Pluripotent Gene Expression in Mesenchymal Stem Cells from Human Umbilical Cord Wharton's Jelly and Their Differentiation Potential to Neural-Like Cells

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Objective: *To explore the expression of pluripotent genes in Wharton's jelly derived MSCs (WJ-MSCs) and their neuronal differentiation potential.*
Material and Method: *Gelatinous connective tissues from umbilical cord Wharton's jelly were digested with trypsin and then cultured in Dulbecco's Modified Eagle's Medium. The expressions of typical MSC markers as well as pluripotent markers were examined by flow cytometry and reverse transcription PCR, respectively. MSCs at passage 3 and 5 were used for in vitro adipogenic, osteogenic and neuronal differentiation by incubation with specific induction media.*
Results: *WJ-MSCs could be easily expanded for more than 20 passages while maintaining their undifferentiated state and their marker expression profiles, being positive for typical MSC markers CD90, CD73, and CD105, and being negative for hematopoietic markers CD34 and CD45. Interestingly, the expression of several pluripotent marker genes including Oct4, Rex1, Sox2, and Nanog was detected in early passages of both cultured WJ-MSCs and BM-MSCs. WJ-MSCs were able to differentiate not only to mesodermal cells, such as adipocyte and osteoblast but also the neural-like cells as characterized by neuronal morphology and the expression of neuronal markers including MAP-2, GFAP, beta-tubulin III and Tau.*
Conclusion: *The present study demonstrates that WJ-MSCs can be readily obtained and expanded in culture while maintaining their typical MSC characteristics. WJ-MSCs and BM-MSCs also expressed several genes associated with pluripotency and exhibited their plasticity by differentiation toward neuronal-cell lineage. Umbilical cord Wharton's jelly might have potential to become an alternative source of MSC for treating nervous system disorders.*

Keywords: *Mesenchymal stem cells, Wharton's jelly, Pluripotency, Neural differentiation*

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Stem cells can be classified as either embryonic stem (ES) cell or adult stem cell. ES cells are pluripotent and can give rise to all specialized cell types of the organisms[1]. Three transcription factors found in mouse and human ES cells play a central role in the regulation of pluripotency and self-renewal. These factors include the POU (Pit/Oct/Unc) domain-containing protein, Oct4[2,3], Sox2[4] and the homeoprotein Nanog[5]. All those transcription factors are expressed in high levels in pluripotent cells and are considered the markers of primitive cells. However, the applications of ES cells are limited by the difficulties in expanding those cells in large quantity required for several applications without losing their properties and the problem in controlling their differentiation to desired cell types.

Adult stem cells are rare cells ($1:10^7$-$1:10^8$ of all cells) thought to be present in all tissues and responsible for maintaining the homeostasis of several tissues. Mesenchymal stem cells (MSCs) are multipotent adult stem cells that differ from hematopoietic stem cells in their characteristics and differentiation potentials[6]. MSCs are capable of differentiating into cells of mesenchymal lineages, including adipose tissues, connective tissues, bone, and

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cartilage[7,8]. MSCs were initially identified in human bone marrow (BM) and later in peripheral blood, muscle, adipose tissue, and connective tissues[8,9]. Although, BM is the most common source of MSCs for clinical applications, however, the frequency number of MSCs in human adult BM is relatively low and their proliferative capacity are decreasing with age[10]. Moreover, the harvesting of the cells from BM remains invasive and painful for the donors. Since umbilical cord, placenta, amniotic membranes, and fluid are usually discarded after birth, they represent non-controversial and potential sources of adult stem cells that could be easily obtained in a large quantity using a non-invasive procedure.

Several previous studies revealed that the matrix cells derived from Wharton's jelly exhibited the characteristics and possessed properties similar to those of bone marrow-derived MSCs. The matrix could be expanded in culture without obvious senescence for more than 80 population doublings (PDs) without signs of cellular senescence. Furthermore, WJ-MSCs also exhibited typical MSC marker expression profiles, being positive for CD90, CD73, and CD105 while being negative for hematopoietic lineage markers CD34 and CD45[11]. In addition, WJ-MSCs have been reported to be able to differentiate into osteoblast-, adipocyte-, and cardiomyocyte-like cells[11-13]. However, the potential of stem cell-based therapies for treating several human and animal diseases emphasizes the importance of comparing the vary sources of stem cells and seeking a better understanding of their proliferative and differentiation capacity. Moreover, very few studies have explored the expression of pluripotent genes in WJ-MSCs as well as their ability to differentiate to extra-mesodermal tissues, such as neural-lineage cells.

