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PROTEIN SYNTHESIS AND SECRETION IN HUMAN MESENCHYMAL STEM CELLS DERIVED FROM AMNIOTIC FLUID, WHARTON'S JELLY AND ENDOMETRIUM

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Abstract: Regenerative medicine uses MSC as a tool to promote tissue regeneration. A major action of MSC to repair pathological degeneration is secreting paracrine secretory factors to reduce inflammation and accelerating tissue regeneration. MSC from different origins produce different significant trophic factors. Using MSC that fits to the need is a strategy for regenerative therapy. Here, we investigated paracrine bFGF TGF-β and IGF, as significant secretory factors, in MSC derived from human amniotic fluid (AF-MSC), Wharton's jelly (WJ-MSC) and endometrium. The work quantified the levels of bFGF TGF-β and IGF gene transcriptions using quantitated real time RT-PCR and the secretory levels of trophic factors using ELISA assay. Our results indicated that bFGF was a significant trophic factor secreted by AF-MSC, whereas, TGF-β was a significant factor in WJ-MSC. AF-MSC secreted bFGF at 3 times over to control human fibroblasts. Secreting of IGF binding protein was found at a low level in AF-MSC, WJ-MSC and EM-MSC as compared to the secreting of control human fibroblast. Moreover, we found that WJ-MSC at late culture had a high level mRNA transcribed in bFGF TGF-β and IGF genes. They showed 2 times of bFGF and TGF-β genes expression and 7 times of IGF-1 gene expression higher than control. However, our study in allelic expression of IGF-2 imprinted gene analysis indicated epigenetic imbalance in WJ-MSC at late cultured passage. We conclude that an optimal MSC type for treatment in different pathologic diseases should be provided regarding a dissimilar significant trophic factor of each MSC origin. We also suggested that MSC in late cultured might great offer in regenerative medicine as cell-free therapy.

***Key words*: amniotic fluid, endometrium, growth factors, paracrine, regenerative medicine Wharton's jelly**

1. INTRODUCTION

Mesenchymal stem cells (MSC) are multipotent progenitor cells. They can be found in almost human tissues and present throughout human development as in amniotic fluid mesenchymal stem cells (AF-MSCs) from fetus, umbilical cord MSC from newborn and bone marrow MSC from adult tissues. MSC have promising tool to promote tissue regeneration. They can migrate into injured tissues and differentiate into specific cell types for tissue repair and regeneration. Moreover, MSC secrete paracrine components to reduce inflammation and promote regeneration of endogenous resident stem cells. However, MSC from different origin have a difference of significant in paracrine secretory components [1,2]. The different secrotome secretion from MSC makes a different effect in tissue regeneration. For example, MSCs derived from human amniotic fluid have been reported to express a wide range of secretory growth factors at a high level [3]. Yoon *et al.*, 2010 [3] showed a releasing of IL-4, an anti-inflammatory cytokine, in AF-MSC, whereas, it cannot found to secrete by other MSC type [3]. Wharton's jelly of umbilical cord is a non-invasive source raising a high quality of MSC. WJ-MSCs expressed various supportive molecules for tissue regeneration [2]. Human endometrium is a dynamic tissue, which undergoes about 400 cycles of regeneration in woman's reproductive years, secreting many cytokines and paracrine factors support embryo implantation and development [4]. Most study general focused on the uses of a specific type of MSC to treat interested diseases, without a studying for which MSC is the best for each therapy.

Three growth factors, which have high effect to tissue regeneration, include transforming growth factor-beta (TGF-β), basis fibroblast growth factor (bFGF) and insulin-like growth factor (IGF). TGF-β associates to regulate cell growth, differentiation, motility and contribute extracellular matrix. TGF-β is a major trophic factor in maintaining self-tolerance of immunoreaction [5]. Shull et al.,1992 [6] described that

TGF-β1 knockout animal presented a widespread autoimmunity. TGF-β is required in articular cartilage formation for treatment of cartilage injury and degeneration [7]. However, using high TGF-β secreting cell should be in awareness. TGF-β has opposing function depending on timing of treatment. bFGF is another major factor impacting in many clinical practice. bFGF has been shown to upregulate in cell growth, angiogenesis and survival. bFGF is distinguish in treatment of wound healing, neurite extension, and neuron and neuronal function [8]. IGF factor has paracrine effect to enhance proliferation of MSC with lower apoptosis [9]. Previous reports demonstrated that IGF has critical role to accelerate regeneration and remodeling of nerve, skeletal muscle and motor neuron [10-11]. IGF-1 activates VEGF to promote angiogenesis, promote muscle cell proliferation and recovery in regenerative skeleton muscle [10]. Moreover, IGF has also autocrine and paracrine action to steroidogenesis [12,13].

