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Assessment the Effect of Human Umbilical Cord Wharton's Jelly Stem Cells on the Expression of Homing Genes: CXCR4 and VLA-4 in Cell Line of Prostate Cancer

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ABSTRACT

Background: Prostate cancer is the second most common cancer in the male that affects the health, social and economic life of person. Different compounds such as Wharton jelly, have been used to treat prostate cancer. Wharton jelly is a tissue rich in cells with mesenchymal morphology. Wharton jelly compound inhibited the growth of various cancer cells, including ovarian, osteosarcoma, breast, and prostate cancers, and also reduced the expression of CXCR4 and VLA-4 genes involved in the metastasis process.

Materials and Methods: To do this research, Wharton jelly stem cells and DU145 cancer cell line were cultured. After cell culture, the effect of Wharton jelly on this cell line was evaluated by scratching and MTT assay. Expression of CXCR4 and VLA-4 genes was also evaluated by Real time PCR.

Results: The results of MTT and Scratching tests showed that Wharton jelly inhibited the growth of DU145 cancer cells and also decreased the expression level of CXCR4 and VLA-4 genes.

Conclusion: The results of this study showed that Wharton jelly can be considered as an effective compound for decreasing metastasis of prostate cancer.

Keywords: Prostate cancer; Wharton jelly; CXCR4 gene; VLA-4 gene.

INTRODUCTION

In developed countries, prostate cancer is the second most common (after skin cancer) and the most deadly cancer (after lung cancer) in men. One in six men has this type of cancer. Epidemiological studies have demonstrated that 10% of prostate cancer are due to genetic factors[1,2]. One of the important abilities of tumor cells is invasive and spread to peripheral tissues which are called metastasis. In fact, metastasis is the spread of cancer from early tumors and development of new tumors in distant organs. Metastasis to distant parts of the body involves several successive steps such as the entry of tumor cells from the primary tissue into the blood vessels, survival in the bloodstream, migration to secondary organs, and the proliferation of cancer cells in the target tissue[3]. Circulatory cancer cells often produce new tumors from the originated tissue. But blood circulation alone does not result in all cases of tumor spread. Clinical evidence suggests that an Implantation mechanism is responsible for some metastatic cases. For example, prostate cancer

and breast cancer often spread to the bone first, and lung cancer often leads to new tumors in the adrenal glands. This junction phenomenon may be related to the identification of the exit site of the circulation by the tumor cell as well as the identification of the location of the new tissue. An example of a nesting phenomenon at the molecular level involves a substance called CXCL12, which is secreted by stromal cells. It absorbs cells that express receptors called CXCR4, which are found in certain types of cancer cells, such as breast cancer cells or chronic myelogenous leukemia (4). Recent studies show that chemokines and their receptors (C-X-C chemokine receptor type 4) play a vital role in the metastasis process and their misstatement can lead to cancer[5]. Dependence of cancer cells to CXCR4 expression at CXCL12-secreting tissues leads to the movement of cancer cells from their main sites of formation [4].

CXCR4 expression significantly increases in breast cancer. Studies have shown that CXCR4 plays an important role in cell survival, migration, proliferation and metastasis of several types of cancer, including breast cancer. Binding of CXCR4 to its ligand (SDF-1) leads to the circulation of cancer cells in the blood, their entry into other tissues, including bone, liver and lung, and the formation of invasive tumors [6].

The integrin family, which includes cell surface receptors for extracellular matrix compounds, is one of the receptors involved in various aspects of adherent leukocytes. VLA family is one of the integrin-related antigens involved not only in extracellular matrix adhesion but also acts as a receptor for fibronectin as well as cell-to-cell adhesion receptors [7].

Various anticancer drugs and compounds that can stimulate tumor-specific immune responses have been studied. There are several reports that confirm hWJSCs have strong tumor-suppressing effects on a variety of cancers such as mammalian adenocarcinoma, ovarian, osteosarcoma, cholangio carcinoma, bladder and lymphoma cancer [8-19]. HWJSCs suppress cancer cells by inducing apoptosis, proliferation and cell cycle inhibition, and the PI3K / Akt signaling pathway inhibition [8, 9, 18]. Numerous studies have shown that hWJSC {[hWJSC-cell lysate (hWJSC-CL) or hWJSC-conditioned medium (hWJSC-CM)] prevents breast cancer and osteosarcoma in vitro and in vivo. Interestingly, hWJSCs, unlike hBMMSCs, do not convert to TAF [8, 20]. HWJSC-CM and hWJSC-CL also inhibit cancer cells growth, so inhibitory mechanisms are inhibited not only by cell-to-cell a but also by hWJSCs [15, 17].