We hypothesize that human umbilical cord Wharton's jelly may be another one source of MSCs that could proliferate and differentiate into neural-like cells. Therefore, the present study aims to study the expression profiles of several pluripotent genes, *Oct4*, *Rex1*, *Sox2*, and *Nanog* in WJ-MSCs as well as their ability to differentiate toward neural-lineage cells in comparison to those of BM-MSCs.

Material and Method

Sample preparation

All procedures were approved by the ethical committee of the Faculty of Medicine Siriraj Hospital, Mahidol University. All subjects participated in the study after giving written informed consent.

Collection, isolation, and culture of human BM-MSCs

Bone marrows were aspirated from sternum or iliac crest of normal healthy volunteers and were processed exactly as previously described[14]. After processing, cells were plated in a tissue culture flask in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Invitrogen, USA) with 10% fetal bovine serum (FBS; Cambrex) and 1% penicillin-streptomycin (Gibco). Cells were maintained at 37°C in 5% CO_2 and were passaged at 90% confluence using 0.25% trypsin/EDTA (Gibco), neutralized with MSC medium, and replated in tissue culture flasks.

Collection, isolation, and culture of human WJ-MSCs

Human umbilical cord Wharton's jelly was aseptically collected from infants delivered by full-term normal labor and was rinsed in phosphate-buffered saline; pH 7.4 (PBS, composed in 140 mM NaCl, 2 mM KCl, 1.5 mM KH_2PO_4), supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin. Umbilical cord (segment 1-3 cm in length) was cut longitudinally to expose the two umbilical arteries and the umbilical vein. The vessels were removed and discarded. The remaining umbilical cord tissues including the Wharton's jelly were cut into pieces of about 1.5 to 2.5 mm^2 using razor blades. The tissues were treated with 0.5% trypsin-EDTA solution (Invitrogen) for 45 minutes at 37°C and then plated on 25-cm^2 tissue culture flasks in DMEM with 10% FBS and 1% penicillin-streptomycin. The cells were cultured under humidified atmosphere of 5% CO_2 in air at 37°C. Culture flasks were observed continuously for the developing colonies of adherent cells and the medium was replaced every three to four days. The adherent cells were passaged using 0.25% trypsin-EDTA when the cell density reached 90% confluence.

MSC characterization

Flow cytometric analysis of MSC surface markers; CD73 (BD Bioscience), CD105 (AbD Serotec), CD90 (AbD Serotec) and hematopoietic cells surface markers; CD34 (BD Bioscience), CD45 (BD Bioscience), were performed. Flow cytometry data were analyzed using CellQuest software (BD Bioscience).

In vitro differentiation

WJ-MSCs and BM-MSCs at the third passage were induced to differentiate into adipocyte, osteoblast, and neural cell.

Adipogenic differentiation

To induce adipocyte differentiation, $8x10^3$ cells/cm^2 MSCs were plated on six well plate in NH AdipoDiff Medium (Miltenyi Biotec) and cultured under 5% CO_2 in air at 37°C with complete change of medium every three days. After three weeks of culture, cytoplasmic inclusions of neutral lipids were observed and stained with Oil-red O (Sigma).

Osteogenic differentiation

To induce osteoblast differentiation, $5x10^3$ cells/cm^2 MSCs were plated on six well plate in NH OsteoDiff Medium (Miltenyi Biotec) and cultured under 5% CO_2 in air at 37°C with complete change of medium every three days. After two weeks of culture, osteogenic differentiation cells were observed and stained for alkaline phosphatase activity (Sigma).

Neural differentiation

To induce neural differentiation, $2.5x10^3$ cells/cm^2 MSCs were plated on six well plate in Advance STEM™ Neural Differentiation Medium (HyClone). The neural like cells were observed at 48 hours and 72 hours after induction and processed for immunocytochemistry and quantitative real-time PCR.

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA were extracted from BM-MSCs, WJ-MSCs and neural-like cells using RNA mini Kit (Invitrogen). For cDNA synthesis, 1 μg of total RNA for each sample was reverse transcribed to cDNA in 20 μl volume using iScript™ Select cDNA synthesis Kit (Bio-Rad Laboratories) according to the manufacturer's instruction. After cDNA synthesis, products were amplified using pluripotency gene specific primers under each optimal condition. The following paired primers were used: *Oct4* (573 bp); forward primer: 5'-CGACCATCTGCCGCTTTGAG-3' and reverse primer: 5'-CCCCCTGTCCCCCATTCC TA-3', *Rex1* (306 bp); forward primer: 5'-CAGATCCT AAACAGCTCGCAGAAT-3' and reverse primer: 5'-GCGTACGCAAATTAAAGTCCAGA-3', *Sox2* (448 bp); forward primer: 5'-CCCCCGGCGGCAA TAGCA-3' and reverse primer: 5'-TCGGCGCCGGGG AGATACAT-3', *Nanog* (426 bp); forward primer: 5'-GCGCGGTCTTGGCTCACTGC-3' and reverse primer: 5'-GCCTCCCAATCCCAAACAATACGA-3'. *Beta-actin* (838 bp); forward primer: 5'-ATCT GGCACCACACCTTCTACAATGAGCTGCG-3' and reverse primer: 5'-CGTCATACTCCTGCTTGC TGATCCACATCTGC-3' was used as the endogenous control for quantitative amount of cDNA template. The molecular size of PCR products was verified by electrophoresis in 1.5% agarose gels.