With this above information, different major component secreted from different type of MSC should be considered for deciding on type of MSC for therapeutic uses. Using suitable type of MSC for each specific disease is a particular regenerative therapy strategy.

To explore paracrine bFGF, TGF-β and IGF secreted by AF-MSCs from fetal amniotic fluid, WJ-MSC from a new born tissue origin and EM-MSCs from adult endometrial tissue, we investigate transcription levels of bFGF, TGF-β and IGF genes in these three MSCs types and also quantified for bFGF, TGF-β and IGFBP-3 protein secretion in their cell culture medium.

2. MATERIALS AND METHODS

2.1 Ethics statement

Human mesenchymal stem cells derived from amniotic fluid, endometrium and Wharton's Jelly were obtained from Stem Cell Research and Development Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. The procedure for tissue manipulation was approved by The Institutional Review Board in accordance with the requirements of the ethics committee approval by Ethics Committee of Siriraj hospital, Mahidol University, Thailand.

2.2 Cell culture

For human MSC preparation, five clonal AF-MSC lines derived from a "starter cell method" as presented in Phermthai et al., 2010, 2011 [14,15] were obtained at the subculture passage 4. The AF-MSC were repeated subculture to passage 7 under a medium containing α--MEM (Gibco) supplemented with 10% ES-FBS (Millipore, Billerica, MA), 1% l-glutamine (Sigma), 1% penicillin/streptomycin (Sigma-Aldrich), 18% Chang B (Irvine Scientific), and 2% serum supplemented with Chang C (Irvine Scientific) and used in experiment. For WJ-MSC, five stem cell lines were expanded under medium containing α-MEM (Gibco), 2mM l-glutamine (Sigma), 1% penicillin/streptomycin (Sigma-Aldrich) and 10% ES-FBS (Millipore). The cell lines at early passage (P7) and late passage (P14-18) were used in experiment. Human foreskin fibroblasts (HF) from a commercial source were used as control cells. For human EM-MSC, five endometrial stem cell lines, which were prepared as presented in previous reports [16] were cultured to passage 5 under culture medium containing DMEM (Gibco, Invitrogen, CA), 2% antibiotic antimycotic solution (200 IU/mL penicillin G sodium, 200 mg/mL streptomycin sulfate, and 0.5 mg/mL amphotericin B; Gibco) and 10% FBS in embryonic stem cell grade (ES-FBS, Millipore) at 37°C in humidified air with 5% CO2 and use in experiment.

2.3 Cell sample and Condition medium preparations

For cell preparation, cell samples were seeded at a density of 7,000 cell/cm2 in T75 tissue culture flask and cultured under their own culture medium until reach a cell density of approximately 80% confluence. At the day of harvest, cells were twice washed with PBS and media was shifted to fresh basal medium. The culture of AF-MSC and WJ-MSC were shifted to α-MEM basal medium (Gibco) in a presence of 1% FBS, whereas, EM-MSC and HF was replaced to DMEM (Gibco) basal medium in a presence of 1% FBS. The cells were incubated at 37°C in humidified air with 5% CO2 for 24 h. The culture medium was collected and prepared for conditioned medium for protein analysis using ELISA. The conditioned media were centrifuged and a 0.22 μM filtered to get rid of cell debris and were then stored at 70˚C until use. The cells were harvested for quantitative real time RT-PCR analysis.

2.4 RNA preparation and quantitative real-time RT-PCR

Cell samples were isolated for total RNA using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Genomic DNA contamination was eliminating using DNase I (Fermentas, Carlsbad, CA). The purity and quantity of the isolated RNA were determined using Micro UV-Vis Fluorescence Spectrophotometer (Malcom ES.2; Malcom.co.Ltd). The total RNA from individual samples was reverse-transcribed to cDNA using a RevertAid First-Strand cDNA Synthesis Kit (Fermentas, EU). The cDNA was applied for gene expression experiment.