Materials and methods

Human Wharton's jelly stem cells

The umbilical cord of the fetus was prepared under sterile conditions and in physiological serum from Tabriz International Hospital and then washed with 70% alcohol. Then, the umbilical cord was crushed into small 2cm pieces and re-washed using PBS and HBSS buffer. The small pieces were then cut and the Wharton jelly was extracted. Du145 Prostate cancer cell line, DMEM Low Glucose, FBS and Pen-Strip (0.25%) were used to culture the Du145 prostate cancer cell line.

Induction of cancer cells by Wharton jelly stem cells

To investigate the apoptosis of induced cells, 15% and 30% doses of Wharton's jelly were added to the Du145 cell culture medium. Apoptosis of induced cells was studied under the microscope and the cell count was performed. In some induced cells, the cells were frozen before apoptosis and then RNA was extracted.

Investigation of cell migration

Scratching test was used to determine the cell migration. DU145 cancer cells were scraped diagonally from the middle and then were treated with 15 and 30% doses of Wharton's jelly. Cell migration was measured at 6, 12, 24 and 48 hours with imaging under the microscope.

Study of gene expression

RNA was extracted from induced cells with 15 and 30% doses of Wharton' jelly. The expression of CXCR4 and VLA-4 genes was evaluated using real-time PCR. The B2M gene was considered to be the internal control gene. Primers used is presented in Table 1.

Table1: CXCR4, VLA-4 and B2M primers

gene	primers	Length
CXCR4	F 5' CGCCACCAACAGTCAGAG 3' R 5' AACACAACCACCCACAAGTC 3'	177bp

VLA-4	F 5' CAAGAATCCAAACTACGGAC 3' R 5' TTGCATTCAGTGTTGTGGGA 3'	145bp
B_2M	F 5'GAGAAGTATGACAACAGCCTC 3' R 5' TGAGTCCTTCCACGATACC 3'	112bp

Statistical analysis

Statistical data were analyzed using SPSS PASW Statistics18 software. All graphs were drawn using GraphPad PRISM version 6.01. The statistical significance was considered with $p < 0.05$.

RESULTS

Cell viability

To evaluate the effect of Wharton's jelly on DU145 cells and the inhibitory concentration of IC50, cancer cells were treated with different doses of Wharton's jelly at 24 and 48h. The cell viability was decreased at the high concentration of Wharton's jelly and in longer times (Figure 1) (Table 2).

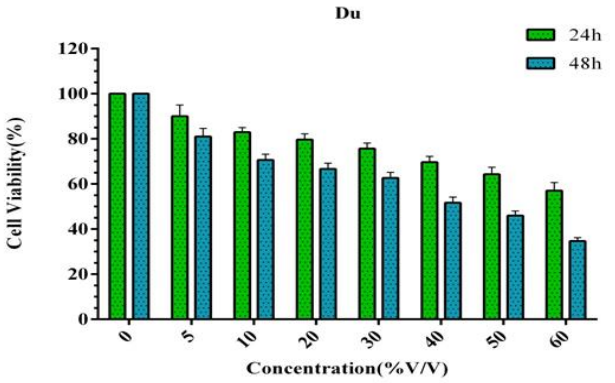

Figure1: Statistical analyzes showed a significant difference in the cell growth of Wharton's jelly-treated cells compared to the control group at 30M and 15M concentrations. This indicated inhibition of DU145 cell growth by Wharton's jelly (p value <0.05).

Table 2: Inhibitory effect of Wharton's jelly on 145 DU cell in prostate cancer

Cell	IC_{50} at 24h	IC_{50} at 48h
DU145	30µM	15µM

Cell migration

Statistical analyzes related to scratching test showed that cell migration decreased compared to the control group at 15 and 30% concentration of wharton's jelly after 12 hours, while at 24 and 48 hours an increase in migration was observed (Figure 2-4).