Quantitative real-time PCR for neuron specific genes expression analysis

The primer sets used for the quantitative real-time PCR were microtubule associated protein 2 (*MAP-2*); forward primer: 5'-CCAATGGATTCCC ATACAGG-3', reverse primer: 5'-CTGCTACAGCC TCAGCAGTG-3', *beta-tubulin III*; forward primer: 5'-AACGAGGCCTCTTCTCACAA-3', reverse primer: 5'-CCTCCGTGTAGTGACCCTTG-3' and glial fibrillary acidic protein (*GFAP*); forward primer: 5'-CCAGTTGCAGTCCTTGACCT-3', reverse primer: 5'-ATCTCGTCCTTGAGGCTCTG-3'. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the endogenous control. Quantitative real-time PCR was carried out using standard protocols with the SYBR® PCR Master Mix (Applied BioSystems). The PCR mix contained SYBR® Green PCR Master Mix, 1 ng DNA template, 1 μM primers, and nuclease free water to reach a final volume of 20 μl. Quantitative real-time PCR was performed using the StepOnePlus™ Real-Time PCR System (Applied BioSystems). A melting curve analysis was performed at the end of each reaction. The gene expression levels were normalized to individual *GAPDH* (internal control). The profile was obtained by plotting relative gene expression levels compared to undifferentiated MSCs.

Immunocytochemistry

To examine neuronal differentiation, WJ-MSCs and BM-MSCs, cultured with or without neural differentiation medium, were rinsed in PBS and fixed with 4% paraformaldehyde in PBS, pH 7.4 for 10 minutes, at room temperature. After washing twice in PBS and incubating 0.1% Triton X-100 in PBS for 10 minutes at room temperature, the cells were incubated for 30 minutes in a blocking solution containing 4% BSA before incubation with the primary antibody [Anti-microtubule-associated protein 2 (Anti-MAP-2; Sigma, USA), Anti-beta tubulin-III (Sigma, USA), and Anti-Tau (Sigma, USA)]. Thereafter, primary antibodies were labeled with appropriate secondary antibody. After immunostaining, cover-slips were mounted cell-side down on microscopic slides using antifade mounting medium (Vectrashield).

Confocal obtained using an Olympus laser scanning confocal microscope. The images were analyzed using Olympus software.

Results

Characterization of WJ-MSCs

After culture for 72 hours, primary cells derived from umbilical cord Wharton's jelly proliferated and generated several outgrowths from the tissue pieces. Those primary cultured cells exhibited a mesenchymal morphology, being spindle-shaped cells that formed cell contact with the adjacent cells. When reach confluence, cells were arranged themselves in parallel arrays (Fig. 1) in a manner similar to those of BM-MSCs.

BM-MSCs and WJ-MSCs were uniformly positive for CD90 (average 94.85% and 95.78%, respectively), CD73 (average 90.65% and 95.68%, respectively), CD105 (average 89.99% and 91.86%, respectively), and negative for CD34 and CD45 (Fig. 2A, B), which indicated that both BM-MSCs and WJ-MSCs were not contaminated with cells of hematopoietic or endothelial origin. WJ-MSCs could be expanded for more than 20 passages while maintaining their undifferentiated state and their marker expression profiles.

Characterization of multi-lineage differentiation potential of BM-MSCs and WJ-MSCs

To determine the multi-lineage differentiation potential of BM-MSCs and WJ-MSCs, cells were cultured in appropriated lineage-specific induction media. After three weeks of adipogenic induction, WJ-MSCs became large cells containing numerous lipid droplets in their cytoplasm. Those lipid droplet-containing cells were positive for Oil-red O staining in a manner similar to that observed in BM-MSCs cultured under the same condition (Fig. 3). In addition, after 10 days of osteogenic induction, several WJ-MSCs had the appearance of refringent crystals in their cytoplasm. Similar to BM-MSCs culture under the same condition, those crystals-containing WJ-MSCs were positive for alkaline phosphatase staining, which indicate that they were osteoblasts (Fig. 4). The untreated control cultures growing in regular medium without adipogenic and osteogenic differentiation stimuli did not show the positive cells for Oil-red O staining (Fig. 3) and alkaline phosphatase activity, respectively (Fig. 4).

