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Comparison of the immunosuppressive capacity of mesenchymal stem cells derived from amniotic fluid, amniotic membrane and Wharton's jelly

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Abstract

Mesenchymal stem cells (MSCs) have promise for clinical therapy due to their differentiation characteristic, in vitro propagation ability and also low immunogenic property. MSCs have low expression of major histocompatability complex (MHC) class I and lack of MHC class II. However, MSCs derived from different sources can exhibit differential immunosuppressive capacity. In this study, we investigated the immunosuppressive capacity of MSCs derived from 3 different sources, including human amniotic fluid (AF), amniotic membrane (AM) and Wharton's jelly (WJ) by assessing the effect on phytohemagglutinin (PHA)-induced peripheral blood mononuclear cell (PBMC) proliferation. MSCs derived from AF (n = 6), AM (n = 10) and WJ (n = 10) were inactivated with mitomycin-C and co-cultured with PHA-induced PBMC in 1:10 ration for 72 hr. The MTT assay was used to calculate the amount of PBMC proliferation, demonstrating immunosuppressive activity of MSCs. The co-cultured of these MSCs and PHA-induced PBMC was analyzed and showed into the percentage of the inhibition rate. After 72 hr of co-culture, AF-MSCs, AM-MSCs and WJ-MSCs suppressed PHA-induced PBMC proliferation. WJ-MSCs showed the highest of the percentage of inhibition rate at the 124.06% ± 24.76%, whereas AF-MSCs showed at the 86.53% ± 8.81% (P value = 0.0033) and AM-MSCs showed 102.69% ± 19.2% (P value = 0.0448). Our result showed that WJ-MSCs had the stronger suppressive effect on PBMC among the 3 populations of MSCs in the co-culture experiments.

Keywords: amniotic fluid, amniotic membrane, immunosuppressive, peripheral blood mononuclear cell, Wharton's jelly

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1. Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells that have been isolated from both adult and fetal tissues. These cells is maintenance of the undifferentiation state for long term culture and can differentiate into various cell lineages when culture under suitable environment [1-5]. For immunosuppressive capacity, MSCs show low expression of major histocompatability complex (MHC) class I and lack of MHC class II [6]. Moreover, MSC suppress proliferation of T cells in co-culture with allogenic peripheral blood mononuclear cells [4, 7-9]. The correlation between MSCs and T cell proliferation is the main interest in regulation of the transplantation rejection [10].

Recently, several studies have demonstrated differential immunosuppressive properties in the different sources of MSCs. MSCs derived from fetal tissues, such as amniotic fluid (AF), Wharton's jelly (WJ) and placenta, had the higher suppressive effect on T cells proliferation than adult tissues, such as bone marrow and adipose tissue [7, 8]. Among all MSCs derived from fetal tissues has been also shown different immune properties [7-9]. The variation of MSCs immunosuppressive properties may occur in culture conditions, environment, human and especially different sources of MSCs. Therefore, the investigation of the immunosuppressive properties of MSCs derived from various sources need to analyze before their clinical use.

In this study, we investigated the immunosuppressive capacity of MSCs derived from 3 different sources, including human amniotic fluid (AF), amniotic membrane (AM) and Wharton's jelly (WJ) by assessing the effect on phytohemagglutinin (PHA)-induced peripheral blood mononuclear cell (PBMC) proliferation. The MTT was used to calculate the amount of PBMC as demonstrating immunosuppressive activity of MSCs. Mesenchymal stem cells (MSCs) are multipotent stem cells that have been isolated from both adult and fetal tissues. These cells is maintenance of the undifferentiation state for long term culture and can differentiate into various cell lineages when culture under suitable environment [1-5]. For immunosuppressive capacity, MSCs show low expression of major histocompatability complex (MHC) class I and lack of MHC class II [6]. Moreover, MSC suppress proliferation of T cells in co-culture with allogenic peripheral blood mononuclear cells [4, 7-9].