The relative expressions of three genes including, bFGF, IGF and TGF-β was determined using quantitative real time RT-PCR. The analysis was assessed using as an endogenous control and SYBR Green-based quantitative RT-PCR. Beta-Actin (ACTB) was used as the housekeeping control gene. The PCR conditions included initial denaturation at 94°C for 5 min and 35 cycles of DNA denaturing at 94°C for 45 sec, annealing at a primer specific temperature for 1 min and an extension at 72°C for 1 min, after which the reaction was extended at 72°C for 10 min. The primers used in experiment were listed in table 1.

Table 1: Primer sequences and restriction enzymes information

Genes	Primer sequences (5' → 3')	Annealing temp. (°C)	PCR product size (bp)	SNP database No.	Restriction enzyme
Human/mouse *bFGF*	F: 5'-GCA GAA GAG AGA GGA GTT GTG TC-3' R: 5'- ACT GCC CAG TTC GTT TCA GT-3'	60	202	-	-
Human *IGF1*	F: 5'-AAG ATG CAC ACC ATG TCC TCC-3' R: 5'-AGC CTC CTT AGA TCA CAG CTC C-3'	57	248	-	-
Human *TGFβ*	F: 5'-GGG ACT ATC CAC CTG CAA GA-3' R: 5'-CCT CCT TGG CGT AGT AGT CG-3'	58	239	-	-
Human/mouse *β-actin*	F: 5'-ATG TGG CCG AGG ACT TTG ATT-3' R: 5'-AGT GGG GTG GCT TTT AGG ATG-3'	58	107	-	-
H19	F: 5'- TTGCACTGGTTGGAGTTGTG -3' R: 5'- GCGTAATGGAATGCTTGAAGGCTGCTC -3'	58	215	rs2839704	*Rsa*I
IGF2	F: 5'-CACGCCAAACACTGAATGTC-3' R: 5'-ATTGGGATTGCAAGCGTTAC-3'	55	151	rs2585	*Alu*I

2.5 Enzyme-linked immunosorbent assay

Condition medium were determined for the levels of accumulated bFGF, IGFBP-3 and TGF-β protein concentrations released by the AF-MSC, EM-MSC, WJ-MSC, and HF using enzyme-linked immunoassay (ELISA) kits. The conditioned medium was measured for concentrations using sandwich ELISA kits for bFGF (cat No. DFB50; R&D Systems, Inc., Minneapolis, MN), TGF-β (cat No. ab100647; Abcam, MA) and IGFBP-3 (cat No. DGB300; R&D Systems, Inc., Minneapolis, MN). Assays were performed according to following manufacturer's instructions. Condition medium of human foreskin fibroblast (HF) was used as control. Two independent repetitions in duplicate were made per sample.

2.6 Allelic expression analysis

For allelic expression analysis, primers were designed to amplify segments of the IGF2 and H19 genes containing single-nucleotide polymorphisms (SNPs). Each primer contained a restriction site for a specific restriction enzyme. The primers and the enzymes used for IGF2 and H19 allelic expression analysis are listed in Table 1. Genomic DNA and cDNA were obtained from ten WJ-MSC lines at late-cultured passage (P14) and then subjected to PCR for 35 cycles with the following conditions: 94oC for 30 s, specific melting temperature for 30 s and extended in 72oC for 45 s. PCR product was determined for 2 parental allele's heterogeneity with a digestion in the specific restriction enzymes using restriction fragment length polymorphism analysis. Gel electrophoresis was performed on 10% polyacrylamide gels.

2.7 Statistical analysis

For statistical analysis, transcription levels of bFGF, TGF-β, IGF genes and protein translation levels of bFGF, TGF-β and IGFBP-3 genes of different studied cell types were assessed using Student's t test. A $P<0.05$ was considered significant (*).

