International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2018): 7.426

Role of Mesenchymal Stem Cells in Breast Cancer Progression *in vitro*

Khalil A. Elhalfawy[1], Bahgat A. Elfiky[2]and Sara Saber[1]

[1]Molecular Biology Department,Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt

[2]Animal Biotechnology Department,Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt

Abstract: ***The role of Mesenchymal stem cells in tumor development is still controversial. MSCs may promote tumor progression through immune modulation, but other tumor suppressive effects of MSCs have also been described. The discrepancy between these results may arise from issues related to different tissue sources, individual donor variability, and injection timing of MSCs. The expression of critical receptors such as Toll-like receptor is variable a teach time point of treatment, which may also determine the effects of MSCs on tumor progression. However, factors released from malignant cells, as well as surrounding tissues and the vasculature, are still regarded as a "black box." Thus, it is still difficult to clarify the specific role of MSCs in cancer development. Whether MSCs support or suppress tumor progression is currently unclear, but it is clear that systemically administered MSCs can be recruited and migrates toward tumors. These findings are important because they can be used as a basis for initiating studies to explore the incorporation of engineered MSCs as novel anti-tumor carriers, for the development of tumor-targeted therapies.***

Keywords: CD105 Mesenchymal stem cells, Bax, Bcl2, P53, CASP9 Tumor markers and Immunophenotyping

1. Introduction

Mesenchymal stem cells are a non-hematopoietic cell population in the bone marrow, which has the ability to self-renew and differentiate into tissues of mesodermal origin (**Horwitz *etal*., 2005)**. Mesenchymal stem cells are progenitors of bone marrow stroma and thus play a crucial role in supporting hematopoiesis **(Calvi *et al*., 2003) ,** by providing hematopoietic progenitors, the necessary cytokines and cell contact-mediated signals to self-renew and differentiate **(Dazzi *et al*., 2005)**. It has been demonstrated that MSCs exhibit a potent immunosuppressive activity, which targets all types of immune cells of lymphoid lineage. There is evidence that such a broad activity results from a selective inhibition of cell cycle at early stages of cell commitment (G0/G1) (**Glennie *et al*., 2005)** and where as cell proliferation is vigorously reduced, most of immune effectors functions are substantially preserved.

MSCs have been tested for therapeutic applications in the field of hematopoietic stem cells transplantation where by preliminary evidence suggests that they improve HSCs engraftmentand suppress graft-versus host disease after allogenic HSCs transplantation **(Le Blanc *et al*., 2004).** Large physiological numbers of MSCs are apparently required for clinical efficacy. Studies revealed a direct effect of stromal fibroblasts in cancer initiation and progression, especially in epithelial tumors (**Allinen *etal*.,2004**).

Although some studies have showed that these cells inhibit tumor growth in rat **(Nakamura *et al*., 2004)** models, others have demonstrated an opposite effect **(Zhu *et al*., 2006)**. Depending on the system used, MSC have been shown to favor tumor growth either by promoting their invasive abilities via the activation of matrix metalloproteinases and neoangiogenesis **(Zhu *etal*., 2006)** or by preventing tumor cells recognition by the immune system. **(Djouad*et al*., 2003**).Regardless of the effect on tumor growth and progression, most studies found a selective migration of MSCs to the tumor site and this property has been successfully exploited in animal models to deliver therapeutic molecules using MSCs transduced with specific genes**(Studeny *et al*., 2004).** So the present study was designed to investigate the role Mesenchymal stem cells in breast cancer development and both of immunological and molecular factors regulating this process *in vitro*.

2. Materials & Methods

2.1 Preparation of CD^{105} Mesenchymal Stem Cells

Umbilical cord blood was obtained from healthy pregnant female after giving birth at department of pediatrics and gynecology, faculty of medicine, Menufyia University according to the esthetical committee roles. Blood samples were transferred to Stem Cells Lab. at Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University. Umbilical cord blood was diluted with running buffer to 3:1, and 9 ml of the diluted blood cells suspension was carefully layered over 3 ml Ficoll Hypaque (1.077 density) in a 15 ml falcon tube and centrifuged at 1500 rpm for 20 minutes at +4°C in a swinging-bucket rotor without brake. The obtained Buffy coat containing mononuclear cells gently collected and aspirated off and transferred into to new 15 ml falcon tube and filled with PBS containing 2 mM EDTA. Gently resuspended using vortex and centrifuged at 1000 rpm for 10 minutes at +4°C. This procedure repeated twice at the same conditions. Then cells proceeding for CD^{105+} Mesenchymal stem cells separation. The final volume of 300 µl / 108 total cells, and then subjected to magnetic labeling.