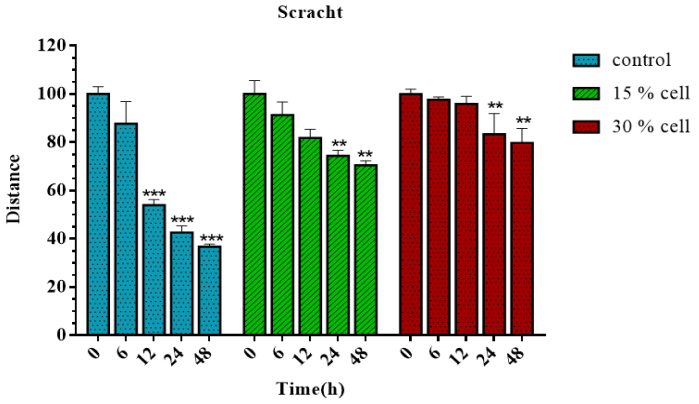

Figure 2. Cell migration at different doses of wharton' s jelly (P <0.001)

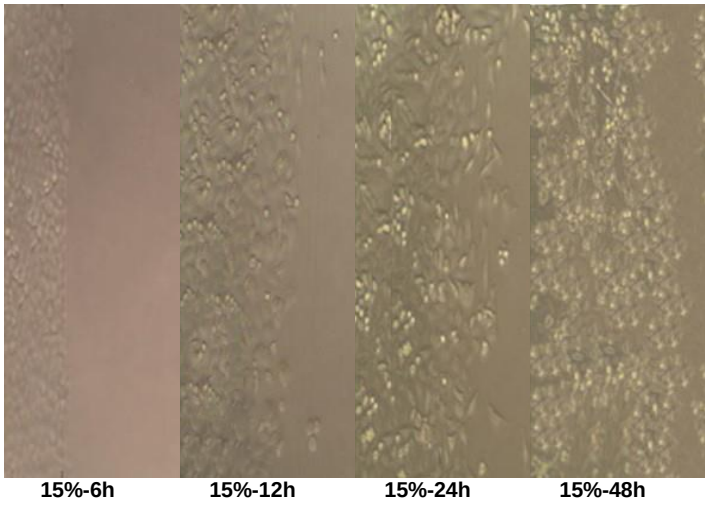

Figure 3. The effect of 15%Wharton's jelly on cancer cell migration (* 400)

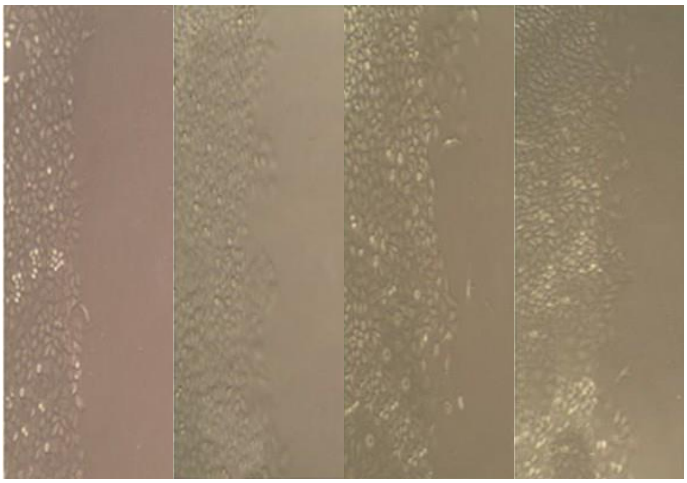

Figure 4. The effect of 30% Wharton's jelly on cancer cell migration (* 400)

The apoptotic effect of Wharton's jelly on Du145 cell line

Microscopic images of DU145 cells apoptotic with Wharton jelly are shown in Figure 5. According to the picture, the cancer cells underwent apoptosis after induction with Wharton jelly.