3. RESULTS

The cell culture was performed and MSC was collected at subculture passage 7 or the last passage before getting into cell senescence. AF-MSC morphology presented in a short and small fibroblastic cell type. WJ-MSC and EM-MSC showed a long spindle shape in fibroblastic cell type (Figure 1A). The population doubling time (PDT), as presenting a character of cell proliferation, were observed throughout the culture from P4 to P7 (Figure 1B). At passage 7, PDT was observed and showed at 1.3±0.12 days in AF-MSC and at 1.6±0.27 days in WJ-MSC. We found that most EM-MSC lines cannot prolong subculture over than passage 6 and the cells got to enlarge cell size. The PDT of EM-MSC was at 2.6±1.19 days in passage 6. Phenotypic surface markers of MSC were analyzed and presented in Table 2.

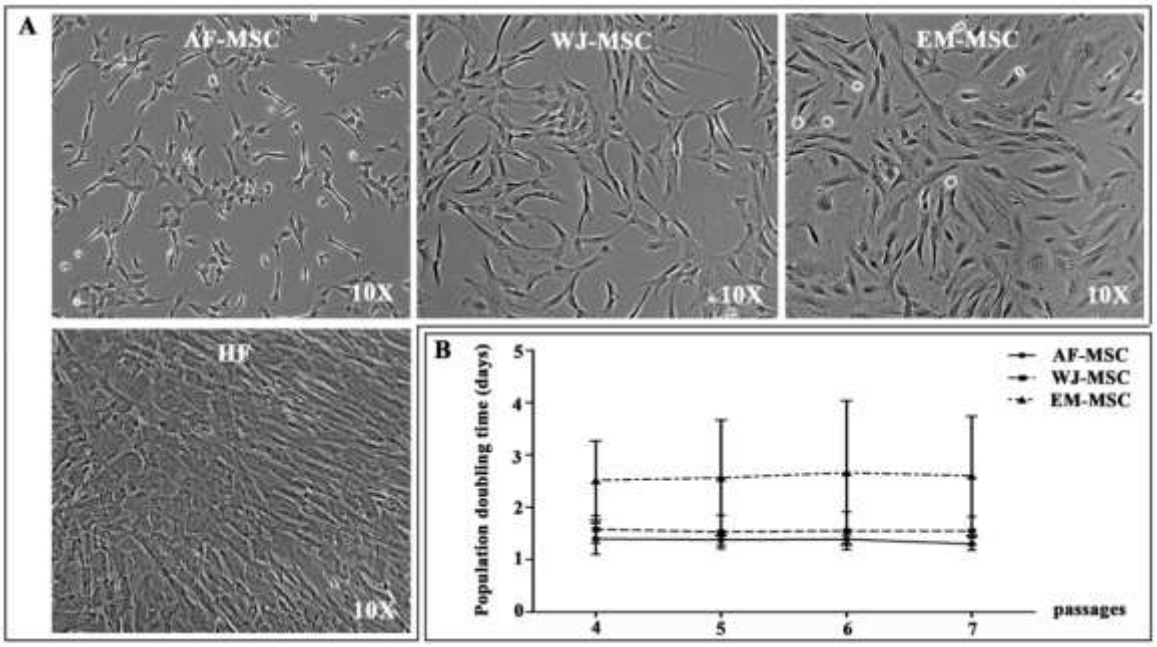

Figure 1. Mesenchymal stem cell morphology and characteristics

(A) Morphologic appearances of MSC from amniotic fluid (AF-MSC), Wharton's Jelly (WJ-MSC) and endometrium (EM-MSC) were observed via inverted microscope. Phase-contrast images of MSC are shown at passage 5 of culture with a 10x magnification. During cell culture P4-P6, MSC was seeding and cultured in a suitable manner depending on each MSC types, low cell seeding in AF-MSC and WJ-MSC culture and high cell seeding in EM-MSC and HF. (B) Proliferation curves of AF-MSC, WJ-MSC and EM-MSC were presented in terms of population doubling times (days) of each MSC type in subsequently subculture passages from 4 to 7.

