-fold decrease after 48 hr in HT-29 and MSCs. On the other hand, we have also found 1.103-fold increase in caspase-3 activity 48 hr after incubation in HCT-116 and MSCs (Figure 2). MRC5 cell line was used to show the relationship between MSCs and healthy cell line. There was almost no important change after 48 hr treatment (*P*-value<0.05).

**Level of phosphatidylserine to detect apoptosis**

Cell death may lead to several changes in components of the inner and outer side of the members such as phosphatidylserine (PS). Normally, PS is located in the cell. While apoptosis starts, PS changes its location through the cell surface. By changes in the PS level, apoptosis can be detected by staining with the green fluorescent dye Annexin V-FITC (BD Pharmingen, Germany). After cells and dyes were suspended and incubated for almost one hour, the mixtures were immediately analyzed using flowcytometry.

**Statistical analysis**

Results are expressed as the mean standard error of the mean (SEM). The data were analyzed using one-way ANOVA. *P*-value<0.05 was considered statistically significant.
Figure 2. Change in Caspase 3 (%) in colon cancer cell lines HT-29, HCT-116, RKO and healthy cell line MRC5

**Level of phosphatidylserine for apoptosis**

PS level was measured by flow cytometry to detect apoptosis after double staining with the green fluorescent dye Annexin V-FITC (BD Pharmingen, Germany). The results demonstrated that highest levels of apoptotic cell death was observed in HCT-116 (7.5%) compared to other cells. Also, 0.15% necrosis was reported in HT-29 cells. Differently, RKO had low level of cell death including necrosis (1.65% for apoptotic cells; 0.10% for necrotic cells) (*P*-value<0.05) (Figure 3).

**Discussion**

MSCs have several abilities due to their differentiation capacity. Not only tissue damage role of MSCs has still being researched, but also growth factor and cytokines production have become a number of area that need to be lighten up by its relation in the pathological conditions such as neoplastic damage, inflammation process, and tissue repair (12). Although, there are some questions about MSCs for the treatments; some preclinical and early clinical trials were come up promising results for cardiovascular area, nervous system, skin and also lung (13,14). Complicatedly, MSCs-related treatments have lots of questions and dilemma including its impact on tumor growth and apoptosis. In this research, for the first time we aimed to find out the apoptosis level of adipose-derived MSCs when given as an anticancer agent in different colon cancer cell lines and also in a healthy cell line.

Khakoo et al. studied antitumorigenic effects of human MSCs at in vivo condition on nude mice in a model of Kaposi’s sarcoma. They injected MSCs into mice with Kaposi’s sarcoma, and isolated and co-cultured MSCs and Kaposi’s sarcoma cells in vitro. They found out decreasing level of proliferation of Kaposi’s sarcoma cells by affecting Akt-signaling (15) Oppositely, Xu et al. worked on osteosarcoma and MSCs by injection of MSCs into nude mice and showed increasing stimulation of tumor growth (16). These different results might be related to different characteristic of derivation area of MSCs such as adipose tissue, bone marrow, and umbilical cord. Furthermore, it also might be due to both tumor type and MSCs characteristics. A limited number of studies have tried to explain underlying mechanism of this interaction (8).

Spaeth et al. focused on MSCs contribution to fibrovascular network expansion and tumor progression on human ovarian carcinoma in tumor-bearing nude mice and observed the larger tumor capacity after treatments (17). Zhu et al. worked on bone marrow MSCs and human colon carcinoma at in vivo condition and showed the stimulation of growth (18). In contrast, our study showed apoptotic cell death in HCT-116 and HT-29 that both are characteristic condition of colon, while no change was observed in MRC5 as healthy cell line. RKO is a colon cancer cell line too although characteristic condition is only carcinoma, resulted instead of apoptotic cell death it ended up low level of necrotic death. In our opinion, it is theoretically thought that MSCs treatments might have a strong relation with the cancer type specifically.

Lanza et al. worked on neuroprotective role and antioxidant capacity of MSCs and successfully showed that MSCs activate the antioxidant system; hence, protection is possible from oxidative stress (19). The study might have pointed out an important key for cancers because of the relation between antioxidant systems.

Hogan et al. showed the role of plasminogen activator inhibitor type 1 (PAI-1) in MSCs on colon cancer using HT-29 and HCT-116 cell lines (20). They focused on secretion of PAI-1 in different concentrations, which resulted in different outcomes on both cell lines. They reported that MSCs-secreted PAI-1 in HCT-116 cells resulted in decreasing proliferation in all concentration, while MSCs-secreted PAI-1 in HT29 cells led to increasing proliferation activity by higher PAI-1 levels (20). Lu et al. studied the growth inhibitory impact of MSCs at in vitro and in vivo conditions on murine hepatoma and lymphoma cells and rat insulinoma cells. They showed upregulation of p21 and caspase 3 in all cell types (21). Similarly, we found out that co-treatment of HCT-116 and MSCs increased caspase 3 activity, while the enzyme activity decreased in other colon cancer cell lines. Again, we ended up with same dilemma that MSCs have effects on cancer cells due to its differentiation characteristic. However, further studies are needed to confirm the possible effects.