2.2 Magnetic labeling of CD^{105+} and Separation with auto MACS Separator (Miltiny Biotech Germany)

Cells were disaggregated by gently pipetting several times, and then passed through 30 µl nylon mesh (Pre -Separation

Filters# 130-041-407) to remove cell clumps. Cell pellet was resuspended in 90 µl running buffer (MACS separation buffer containing 0.5% bovine serum albumin, phosphate buffered saline, pH 7.2, and 2 mM EDTA and 0.09% sodium azide), 10 µl of CD^{105+} Progenitor Cell Isolation Kit was added to the cell suspension and mix well, then incubated for 30 minutes in the refrigerator at +4°C), washed by adding 1-2 mL of buffer and centrifuged again in +4°C cooling centrifuge at 1000 rpm for 10 minutes. Magnetic separation column was placed in the magnetic field of MACS Separator. Cell suspension was then applied on to the column, where CD^{105+} cells were attached to the column and non-attached cells were eluted. After complete separation, the separation column was separated from the column and CD^{105+} was eluted by using running buffer to undergo proliferation *in vitro*.

MCF-7 Cell Line

MCF-7 was obtained from the American Type Culture Collection (ATCC)]. Cells were maintained in RPMI 1640 growth medium (Invitrogen-Gibco), supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Invitrogen-Gibco) and incubated in humidified atmosphere containing 5% CO2 in 37^{O}C.

MTT cell viability assay

MTT assay was performed for MCF-7 according to the method described before **(*Liu et* al., 2006)** Cells were allowed for confluency in 96-well microtitre plat and were treated with different concentrations of MSCs, and incubated at 37°C in 5% CO 2 for 48 hours. MTT reagent was added to each well, and incubated for 2 hours and then 100 µL DMSO was added into the wells to solubilize the produced formazan. The MTT formazan absorbance was measured at 540 nm by a microplate reader (Model APW-100; Biotech, Hangzhou, China). IC 50value was estimated.

Real-time PCR

MCF-7 Cells have been allowed to grow for a day, and then treated with MSCs0. Untreated cells served as a control. RNA was obtained from cells via Qiagen RNeasy. cDNAs were synthesized from RNA, via GeNeiTM, Bangalore kit. For RT-PCR reaction, cDNAs was used as a template for amplification to quantify the steady-state mRNA levels of the tested genes. GAPDH gene was amplified as an internal control. The expression level of the apoptotic markers under the effect of MSCs was also calculated in terms of relative fold change.

Protein extraction and immune-blotting analysis

Blot analysis for investigating the effect of MSCs on the regulation of apoptotic proteins was performed as reported previously **(Zhang*et al*., 2011)**. Cells were harvested after treatment with MSCs for 24 hour and lysed in ice-cold lysis buffer for 2 h, followed by centrifugation for 30 min at 4°C. Supernatants were aliquot, heated and loaded on SDS-PAGE, following which proteins in gels were transferred to polyinylidenedifluoride membranes (Millipore, Bedford, MA). Membranes were firstly blocked and incubated with corresponding primary antibodies for P53, Bax, Bak, Bcl-2, caspase-9 at 4°C and later incubated for 2 h with HRP-conjugated secondary antibody. Protein bands were visualised using enhanced chemiluminescent reagent kit (Amersham, ECL advance, Western blotting detection kit, UK), as per the manufacturers protocol. Bands were then recorded by a digital camera. The monoclonal primary antibodies used in this study were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Secondary antibodies were peroxidase-conjugated affinity-purified anti rabbit or mouse IgG (R.

Cell Cycle analysis

ab139418 is designed for quantitative DNA content analysis in tissue culture cells using the nucleic acid stain propidium iodide followed by flow cytometry analysis. **Principle**: Transfer the previously prepared cells from 4ºC to the bench-top and equilibrate to room temperature, gently re-suspend cells by inverting the tube or by gentle up and down pipetting, may be visible thin salt crystals in the tube but this will not affect the sedimentation of the cells, Pellet the cells at 500 x g for 5 minutes carefully aspirate the supernatant without disrupting the pellet, wash the cells by gently resuspending in 1 mL 1X PBS. Again, pellet the cells at 500 x g for 5 minutes and carefully remove the supernatant, gently resuspend the cell pellet in 200 µL 1X Propidium Iodide + RNase Staining Solution. Ensure that the cells are fully resuspended Incubated at 37ºC in the dark for 20 – 30 minutes, Place tubes on ice (still in the dark) and prepare for flow cytometry analysis.