Table 2. Phenotypic antigenic markers of AF-MSC, WJ-MSC and EM-MSC using flow cytometry analysis

Cell surface markers	AF-MSC (n=5) (mean ± SD)	WJ-MSC (n=5) (mean ± SD)	EM-MSC (n=5) (mean ± SD)
CD29	93.56 ± 9.67	97.76 ± 1.53	96.13 ± 6.22
CD34	1.68 ± 1.98	0.70 ± 0.33	0.61 ± 0.51
CD45	1.49 ± 1.08	0.38 ± 0.26	0.82 ± 0.77
CD73	97.56 ± 4.40	97.68 ± 3.42	98.51 ± 2.25
CD90	86.27 ± 7.30	99.71 ± 0.51	78.34 ± 18.07
CD105	91.84 ± 10.79	99.78 ± 0.39	99.82 ± 0.12

Human foreskin fibroblast (HF) was used as controls cells to investigate for gene transcription and protein expression levels of paracrine bFGF, TGF-β and IGF secretory growth factors regarding their effectiveness to be used as feeder cell to facilitating paracrine bFGF ad TGF-β for supporting ESCs/iPSCs culture. It presented secretory bFGF protein at a concentration of 16.5 pg/mL and TGF-β protein at a concentration of 6,805.6 pg/mL in conditioned medium of human fibroblast culture.

The transcription levels of bFGF, TGF-β and IGF genes were investigated in MSCs derived from amniotic fluid, Wharton's jelly in umbilical cord and endometrial tissues. For bFGF gene expression, we found that the highest level of bFGF gene expression in WJ-MSC. bFGF gene transcription was found in WJ-MSC as 2 times over than the expression in AF-MSC, EM-MSC and control HF (Figure 2A). For transcription of TGF-β gene, expressions found in AF-MSC and EM-MSC were significant lower (p-value <0.05) than HF control. WJ-MSC showed a level of TGF-β expression at 2 times less than HF control. The AF-MSC and EM-MSC showed a similar level of TGF-β expression. WJ-MSC showed 2 times TGF-β overexpressed as compared to AF-MSC and EM-MSC (Figure 2A). For IGF1 gene expression, AF-MSC, WJ-MSC and EM-MSC showed a comparable IGF1 gene expression level and also similar to the expression level found in control human fibroblast (Figure 2A).

We also studied bFGF, TGF-β and IGF genes expression in AF-MSC, WJ-MSC and EM-MSC at late cultured passages. We found that WJ-MSC at high repeated subculture passage had overexpression of bFGF, TGF-β and IGF genes as compared to WJ-MSCs in early subculture passage, whereas, the overexpression was not found in AF-MSC and EM-MSC in late culture. bFGF gene expression in WJ-MSC at late passage (P14-17) showed a highly significant 5-times higher than the expression in HF and a significant higher (p-value <0.05) than WJ-MSCs at early passage (Figure 2A). WJ-MSC at late passage had a comparable expression level of TGF-β to HF control. It also showed a 6 times overexpression of IGF to early passage WJ-MSCs.

Although, the mRNA of bFGF gene was transcribed at high in WJ-MSC, we found that the highest presence of bFGF protein was secreted by AF-MSC. The result showed that bFGF protein was found in AF-MSC condition medium at a concentration of 59.9 pg/mL, which was a 3

times over than in control HF (a concentration of 16.5 pg/mL), and over than the presence in WJ-MSC (a concentration of 39.4 pg/mL). bFGF protein was prominently expressed in AF-MSC (Figure 2B). For paracrine TGF-β detected in condition medium, we found that all cell studied secreted TGF-β at a high level. The highest concentration of TGF-β was observed in condition medium of WJ-MSC at a concentration of 9,527.8 pg/mL. AF-MSCs secrete TGF-β protein (7,361.1 pg/mL) in a similar level to control HF (6,805.6 pg/mL) (Figure 2B). Both analysis of bFGF and TGF-β protein secretion, EM-MSC was not analyzed, owing to their low quality of cell proliferation and getting senescence in very early cultures passage resulting to fluctuating results in ELISA assessment. For investigation for the action of IGF1, we examined on Insulin-like growth factor binding protein type 3 (IGFBP-3), which is prolonging the half-life and paracrine regulators of IGF action. The result showed that AF-MSC, WJ-MSC and EM-MSC secrete a same level of IGFBP-3 at the concentrations of 93.1 pg/mL, 70.9 pg/mL and 93.5 pg/mL, in respectively. The IGFBP-3 found in condition medium of AF-MSCs and WJ-MSCs was significant lower than (P-value<0.05) than those found in condition medium of control HF (151.8 pg/mL) (Figure 2B).