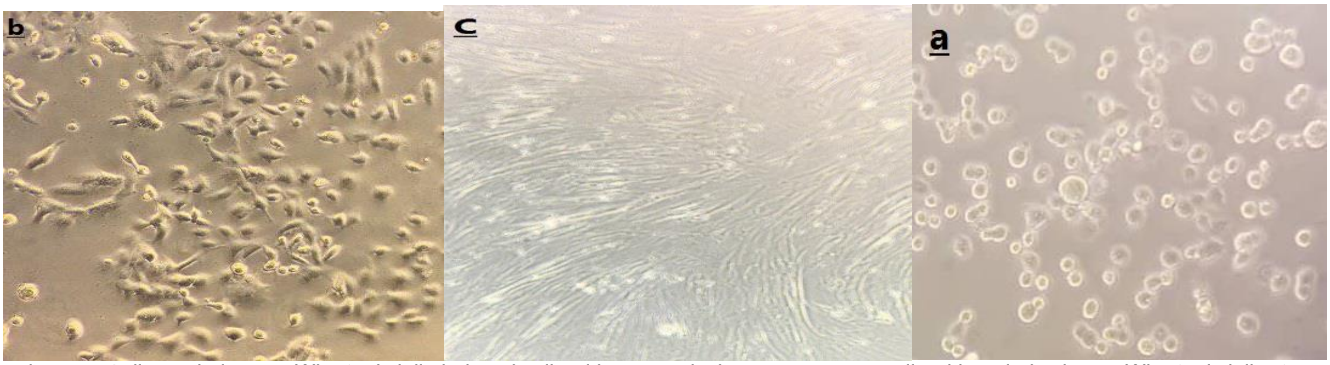

Figure 5. Cell morphology. a: Wharton's jelly-induced cells with apoptosis. b: DU145 cancer cells without induction. c: Wharton's jelly stem cells (* 1000).

Expression of CXCR-4 and VLA-4 genes in DU145 cell line

The expression of CXCR4 and VLA-4 genes was decreased significantly after treatment of DU145 cell line with 15 and 30% concentrations of Wharton's jelly (P-value <0.001). The expression of these genes was decreased in a dose depended manner of Wharton's jelly, ttherefore, the decrease in expression was significant only at 30% concentrations of Wharton's jelly and no significant decrease was observed at 15%. These results indicated that at higher concentrations of Wharton's jelly, the expression of CXCR4 and VLA-4 genes decreased (Figure 6).

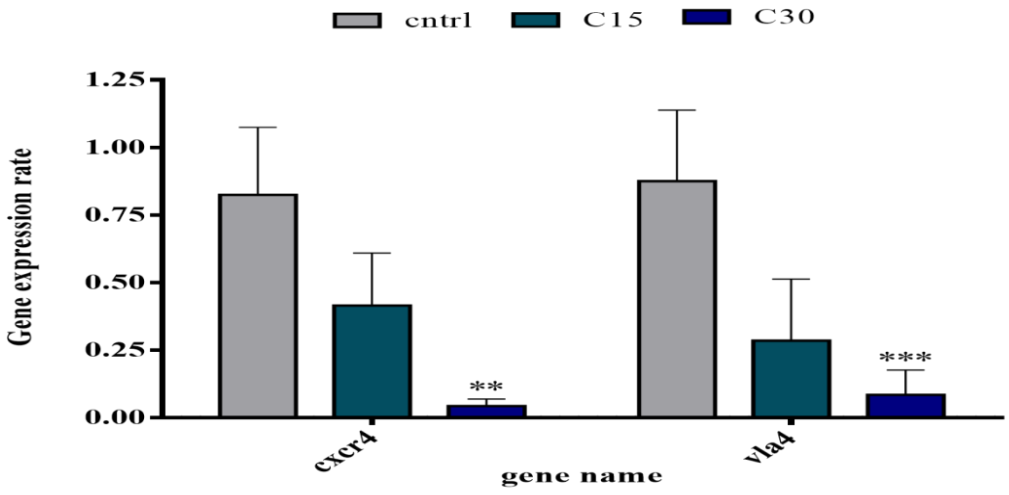

Figure 6. The expression of CXCR-4 and VLA-4 genes in the DU145 cell after treatment with Wharton 's jelly.

DISCUSSION

In our study, DU145 cells growth was decreased at 15 and 30% concentrations Wharton's jelly which indicated that Wharton's jelly has a high inhibitory effect on the growth and proliferation of cancer cells. DU145 cells growth was reduced in a dose- and time-dependent manner that is demonstrated the anti-tumor effect of Wharton's jelly.

We also showed that CXCR4 expression has a significant decreases gene at 30% concentration of Wharton's jelly which indicated that CXCR4 expression is depended on Wharton's jelly high doses. CXCR4 is activated in the metastatic pathway and according to our results, it can be said that Wharton's can reduce cell metastasis jelly in a dose-dependent manner. VLA-4 expression in DU145 cell line was decreased after treatment with 30%Wharton's jelly, which is indicated that VLA-4 is decreased at high doses of Wharton's jelly.

We also studied DU145 cell apoptosis after treatment with Wharton's jelly by microscope witch DU145 cells was affected by apoptosis with different doses of Wharton's jelly stem cells.