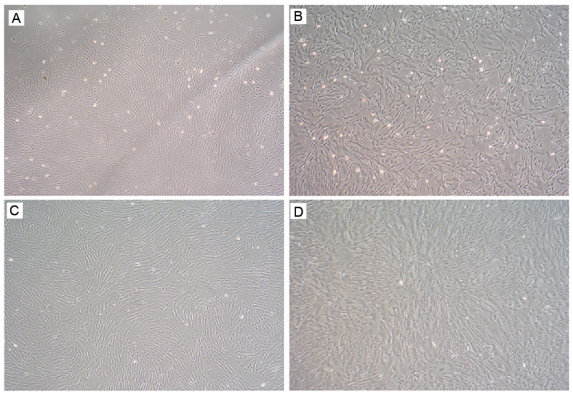

Fig. 1 Characterization of human umbilical cord Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs). WJ-MSCs at primary culture (A), passage 1 (B), passage 2 (C) and passage 3 (D) appeared as spindle-shaped cells. Magnification x4.

Pluripotent gene expression in BM-MSCs and WJ-MSCs

The expression of several pluripotent genes including *Oct4*, *Rex1*, *Sox2*, and *Nanog* in cultured BM-MSCs and WJ-MSCs was determined by RT-PCR. The results showed that the expression of *Oct4*, *Rex1*, *Sox2* and *Nanog* were detected in both BM-MSCs and WJ-MSCs at passage 2 and 3 (Fig. 5), while the expression level of *Oct4*, *Rex1* and *Nanog* in BM-MSCs and WJ-MSCs were similar.

Differentiation of MSCs after neural induction

Both WJ-MSCs and BM-MSCs used for neural differentiation were phenotypically homogeneous in terms of their morphology and marker expression before the time of neuronal induction. After culture in neural induction medium for 48 to 72 hours, MSCs from both sources underwent very rapid morphological changes: most cells retracted their cytoplasm, forming spherical cell body and exhibited several cellular protrusions which looked-like neural processes (Fig. 6). At the end of induction process, some cultured BM-MSCs and WJ-MSCs appeared as sharp, elongated bi- or tri-polar cells with primary and secondary processes (Fig. 6), highly expressed several neural markers such as MAP-2 (Fig. 7F, H), beta-tubulin III (Fig. 7J, L) and Tau (Fig. 7N, P). In contrast, MSCs from untreated group did not exhibit any morphologically reminiscent of neurons (Fig. 7A, C) and did not express any of the neuronal markers examined (Fig. 7E, G, I, K, M, O). In agreement with the immunofluorescent study, the expression level of *MAP-2*, *beta-tubulin III* and *GFAP* genes in both WJ-MSCs and BM-MSCs were up-regulated after

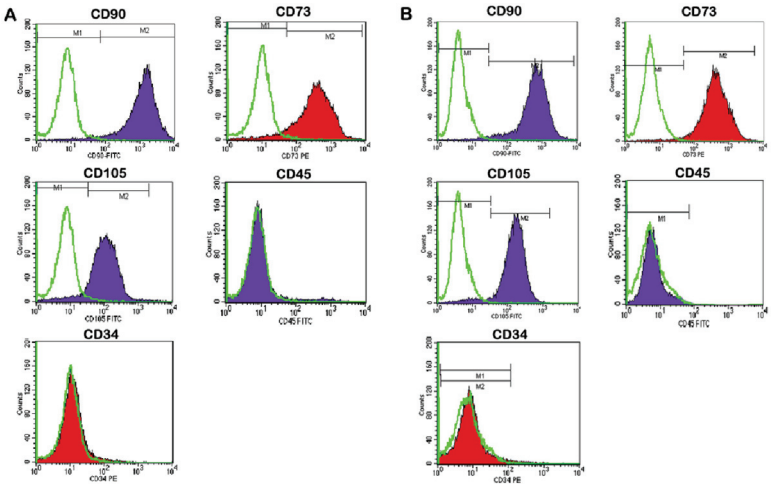

Fig. 2 Flow cytometric analysis of phenotype of human BM-MSCs (A) and WJ-MSCs (B). Representative histograms for CD90, CD73, CD105, CD45 and CD34 are presented. The empty profiles in the histogram indicate the background cell staining by isotypic control. These data shown are representative of those obtained in three different experiments.