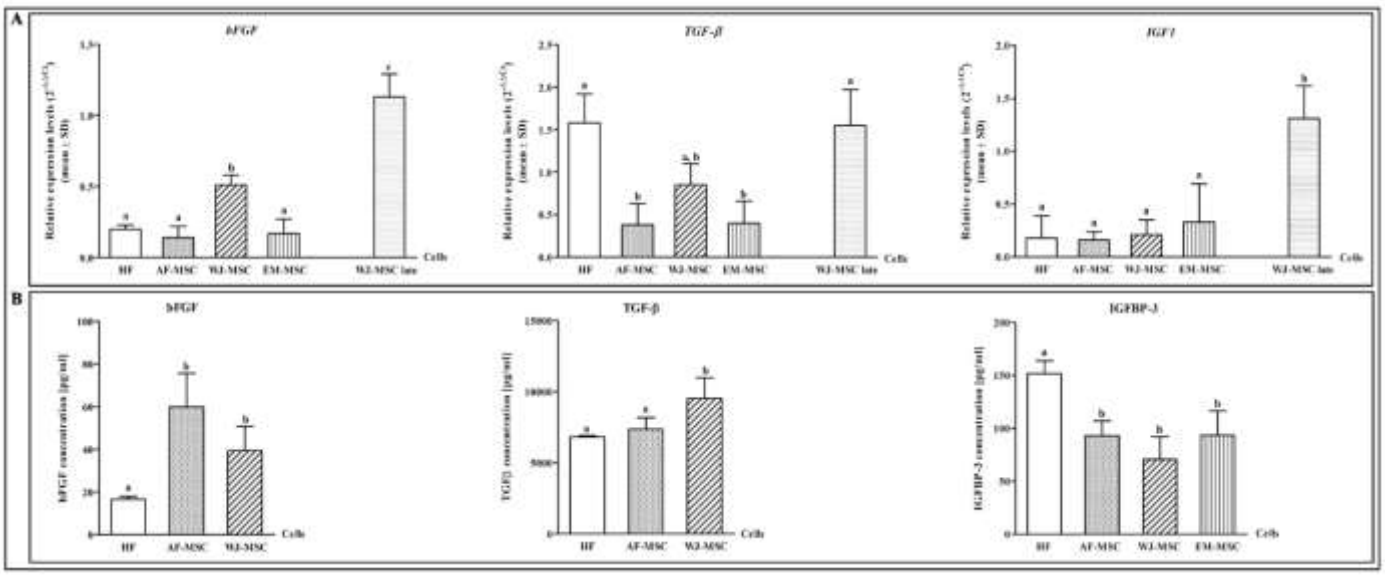

Figure 2. Trophic factors secreting from different type MSC

MSC form different origins facilitate significant paracrine growth factors. Levels of bFGF, TGF-β and IGF gene expression in MSC were performed using real-time RT-PCR (A). The trophic factors secreted into condition medium were quantified using ELISA and presented in mean±SD and plotted in graph (B). P-value <0.05 was considered to be statistically significant difference. The different alphabet (a,b,c) in bar graph indicates statistical significant difference in each other.

Regarding high expression of bFGF, TGF-β and IGF1 found in late cultured passage WJ-MSC, epigenetic analysis was investigated via the action of gene imprinting. Epigenetic alteration in late passages of WJ-MSC was examined for allelic expression on ICR of IGF2 and H19 genes at domain 11p15.5. For allelic expression analysis, the presence of SNPs in the IGF2 and H19 genes was analyzed in ten late-cultured passage WJ-MSC lines. These polymorphisms were used to distinguish the maternal and paternal alleles. For IGF2, we found five WJ-MSC lines to carry SNPs. The WJ-MSC lines comprising SNPs were used for allelic expression analysis. At the early-cultured passage (P4), we found that three from five WJ-MSC lines exhibited expression in mono-allelic pattern and two from five lines showed a bi-allelic expression pattern. At the late-cultured passage (P14), we found that one of three cell lines in a typical genetic imprinting, with monoallelic expression, became biallelic expression pattern for IGF2 (Figure 3). For H19, six WJ-MSC lines carried SNPs. The WJ-MSC lines comprising SNPs were allelic expression analyzed. We found that all six WJ-MSC lines exhibited a typical genetic imprinting pattern in mono-allelic expression in both of the early-cultured passage (P4) and late-cultured passage (P14) (Figure 3).