3. Results

Table 1

Sample data			Bax							
			Control cells			Test cells				FLD
Ser	Sample code	Conc. uM	B Actin	Bax	ΔCTC	B Actin	Bax	ΔCTE	ΔΔ CT	2^ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
1	CD105+		27.07	34.76	7.69	27.34	30.86	3.52	-4.17	13.15207
2	CD105-		27.07	34.76	7.69	27.29	32.49	5.2	-2.49	4.657752
3	MCF7 control		27.07	34.76	7.69	27.07	34.76	7.69	0	1

Table 2

Sample data			Bcl2							
			Control cells			Test cells				FLD
Ser	Sample code	Conc. uM	B Actin	Bcl2	ΔCTC	B Actin	Bcl2	ΔCTE	ΔΔ CT	2^ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
1	CD105+		27.07	31.26	4.19	27.34	32.85	5.51	1.32	0.44237
2	CD105-		27.07	31.26	4.19	27.29	31.91	4.62	0.43	0.766676
3	MCF7 control		27.07	31.26	4.19	27.07	31.26	4.19	0	1

Table 3

Sample data			p53							
			Control cells			Test cells				FLD
Ser	Sample code	Conc. uM	B Actin	p53	ΔCTC	B Actin	p53	ΔCTE	ΔΔ CT	2^ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
1	CD105+		27.07	34.31	7.24	27.34	29.72	2.38	-4.86	20.14426
2	CD105-		27.07	34.31	7.24	27.29	32.67	5.38	-1.86	3.155874
3	MCF7 control		27.07	34.31	7.24	27.07	34.31	7.24	0	1

Table 4

Sample data			Casp9							
			Control cells			Test cells				FLD
Ser	Sample code	Conc. uM	B Actin	Casp9	ΔCTC	B Actin	Casp9	ΔCTE	ΔΔ CT	2^ ΔΔCT
			HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
1	CD105+		27.07	34.64	7.57	27.34	30.57	3.23	-4.34	14.60873
2	CD105-		27.07	34.64	7.57	27.29	32.73	5.44	-2.13	3.72883
3	MCF7 control		27.07	34.64	7.57	27.07	34.64	7.57	0	1

Table 5

Bcl2								
Sample	Conc. uM	Control cells			Test cells				FLD
		B Actin	Bcl2	ΔCTC	B Actin	Bcl2	ΔCTE	ΔΔ CT	2^ ΔΔCT
		HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
CD105+		27.07	31.26	4.19	27.34	32.85	5.51	1.32	0.44237
CD105-		27.07	31.26	4.19	27.29	31.91	4.62	0.43	0.766676
MCF7 control		27.07	31.26	4.19	27.07	31.26	4.19	0	1

Table 6

Sample data		p53							
		Control cells			Test cells				FLD
Sample code	Conc.uM	B Actin	p53	ΔCTC	B Actin	p53	ΔCTE	ΔΔ CT	2^ ΔΔCT
		HC	TC	TC-HC	HE	TE	TE-HE	ΔCTE-ΔCTC	Eamp=1.855
CD105+		27.07	34.31	7.24	27.34	29.72	2.38	-4.86	20.14426
CD105-		27.07	34.31	7.24	27.29	32.67	5.38	-1.86	3.155874
MCF7 control		27.07	34.31	7.24	27.07	34.31	7.24	0	1

Table 7

Sample data		Results			
		Fold Change			
Ser	Sample code	Bax	Bcl2	p53	Casp9
1	CD105+	13.15207	0.44236	20.14426	14.60873
2	CD105-	4.657752	0.76657	3.155874	3.72883
3	MCF7 control	1	1	1	1

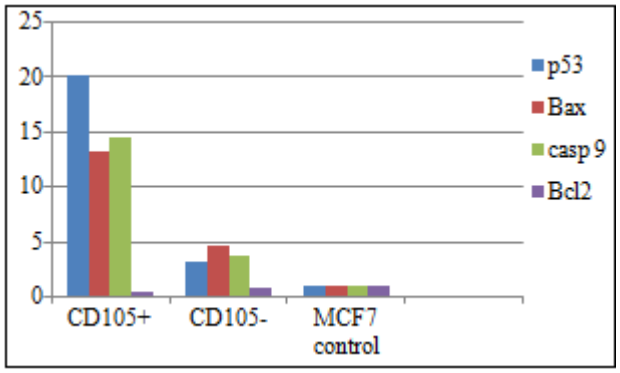

Figure 1: Data showed CD105+ detected high exepretion of P53 ,Casp 9,Bax and very low exeprtion of Bcl2 compared to MCF7control

CD105- detected moderate exepretion of Bax,Casp 9,P53, Bcl2 respectivly compared to MCF7 control.