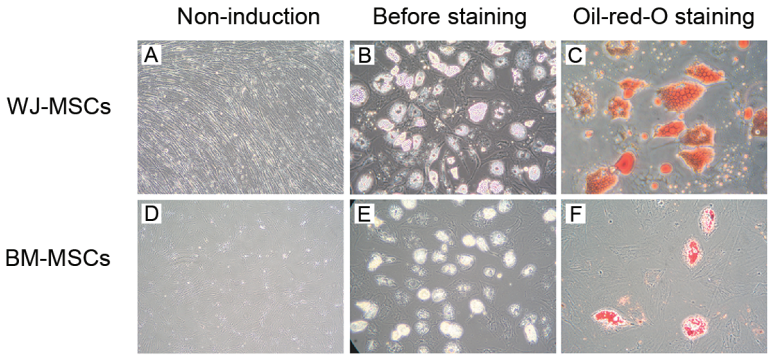

Fig. 3 Adepogenic differentiation of WJ-MSCs and BM-MSCs. Adipocytic vesicles (B and E) are stained with Oil-red O (C and F). No lipid droplet was observed in the cytoplasm of WJ-MSCs (A) and BM-MSCs (D) cultured in DMEM supplemented with 10% FBS. This is representative of three independent experiments.

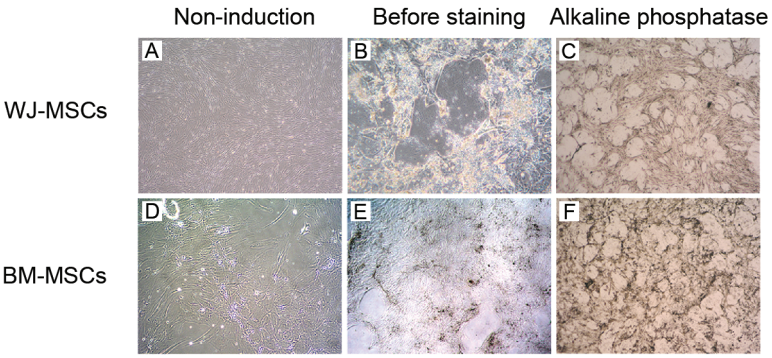

Fig. 4 Osteogenic differentiation of WJ-MSCs and BM-MSCs. After induction, both WJ-MSCs and BM-MSCs had the appearance of refringent crystals (B and E) and the positive for alkaline phosphatase activity (C and F). No alkaline phosphatase-positive aggregates was found in cytoplasm of WJ-MSCs (A) and BM-MSCs (D) cultured in DMEM supplemented with 10% FBS. This is representative of three independent experiments.

cultured in neural induction media for three days. The expression level of *MAP-2*, *beta-tubulin III*, and *GFAP* genes in BM-MSCs cultured in neural induction medium was 1.22, 1.68, and 3.27 fold higher than those of BM-MSCs cultured in control medium, respectively (Fig. 8). Similar to BM-MSCs, the expression level of *MAP-2*, *beta-tubulin III*, and *GFAP* genes in WJ-MSCs cultured in neural induction medium was 1.93, 2.50, and 3.74 fold higher than those of WJ-MSCs cultured in control medium, respectively (Fig. 8).

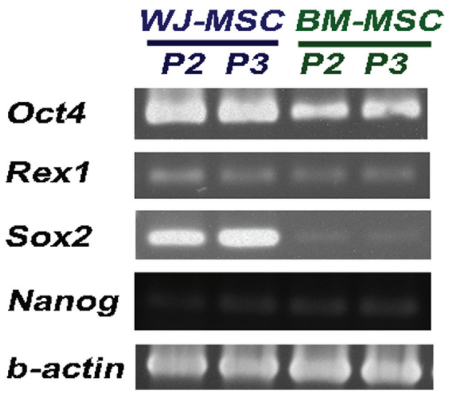

Fig. 5 Representative panel of reverse transcription PCR analysis of WJ-MSCs and BM-MSCs at passage 2 (P2) and passage 3 (P3). Cultured WJ-MSCs and BM-MSCs showed the expression of pluripotent genes; *Oct4*, *Rex1*, *Sox2* and *Nanog*.

Discussion

Although bone marrow has been the main source of MSCs, the use of BM-MSCs is limited because of a high degree of viral exposure, donor morbidity, and substantial decrease in cell number and proliferation/differentiation capacity with age. In addition, an invasive and painful procedure is used to obtain BM. Thus, investigators have searched for a good substitute for bone marrow as an MSC source. Recently, some studies[15,16] demonstrated that MSC from umbilical cord Wharton's jelly are a good substitute for bone marrow as an MSC source for cell-based therapy. As compared with bone marrow, MSC can be non-invasively obtained from Wharton's jelly after delivery.