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Numerous studies on chemokines and their receptors have indicated their role in cancer cell metastasis and tumor spread. CXCR4 plays an important role in cell survival, proliferation, migration and metastasis[5,21.] So that, CXCR4 antagonists can be a significant factor in the prevention and treatment of prostate cancer. Today, various CXCR4 inhibitors have been reported, including Wharton's jelly. Wharton's jelly has an anti-tumor effect which various studies have reported that Wharton's jelly has a role in various messaging mechanisms, including initiation of transcription, differential expression of functional genes, and in reprogramming specific cell types[22]. VLA-4 is involved in extracellular matrix adhesion as well as a receptor for fibronectin and a cell-to-cell binding. It is one of the genes involved in the humming process and decreased expression leads to reduce metastasis[23]. According to our study, the appropriate dose to reduce the VLA-4 expression was 30% Wharton's jelly, so it can be said that a 30% dose of Wharton's jelly inhibit the metastasis in prostate cancer. Various studies have been performed on the role of VLA-4, CXCR4 and Wharton's jelly in cancer.

CXCR4 antagonists inhibit the growth and spread of cancer. Numerous studies showed that CXCR4 receptors are essential for the invasion, proliferation and metastasis in breast cancer. Therefore, CXCR4 is considered as a diagnostic marker and an important therapeutic target [24]. Studies have shown that the SDF-1 / CXCR-4 signaling pathway is active in most cancer cells [25]. Depending on our results, Wharton's gel inactivates the SDF-1 / CXCR4 pathway by decreasing CXCR-4 expression, which leads to apoptosis in prostate cancer. So other studies showed that VLA-4 expression has significantly increased in tumor cells [26, 27]. It was shown that the activation of P38MPK and NF-β pathway leads to metastasis by positively regulating VCAM-1 / VLA-4 expression [28]. Kalamegam et al. demonstrated that Wharton's jelly, in addition to inhibiting the breast cancer, also had anti-cancer effects in other cancers, including ovarian cancer and osteosarcoma. An interesting point in their studies was that the anti-cancer effect varied between cell lines, which was more severe on osteosarcoma and less on ovarian cancer[22]. Evidence have shown that human Bone Marrow MSCs and other types of MSCs inhibit tumor growth in vitro and in vivo. Khakoo et al. showed that injection of Wharton's jelly-derived hBMMSC into Kaposi sarcoma mouse model inhibited tumor growth in a dose-dependent manner [29]. Zhang et al. studied the effect of fetal umbilical cord stem cells on the MDA-MB-231 cancer cells and showed that fetal umbilical cord cells are involved in inducing apoptosis [30].

CONCLUSION

The aim of our study was to assess the antitumor effect of Wharton's jelly on prostate cancer. Our results showed that different concentration of wharton jelly may change the cell migration and dysregulate the expression of CXCR-4 and VLA-4 in a time dependent manner. Wharton's jelly at high doses significantly was decreased the

migration of DU145 cells and expression of CXCR4, VLA-4. Considering that CXCR4 and VLA-4 are involved in the metastasis pathway, it can be said that Wharton's jelly prevents tumor metastasis. Although the results of the present study and previous studies suggest that Wharton's jelly may be considered as a treatment option for invasive prostate cancer, more studies are needed to understand its anti-metastatic effects.