Cell Cycl analysis

Table 8

ser.	Sample code	%G1	%S	%G2/M	%Pre\ G1	Comment
1	CD105+	22.64	31.42	45.94	23.19	Pre-G apoptosis & Cell growth arrest@G2/M
2	CD105-	45.09	38.34	16.57	9.66	
3	MCF7 control	53.77	42.34	3.89	1.49	

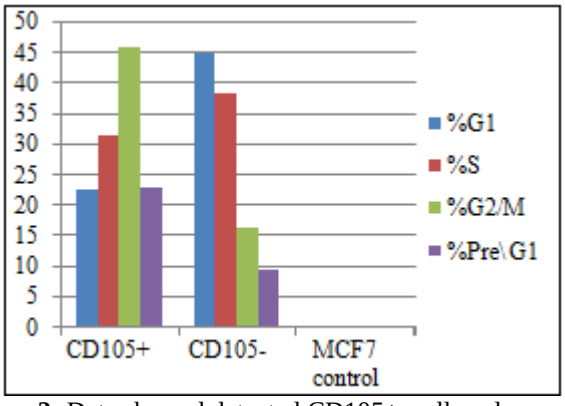

Figure 2: Data showed detected CD105+ cell cycle arrested in G2/M then S then pre/G1 then G1 compare to MCF7 controlled.

CD105- cell cycle arrested in G1 then S then G2/M then Pre/G1 compare to MCF7 controlled.

Table 9: Apoptosis

		Apoptosis			
		Total	Early	Late	Necrosis
1	CD105+	23.19	5.23	14.9	3.06
2	CD105-	9.66	4.38	3.14	2.14
	MCF7 control	1.49	0.84	0.47	0.18

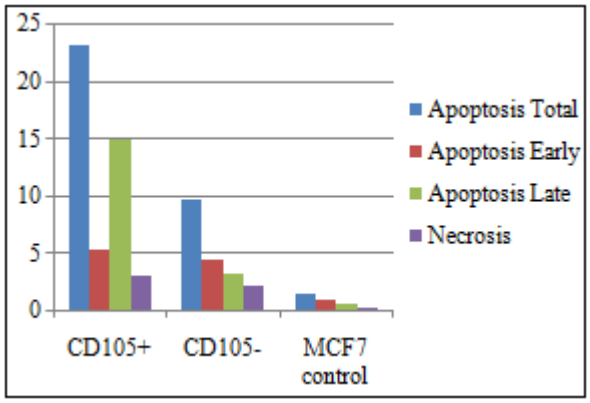

Figure 3: Data showed detected higher exepretion of Apoptosis than necrosis from CD105+ to CD105- respectively compare to MCF7 controlled

4. Discussion

Since the data reported by Tulare *et al*. (2007) that sarcoma developed following transplantation of MSCs into animals, determination of their therapeutic efficacy and safety is now required for clinical applications. From a practical perspective, MSCs seem to be a very promising cell source for use in stem cell therapies for tissue impairment, given that MSCs can home to inflamed or injured tissues, as well as tumors, likely without differentiating into somatic cells. It is important to identify the utility of MSCs in clinical settings, in the context of an understanding of their complicated mechanisms as immune and inflammatory regulators. As discussed in this chapter, the most promising clinical aspects of MSCs might be immune-modulatory and anti-inflammatory effects. However, major challenges remain in our understanding of both the actual benefits, as well as the side effects of these cells in human disease.

The data in the present study discussed key modulators regarding the importance of the migration capacity of MSCs. Controlling the level of these key factors in target tissues may be a way to increase the specificity of MSCs applications in these tissues, which may also lead to a reduction in the total cell number needed for the therapy, and, in concert, may reduce potential side effects, such as malignant transformation. Receptors for the reviewed key factors expressed on MSCs, including TLR and CXCR4, can also be potentially modified genetically via transfection, which may augment the efficacy of MSCs in clinical settings and decrease the migration of MSCs to non-targeted sites.