In the present study, WJ-MSCs were successfully isolated and could be expanded efficiently for more than 20 passages while maintaining their undifferentiated state and their marker expression profiles, being positive for typical MSC markers CD90, CD73, CD105 and being negative for hematopoietic markers CD34 and CD45. This result is in agreement with the characteristics of Wharton's jelly-derived cells described previously[17]. To further characterize the isolated WJ-MSCs multi-lineage differentiation assays toward osteogenic and adipogenic-lineages were performed. Similar to BM-MSCs, WJ-MSCs were capable of differentiating toward adipogenic and osteogenic lineages.

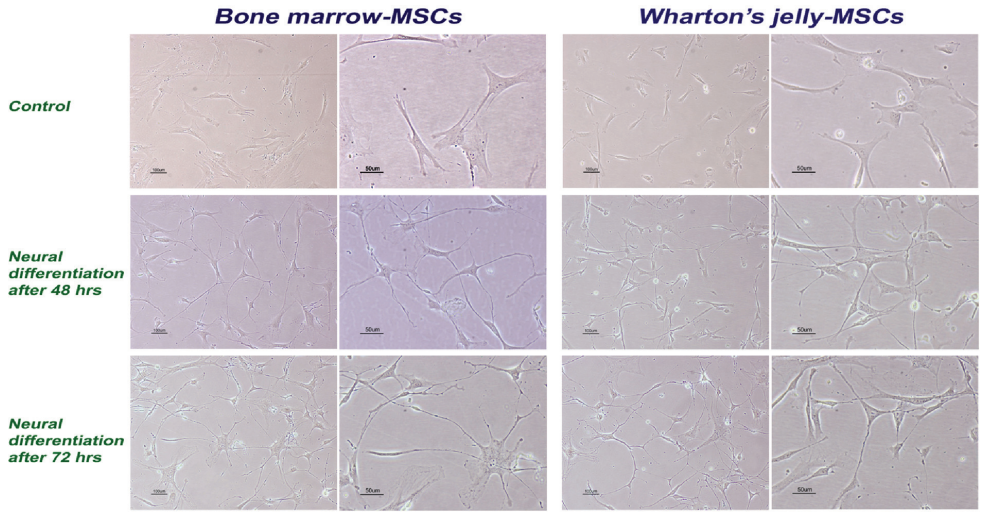

Fig. 6 Representative photomicrographs of neural differentiation of WJ-MSCs and BM-MSCs after 48 hours and 72 hours induction into neural differentiation. Spindle-shaped MSC from Wharton's jelly showed similar morphology to MSCs derived from bone marrow in basal conditions as non-induction control. Scale bar = 100 μm (left-handed panel) and 50 μm (right-handed panel).