Similar to other researches, we need to examine its potential effects on colon cancer and also underlying mechanistic pathways and the possible role of cytokines and chemokines until their role is clearly lightened up.

**Conclusion**

There are several doubtful questions on many
researches about MSCs. On the other hand, differentiation potential and repair capacity of MSCs may have beneficial therapeutic effect in some diseases including cancer. We suggest that, after working on detailed pathways of HCT-116, HT-29, and RKO cell lines, it might be considered as a new treatment option. Prospectively, the results would lead to new treatment options by a view on lack of immunosuppression using MSCs of patients isolated from their own adipose tissue.

Acknowledgements

This work was funded by Istanbul University Scientific Research Project number 39247. We would like to thank Mr David F. Chapman for editing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Nakaji S, Umeda T, Shimoyama T, Sugawara K, Tamura K, Fukuda S, et al. Environmental factors affect colon carcinoma and rectal carcinoma in men and women differently. Int J Colorectal Dis 2003; 18: 481–486.

2. Saif MW, Chu E. Biology of colorectal cancer. Cancer J 2010;16:196–201.

3. Koliaraki V, Pallangyo CK, Greten FR, Kollias G. Mesenchymal cells in colon cancer. Gastroenterology 2017; 152:964-979.

4. Niv Y, Goel A, Boland CR. JC virus and colorectal cancer: a possible trigger in the chromosomal instability pathways. Curr Opin Gastroenterol 2005; 21: 85–89.

5. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr. 2001;131:3 109–120.

6. Slattery ML. Diet, lifestyle, and colon cancer. Semin Gastrointest Dis 2000;11:142–146.

7. Mishra J, Drummond J, Quazi SH, Karangi SS, Shaw JJ, Chen B, et al. Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis. Crit Rev Oncol Hematol 2013; 86: 232–250.

8. Bergfeld SA, DeClerck YA. Bone marrow-derived mesenchymal stem cells and the tumor microenvironment. Cancer Metastasis Rev 2010; 29: 249–261.

9. Sunay O, Can G, Cakir Z, Denek Z, Kozanoglu I, Erbil G, et al. Autologous rabbit adipose tissue-derived mesenchymal stromal cells for the treatment of bone injuries with distraction osteogenesis. Cytotherapy 2013; 15: 690-702.

10. Bieback K, Kem S, Kocaomer A, Ferlìk K, Bugert P. Comparing mesenchymal stromal cells from different human tissues: bone marrow, adipose tissue and umbilical cord blood. Biomed Mater Eng 2009; 18: 71–76.

11. Kozanoglu I, Boga C, Ozloglu H, Sozer O, Maytalman E, Yazici AC, et al. Human bone marrow mesenchymal cells express NG2: possible increase in discriminative ability of flow cytometry during mesenchymal stromal cell identification. Cytotherapy 2009;11:527-533.

12. Kalinina NI, Sysoeva YV, Rubina KA, Parfenova YV, Tkachuk VA. Mesenchymal stem cells in tissue growth and repair. Acta Naturae 2011; 3: 30-37.

13. Williams AR, Hare JM. Mesenchymal stem cells: Biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res 2011;109: 923-940.

14. Hong HS, Kim YH, Son Y. Perspectives on mesenchymal stem cells: Tissue repair, immune modulation, and tumor homing. Arch Pharm Res 2012;35:201.

15. Khakoo AV, Pati S, Anderson SA, Reid W, Elshal MF, Novira II, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi’s sarcoma. J Exp Med 2006; 203: 1235–1247.

16. Xu WT, Bian ZY, Fan QM, Li G, Tang TT. Human mesenchymal stem cells (hMSCs) target osteosarcoma and promote its growth and pulmonary metastasis Cancer Lett 2009; 281: 32–41.

17. Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klop A, Hall B, et al. Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS One 2009; 4:e4992.

18. Zhu W, Xu W, Jiang R, Qian H, Chen M, Hu J, et al. Mesenchymal stem cells derived from bone marrow favor tumor cell growth in vivo. Exp Mol Pathol 2006;80: 267–274.

19. Lanza C, Morando S, Voci A, Canesi L, Principato MC, Serpero LD, et al. Neuroprotective mesenchymal stem cells are endowed with a potent antioxidant effect in vivo. J Neurochem 2009; 110:1674–1684.

20. Hogan NM, Joyce MR, Murphy JM, Barry FP, O’Brien T, Kerin MJ, et al. Impact of Mesenchymal Stem Cell secreted PAI-1 on colon cancer cell migration and proliferation. Biochem Biophys Res Commun 2013; 435: 574–579.

21. Lu YM, Yuan Y, Wang XJ, Wei LL, Chen YN, Cong C, et al. The growth inhibitory effect of mesenchymal stem cells on tumor cells in vitro and in vivo. Cancer Biol Ther 2008; 7: 245–251.