However, the clinical application of MSCs for cancer treatment is still challenging. This review described the migratory potential of MSCs to malignant tissues, which is largely similar to MSCs migration into inflammatory tissue. However, factors released from malignant cells, as well as surrounding tissues and the vasculature, are still regarded as a “black box.” Thus, remains difficult to provide a specific role for MSCs in cancer development after they migrate and home into different tissues. Although some reports have demonstrated a tumor suppressive effect of MSCs, others described a tumor supportive potential. In any case, these reports encourage the notion that MSCs may play a critical role in cancer development and may be useful as a novel therapeutic delivery system that can target malignant tumors, potentially superior to existing therapeutic molecular therapies. While MSCs can react to surrounding microenvironments, molecular therapies cannot. Thus, it is imperative that scientists continue to investigate the roles and mechanisms of MSCs in tumor progression in order to harness the therapeutic potential of MSCs to regulate both inflammatory and metastatic diseases.

For clinical applications, the methodology of administration of MSCs is crucial to determine their efficacy, since there are several reports describing the risk of capillary embolism by MSCs after intravascular administration **(Furlani *et al.*, 2009;Tatsumi *et al*., 2013)**. Additional strategies, such as co-administration of anti-coagulant or adhesion factors **(Tatsumi *et al*., 2013),**as well as engineering approaches **(Karoubi *et al.*, 2009**; **Houtgraaf *et al.*, 2013),** might attenuate these risks.

References

[1] **Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H and *et al*.(2004):** Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell; 6: 17-32.

[2] **Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC and *et al*.(2003):** Osteoblastic cells regulate the haematopoietic stem cell niche. Nature; 425: 841-846.

[3] **Dazzi F, Ramasamy R, Glennie S, Jones SP. and Roberts I. (2005):**The role of mesenchymal stem cells in haemopoiesis. Blood Rev; 20: 161-171.

[4] **Djouad F, Plence P, Bony C, Tropel P, Apparailly F, SanyJ and*et al.* (2003):**Immunosuppressive effect of mesenchymal stem cells favors tumorgrowth in allogeneic animals. Blood; 102: 3837–3844.

[5] **Furlani D., Ugurlucan M., Ong L., Bieback K., Pittermann E., Westien I., et al. (2009): Is** the intravascular administration of mesenchymal stem cells safe? Mesenchymal stem cells and intravital microscopy.Microvasc. *Res.* 77 370–376

[6] **Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F.(2005):** Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood; 105: 2821–2827.

[7] **Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC and et *al.* (2005):** Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy; 7: 393-395.

[8] **Karnoub A. E., Dash A. B., Vo A. P., Sullivan A., Brooks M. W., Bell G. W., *et al*. (2007):** Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 449 557-563

[9] **Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M and et *al*.(2004):** Treatment of severe acute graft-versus-host diseasewith third party haploidentical mesenchymal stem cells. Lancet; 363: 1439- 1441.

[10] **Liu H, Zang C, Fenner MH, Liu D, PossingerK,and*et al*. (2006) :** Growth inhibition and apoptosis in human Philadelphia chromosome-positive lymphoblastic leukemia cell lines by treatment with the dual PPARalpha/gamma ligand TZD18. Blood 107: 3683-3692.

[11] **Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H and *et al*.(2004):** Antitumor effect of genetically engineered mesenchymalstem cells in a rat glioma model. Gene Ther; 11: 1155-1164.

[12] **Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN and *et al*.(2004):** Mesenchymal stem cells: potentialprecursors for tumor stroma and targeted-delivery vehicles foranticancer agents. J Natl Cancer Inst; 96: 1593-1603.

[13] **Tatsumi K., Ohashi K., Matsubara Y., Kohori A., Ohno T., Kakidachi H., *et al.* (2013):**Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. *Biochem. Biophys. Res. Commun.* 431 203-209**.**

[14] **Tolar J., Nauta A. J., Osborn M. J., Panoskaltsis Mortari A., McElmurry R. T., Bell S., *et al.* (2007):**Sarcoma derived from cultured mesenchymal stem cells. *Stem Cells* 25 371-379.

[15] **Zhang CZ, Zhang HT, Chen GG.and Lai PB (2011)**: Trichostatin A sensitizes HBx-expressing liver cancer cells to etoposide treatment. Apoptosis 16: 683-695.

[16] **Zhu W, Xu W, Jiang R, Qian H, Chen M, Hu J and et *al.* (2006):** Mesenchymal stem cells derived from bone marrow favor tumor cell growth *in vivo*. Exp Mol Pathol; 80: 267-274.