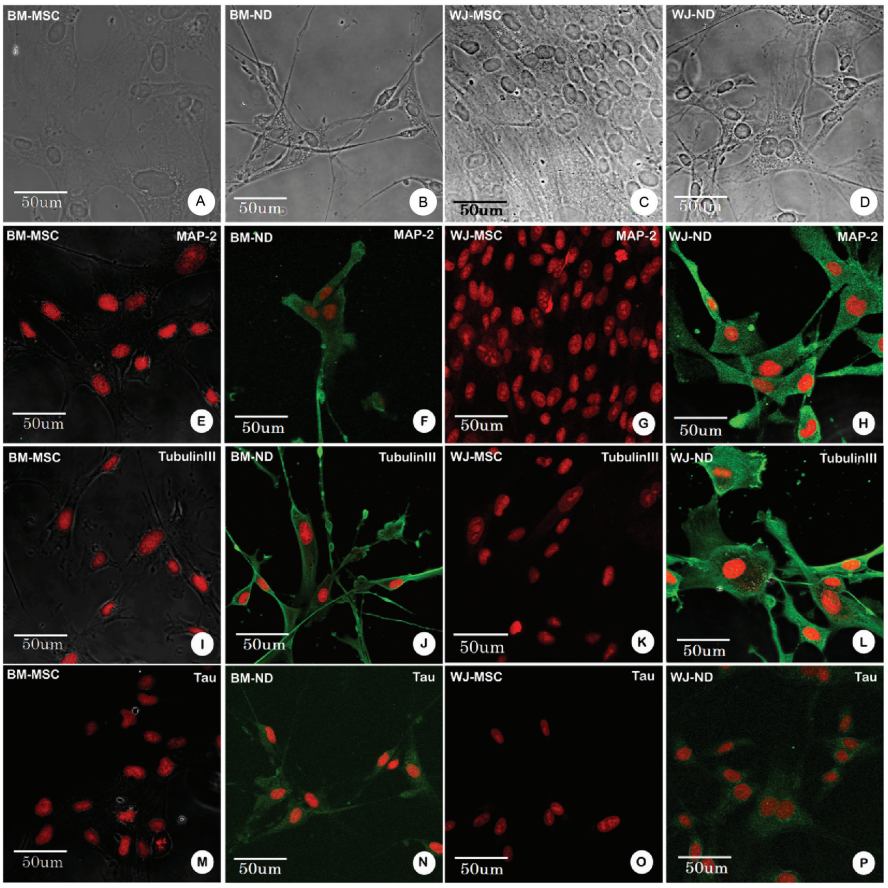

Fig. 7 Representative photomicrographs of neural differentiation of WJ-MSCs and BM-MSCs. Spindle-shaped MSCs from Wharton's jelly (C, G, K, O) showed similar morphology to MSCs derived from bone marrow (A, E, I, M) in basal conditions. Dramatic changes after the exposure to neural differentiation medium in terms of morphology and phenotype were observed on MSCs from Wharton's jelly (D, H, L, P) and bone marrow (B, F, J, N). Immunostaining for MAP-2 (E-H), beta-tubulin III (I-L) and Tau (M-P) on WJ-MSCs and BM-MSCs in neural differentiation medium showed positive staining whereas in basal medium reveals negative staining. Scale bar = 50 μm.

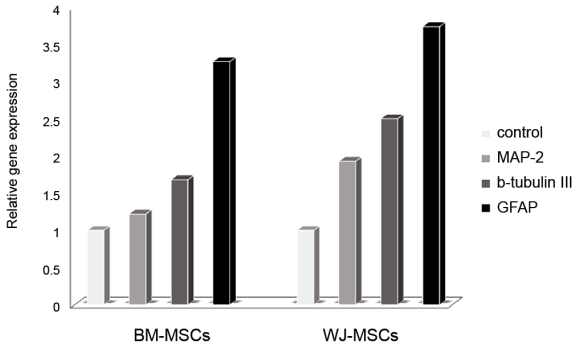

Fig. 8 Relative gene expression value of *MAP-2*, *beta-tubulin III* and *GFAP* in differentiated and undifferentiated BM-MSCs and WJ-MSCs at day 3 of induction by quantitative real-time PCR. BM-MSCs and WJ-MSCs cultured in basal condition served as a negative control.

Considering the easily obtained WJ-MSCs as an alternative source of stem cells for clinical applications, we studied the expression of pluripotent genes in WJ-MSCs and further investigated whether WJ-MSCs have the capacity to differentiate to extra-mesodermal cells, such as neural-lineages and compared the results with those of BM-MSCs. Pluripotent-associated transcription factors, such as Oct4, Sox2, Rex1, and Nanog have been demonstrated as critical regulators for establishing and maintaining pluripotency in embryonic stem cells. Oct4 is known to bind to a classical octamer sequence, ATGCAAAT, and in ES cells, it often binds in partnership with Sox2, which binds to a neighboring sox element[18]. Nanog, a homeodomain-containing protein, was identified as a factor that can sustain pluripotency of ES cells even

in the absence of leukemia inhibitory factor[5]. Furthermore, overexpression of *Nanog* is capable of maintaining the pluripotency and self-renewing characteristics of ES cells cultured in differentiation-inducing culture conditions[5]. During development, Nanog function is required at a later point than the initial requirement for Oct4, but both are required for the maintenance of pluripotency. In addition, *Rex1* gene is a developmentally regulated acidic zinc finger gene (Zfp-42) and a well-recognized marker for the pluripotent state of both ES cells and embryonic carcinoma cells[19]. Our work demonstrated the expression of *Oct4*, *Rex1*, *Sox2* and *Nanog* in both BM-MSCs and WJ-MSCs. The expression of these pluripotent-associated genes, which has rarely been reported in adult cells[20] in BM-MSCs and WJ-MSCs, might suggest that the differentiation potential of those cells might be higher than previously described.

However, the present study focused especially on the ability of BM-MSCs and WJ-MSCs to differentiate along ectodermal-derived neural lineages. Evidence of neural differentiation has been obtained by several groups studying BM-MSCs[21]. In addition, the potential to differentiate along neuronal lineages has also been demonstrated with MSCs from other sources such as adipose tissue[22], placenta[23], umbilical cord blood[24], and amnion[25]. In this study, we demonstrated that WJ-MSCs could be differentiated to neural-likes cells as demonstrated by the morphological characteristics, the up-regulation of neural-specific genes, and the expression of neural-specific proteins. Taken together, this study demonstrated that WJ-MSCs possessed the same differentiation properties to several mesodermal lineages as those of BM-MSCs. In addition, WJ-MSCs could also trans-differentiate to cells that exhibited the morphological characteristics and the marker expression profiles of neuron. The future studies will explore the differentiation capability of WJ-MSCs into other extra-mesodermal lineages such as endodermal derivatives.