REFERENCES

1. Jemal A, Murray t, Ward E, et al. CA Cancer J Clin . 2005;55(1):10-30
2. Carter H, Partin A, Retik A, et al. Diagnosis and staging of prostate cancer. Campbell, s urology. Sydney: Elsevier science publishers; 2002.
3. Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat Rev Cancer . 2002;2(8):563-72.
4. [Available from: https://www.britannica.com/science/metastasis.
5. Xu C, Zhao H, Chen H, et al. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. Drug Des Devel Ther. 2015;9:4953-64.
6. Müller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature. 2001;410(6824):50-6.
7. Larson RS, Corbi AL, Berman L, et al. Primary structure of the leukocyte function-associated molecule-1 alpha subunit: an integrin with an embedded domain defining a protein superfamily. J Cell Biol. 1989;108(2):703-12.
8. Fong CY, Subramanian A, Gauthaman K, et al. Human umbilical cord Wharton's jelly stem cells undergo enhanced chondrogenic differentiation when grown on nanofibrous scaffolds and in a sequential two-stage culture medium environment. Stem Cell Rev Rep. 2012;8(1):195-209.
9. Lin HD, Bongso A, Gauthaman K, et al. Human Wharton's jelly stem cell conditioned medium enhances freeze-thaw survival and expansion of cryopreserved CD34+ cells. Stem Cell Rev Rep. 2013;9(2):172-83.
10. Rachakatla RS, Marini F, Weiss ML, et al. Development of human umbilical cord matrix stem cell-based gene therapy for experimental lung tumors. Cancer Gene Ther. 2007;14(10):828-35.
11. Ayuzawa R, Doi C, Rachakatla RS, et al. Naive human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells in vitro and in vivo. Cancer Lett. 2009;280(1):31-7.
12. Tamura M, Kawabata A, Ohta N, et al. Wharton's jelly stem cells as agents for cancer therapy. Tissue Eng Regen Med .2011;4:39-47.
13. Maurya DK, Doi C, Kawabata A, et al. Therapy with un-engineered naive rat umbilical cord matrix stem cells markedly inhibits growth of murine lung adenocarcinoma. BMC Cancer. 2010;10:590.
14. Chao KC, Yang HT, Chen MW. Human umbilical cord mesenchymal stem cells suppress breast cancer tumourigenesis through direct cell–cell contact and internalization. J Cell Mol Med . 2012;16(8):1803-15
15. Liu J, Han G, Liu H, et al. Suppression of cholangiocarcinoma cell growth by human umbilical cord mesenchymal stem cells: a possible role of Wnt and Akt signaling. PLoS One. 2013;8(4):e62844.
16. Ma Y, Hao X, Zhang S, et al. The in vitro and in vivo effects of human umbilical cord mesenchymal stem cells on the growth of breast cancer cells. Breast Cancer Res Treat. 2012;133(2):473-85
17. Wu S, Ju GQ, Du T, et al. Microvesicles derived from human umbilical cord Wharton's jelly mesenchymal stem cells attenuate bladder tumor cell growth in vitro and in vivo. PLoS One. 2013;8(4):e61366.
18. Lin HD, Fong CY, Biswas A, et al. Human Wharton's jelly stem cells, its conditioned medium and cell-free lysate inhibit the growth of human lymphoma cells. Stem Cell Rev Rep. 2014;10(4):573-86.
19. Kawabata A, Ohta N, Seiler G, et al. Naive rat umbilical cord matrix stem cells significantly attenuate mammary tumor growth through modulation of endogenous immune responses. Cytotherapy. 2013;15(5):586-97.
20. Subramanian A, Shu-Uin G, Kae-Siang N, et al. Human umbilical cord wharton's jelly mesenchymal stem cells do not transform to tumor-associated fibroblasts in the presence of breast and ovarian cancer cells unlike bone marrow mesenchymal stem cells. J Cell Biochem. 2012;113(6):1886-95.
21. Kim JH, Lee HJ, Song YS. Stem cell based gene therapy in prostate cancer. Biomed Res Int. 2014;2014:549136.
22. Gauthaman K, Yee FC, Cheyyatraivendran S, et al. Human umbilical cord Wharton's jelly stem cell (hWJSC) extracts inhibit cancer cell growth in vitro. J Cell Biochem. 2012;113(6):2027-39.
23. Abe M, Hiura K, Ozaki S, et al. Vicious cycle between myeloma cell binding to bone marrow stromal cells via VLA-4–VCAM-1 adhesion and macrophage inflammatory protein-1α and MIP-1β production. J Bone Miner Metab. 2009;27(1):16-23.
24. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. Semin Cancer Biol. 2004;14(3):171-9.

25. Libura J, Drukala J, Majka M, et al. CXCR4–SDF-1 signaling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. Blood. 2002;100(7):2597-606.
26. Honn KV, Tang DG. Adhesion molecules and tumor cell interaction with endothelium and subendothelial matrix. Cancer Metastasis Rev. 1992;11(3-4):353-75.
27. Albelda S. Role of integrins and other cell adhesion molecules in tumor progression and metastasis. Lab Invest. 1993;68(1):4-17.
28. Diao XW, Feng JY, Wang QW, et al. SDF-1/CXCR4 axis promotes prostate cancer cell invasion and bone metastasis through p38, NF-κB and HIF-1α pathways. Int J Clin Exp Pathol. 2016;9(2):2706-17.
29. Khakoo AY, Pati S, Anderson SA, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med. 2006;203(5):1235-47.
30. Zhang C, Yang SJ, Wen Q, et al. Human-derived normal mesenchymal stem/stromal cells in anticancer therapies. J Cancer. 2017;8(1):85-96.