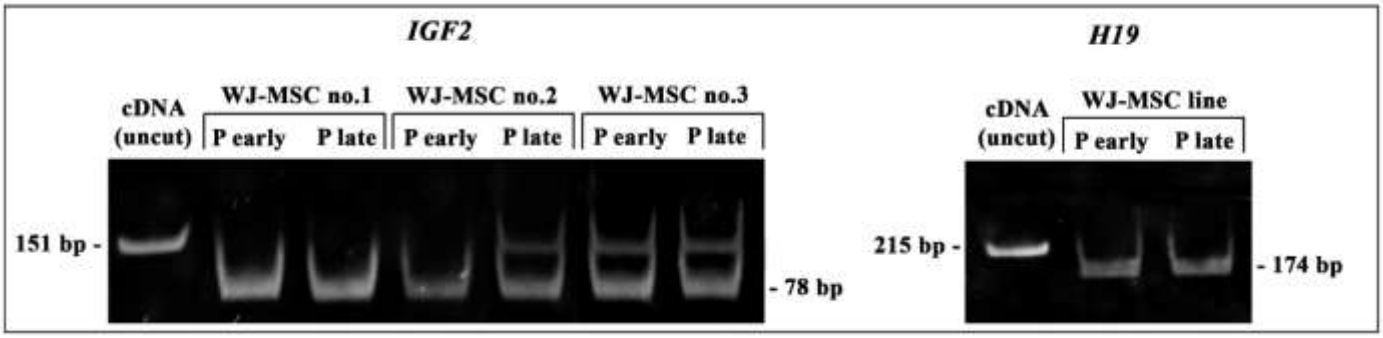

Figure 3. Allelic expression patterns of imprinted IGF2 and H19 genes in the early- and late-cultured passage WJ-MSC

WJ-MSC at late passage of cultured was determined for the bias of parental allele expression in cell lines carrying SNPs in IGF2 and H19 using a biallelic expression analysis. The cell lines presented the heterogeneous alleles on IGF2 and H19 at the site of the SNP were used. The cDNA lane shows PCR product before restriction enzyme digestion. Expression of IGF2 was observed in monoalleclic and biallelic patterns, indicating the loss of gene imprinting during in vitro cell culture, whereas, H19 was found a typical expression in monoallelic pattern.

4. DISCUSSION

MSC are promising tools for regenerative medicine due to its two actions in migration to injured tissues to differentiate to be specific cells and secreting cytokines or paracrine to accelerate endogenous stem cells to repair, remodel and regenerate tissues. MSC secrete various paracrine growth factors including bFGF, TGF-β and IGF. However, MSC from different origins are prior to secrete a distinctive profile of cytokines. Attempting to address the most suitable type of MSC to fit the needs in specific disease for therapeutic uses, we explore bFGF, TGF-β and IGF production and secretion in MSC in a fetal MSC; AF-MSC, newborn MSC; WJ-MSC and adult MSC; EM-MSC to find suitable MSC type. Human HF and MEF were used as control cells. Our results indicated that AF-MSC and WJ-MSC secreted bFGF and TGF-β at a high level. AF-MSCs had significance in expression of soluble bFGF protein, whereas, a significant soluble TGF-β protein was found in WJ-MSC. EM-MSC secreted a comparable IGF-binding protein 3 (IGFBP-3) concentrations to AF-MSC and WJ-MSC. We also found that transcriptions of bFGF, TGF-β and IGF genes in WJ-MSC were elevated when cultured for long-period. The expression of bFGF, TGF-β and IGF genes in late-cultured WJ-MSC (P14) presented 2-times higher than in early passage (P 6-7). IGF gene expression in late-passage WJ-MSC showed 7-times higher than in early passage.