Conclusion

In conclusion, the WJ-MSCs isolated in this study expressed several pluripotent genes and exhibited the classical stem-cell traits of long-term self-renewal, multi-lineage differentiation, as well as the trans-differentiation capacity toward neural-lineages. The present study suggests that umbilical cord Wharton's jelly is an alternative source of MSCs that could be easily obtained by non-invasive procedure and might be used as a substitute for BM-MSCs in several clinical applications.

Acknowledgements

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Potential conflicts of interest

None.

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การแสดงออกของจีนที่เกี่ยวข้องกับความสามารถในการเจริญพัฒนาไปเป็นเซลล์หลายชนิดในเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จาก Wharton's jelly ในสายสะดือ และศักยภาพในการเจริญพัฒนาเป็นเซลล์ประสาท

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วัตถุประสงค์: เพื่อตรวจสอบดูการแสดงออกของจีนที่เกี่ยวข้องกับความสามารถในการเจริญพัฒนาไปเป็นเซลล์หลายชนิด (pluripotent gene) ในเซลล์ต้นกำเนิดชนิดมีเซนไคม์ที่ได้จาก Wharton's jelly ภายในสายสะดือ (WJ-MSCs) และศักยภาพในการเจริญพัฒนาเป็นเซลล์ประสาท

วัสดุและวิธีการ: แยกสกัดเอาเนื้อเยื่อเกี่ยวพันที่มีลักษณะเป็นเจลภายในสายสะดือ นำไปย่อยด้วยเอนไซม์ทริปซิน และเลี้ยงในอาหารเลี้ยงเซลล์ชนิด Dulbecco's Modified Eagle's Medium (DMEM) จากนั้นตรวจสอบดูการแสดงออกของ MSC markers ด้วยวิธี flow cytometry และการแสดงออกของ pluripotent genes โดยวิธี reverse-transcription PCR รวมถึงความสามารถในการเจริญพัฒนาไปเป็นเซลล์ไขมัน เซลล์กระดูก และเซลล์ประสาทของเซลล์ MSCs ที่ได้จาก Wharton's jelly และไขกระดูก (BM-MSCs) ใน passage 3-5 ที่เลี้ยงในอาหารเลี้ยงเซลล์ชนิดที่จำเพาะ

ผลการศึกษา: เซลล์ WJ-MSCs สามารถเพาะเลี้ยงเพิ่มจำนวนได้ง่ายและมากถึง 20 passages ซึ่งยังคงสภาพความเป็นเซลล์ต้นกำเนิดไม่เปลี่ยนแปลง โดยมีการแสดงออกของ MSC markers (CD90, CD73 และ CD105) และไม่พบการแสดงออกของ hematopoietic markers (CD34 และ CD45) สิ่งที่น่าสนใจคือ WJ-MSCs และ BM-MSCs ที่เลี้ยงใน passage แรก ๆ มีการแสดงออกของ pluripotent genes ได้แก่ Oct4, Rex1, Sox2 และ Nanog เซลล์ WJ-MSCs นอกจากสามารถเจริญพัฒนาไปเป็นเซลล์ที่พัฒนามาจากเนื้อเยื่อชั้นกลางได้แก่ เซลล์กระดูกและเซลล์ไขมันได้เช่นเดียวกับ BM-MSCs แล้วยังสามารถถูกเหนี่ยวนำให้เจริญพัฒนาไปเป็นเซลล์ที่มีลักษณะคล้ายเซลล์ประสาททั้งทางด้านรูปร่างและการแสดงออกของ neural markers คือ MAP-2, GFAP, beta-tubulin III และ Tau อีกด้วย

สรุป: จากการศึกษาครั้งนี้แสดงให้เห็นว่า WJ-MSCs สามารถเก็บและเพาะเลี้ยงเพิ่มจำนวนได้ง่ายและจำนวนมากครั้ง โดยยังคงมีคุณสมบัติความเป็น MSCs นอกจากนี้ BM-MSCs และ WJ-MSCs มีการแสดงออกของ pluripotent genes หลายชนิด และแสดงให้เห็นถึงความสามารถที่ถูกเหนี่ยวนำให้เจริญพัฒนาไปเป็นเซลล์ประสาทได้ ดังนั้น WJ-MSC จึงอาจเป็นแหล่งของเซลล์ทางเลือกหนึ่งที่มีศักยภาพใช้ในการรักษาโรคต่าง ๆ ที่มีความผิดปกติในระบบประสาทในอนาคต