Although, WJ-MSC at late-cultured passage can secret a high level of paracrine trophic factors, our results in allelic gene analysis indicated that aberrant allelic gene imprinting pattern, as the loss of parent specific imprinting of the IGF2 gene, was observed in 2 of five WJ-MSC lines at late cultures. Genomic imprinting is a key mechanism in epigenetic control system, which acts via the expression of parent-of-origin-specific manner and has a role in cell development [17]. Aberrance of IGF2 imprinted gene expression has been reported in association with various disease, such as cancer [18,19]. Martinez-Quetglas et al., 2016 presented that overexpression of IGF2 gene was found in a large proportion of hepatocellular carcinoma [19]. Our finding in imbalance allelic expression of imprinted genes in late-passage MSC is consistent to the finding by previous works [20,21]. Phermthai et al., 2013 [21] exhibited loss of imprinting of IGF2 in MSC culture in long term. With our present result, we suggest that late cultured WJ-MSC transcribed high levels of paracrine bFGF, TGF-β and IGF should not be used for transplantation in cell based therapy. However, it may be applied to use as supportive feeder cell for stem cell culture due to its high level of paracrine trophic factors. Late cultured passage WJ-MSC might be a good source for production of micro-vesicular and exosomal components for regenerative medicine.

In a character of paracrine action, bFGF and TGF-β are important paracrine signals to support a stemness, self-renewal, pluripotency, adhesion molecules and extracellular matrices for a pluripotent cell growth in an in vitro. bFGF and TGF-β secreted by fibroblast feeder cells facilitate cell attachment and prevent spontaneous differentiation for human ESCs/iPSCs [22,23]. Widely used fibroblast feeder cells are primary human foreskin fibroblasts (HF) from infant foreskin [24].

In aspect of using MSC for regenerative medicine, there are attempting to provide ideal cell source for different pathological situation. MSC isolated from different origins showed similar morphology, multipotent cell surface markers, but it can secrete different amount and types of significant growth factors and showed incomparable effective in medical treatment. For example, secretory factors from MSC, which secretes IGF as a major trophic factor, may act to steroidogenesis, whereas, the secrotomic factors from MSC, which secretes TGF-β as a major trophic factor, may prohibit steroidogenesis [13,25]. Furthermore, using MSC in unsuitable sources may result to adverse effects in medical treatment. For example, TGF-β inhibit the proliferation of transformed cell and acts as a tumor suppressor during early stages of tumorigenesis, but it suppresses the immune system by inhibiting NK-cell activity and dendritic cell maturation in advance stages of malignancies [5]. The treatment with MSC releasing a high concentration TGF-β should be considered in this case. Using ideal MSC can advantage to outcome of cell-based therapy. For example, the in vivo study of Kehl et al., 2019 [2], which examined induction of vascularization with trophic factors released from human WJ-MSC, bone marrow-MSC and adipose-MSC and demonstrated that WJ-MSC is the most potent human MSC source for inflammation-mediated angiogenesis induction. They described that trophic factors secreted from WJ-MSC composed with complete network proteins relating to angiogenic function, whereas, secretory factors from adipose-MSC lacked a central angiogenic proteins. This work was consistent to the finding by Hsieh et al., 2013, who presented that WJ-MSC had more potential action for treatment of myocardial infarction as compared to bone marrow-MSC [26].

bFGF has been known a major role to accelerate cell growth, proliferation and neuron function. Our finding presented that bFGF secreting was superior in MSC derived from amniotic fluid. It is consistent to a previous presence, which found a high level of bFGF in AF-MSC [27]. This is compromising of bFGF function to tissue origin of AF-MSC, which is in stage of fetal growth and enlargement of amniotic sac. TGF-β has been known as a major regulator of cartilage homeostasis and important to produce chondrocyte and repair pathologic osteoarthritis cartilage. Several studies presented that TGF-β can be secreted from various MSC types. The work by Kamprom et al., 2016 [1] revealed a high expression of TGF-β in WJ-MSC. In this study, our study indicated that the highest level of TGF-β was found in WJ-MSC originating in umbilical cord. Providing of growth factor secretory levels in various MSC are very useful to select ideal MSC source for treatment in specific pathological issue. It is an important strategy to achieve in cell therapy and regenerative medicine.

CONCLUSIONS

In conclusion, our study provided information of significant trophic factor in MSC. We demonstrate that bFGF was a significant trophic factor of AF-MSC and TGF-β was a significant trophic factor of WJ-MSC. IGFBP-3 was secreted in a comparable level among AF-MSC, WJ-MSC and EM-MSC. Furthermore, we displayed that late-cultured WJ-MSC secrete significant higher levels of bFGF, TGF-β and IGF, but they often carried epigenetic alteration. We suggest that MSC at late-passage should not be used in cell-based therapy, but it might be used for cell source for cell-free tools in regenerative medicine.

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