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2. Materials and methods

2.1 Samples

The MSCs derived from 3 different sources, including AF (n=6), AM (n=10) and WJ (n=10), were provided from Stem Cell Research and Development Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. These cells were expanded in culture medium containing α-minimum essential medium (α-MEM, Gibco, Invitrogen, CA) supplemented with 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin and streptomycin (Gibco). Cultures were incubated under humidified 5% CO_2 at 37˚C.

2.2 Phenotyping Analysis

The AF-MSCs, AM-MSCs and WJ-MSCs at passage 3-5 were characterized for phenotypic characteristics using flow cytometry. The cells were harvested and washed in PBS (pH 7.4) and stained with the monoclonal antibodies; including CD29, CD73, CD90 and CD105 (eBioscience, Sadiego, CA) and CD34, CD45 (Becton Dickinson, Franklin lakes, NJ). After 15 min incubation in the dark at room temperature, the cells were washed with PBS and fixed in 1% paraformaldehyde. Analyzed data were acquired and measured with a CELL Quest software in a FACSCalibur flow cytometer (Beckton Dickinson).

2.3 Differentiation Potential

To investigate the in vitro differentiation potential, AF-MSCs, AM-MSCs and WJ-MSCs at passage 5 in 35 mm tissue culture dishes were cultured in induction media for adipogenic and osteogenic differentiation. For adipogenic differentiation, the cells (50% confluency) were incubated under adipogenic induction medium containing α-MEM (Gibco), 10% FBS (Gibco), 1 µmol/L dexamethasone, 5 µg/ml insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine and 60 µmol/L indomethacin (Sigma-Aldrich, MO) for 3 weeks. The cultures were incubated at humidified 5% CO_2 at 37˚C and medium was changed twice a week. After 3 weeks, adipocyte-like cells were fixed with 10% formalin and stained with Oil red O working solution to test for lipid reactivity. The Oil red O working solution was prepared by dissolving 0.35% w/v Oil red O (sigma) in isopropanol and diluted in distilled water (6/4 ratio) followed by passed through a 0.22 µm filter. Oil red O staining was detected through the use of light microscopy. For osteogenic differentiation, the cells (50-70% confluency) were incubated under osteogenic induction medium containing α-MEM (Gibco), 10% FBS (Gibco), 0.1 µmol/L dexamethasone, 10 mmol/L glycerol-2-phosphate and 50 µmol/L ascorbic acid (Sigma-Aldrich) for 4 weeks. The cultures were incubated at humidified 5% CO_2 at 37˚C and medium was changed twice a week. After 4 weeks, osteoblast-like cells were fixed with 10% neutral buffered formalin including 4 g/L NaH_2PO_4, 6.5 g/L Na_2HPO_4 (Sigma), 100 ml formaldehyde 37-40% (w/v) and 900 ml distilled water. The examination was identified by alkaline phosphatase activity staining.

2.4 Gene expression

The AF-MSCs, AM-MSCs and WJ-MSCs at passage 5 were extracted for total RNA using Trizol reagent. The RNA was reverse transcribed to complementary DNA (cDNA) with the use of a RevertAid First-Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). The cDNA was amplified using the selective primers as follow:

HLA-ABC (394 bp) Forward: 5'-GTATTTCTTCA CATCCGTGTCCCG-3' and Reverse: 5'-GTCCG-CCGCGGTCCAAGAGCGCAG-3'

HLA-DR (220 bp) Forward: 5'-CTGATGAGCGC TCAGGAATCATGG-3' and Reverse: 5'-GACTT-ACTTCAGTTTGTGGTGAGGGAAG-3'

β-actin (107 bp) Forward: 5'-ATGTGGCCGAGGAC TTTGATT-3' and Reverse: 5'-AGTGGGGTGG-CTTTTAGGATG-3'

cDNA amplification was performed using the following PCR conditions: initial denaturation at 95˚C for 10 min and 35 cycles of DNA denaturing at 95˚C for 30 sec, annealing for 45 sec, extension at 72˚C for 1 min and final extension at 72˚C for 10 min. The annealing temperatures used were 53˚C for HLA-ABC and HLA-DR and 57˚C for β-actin.

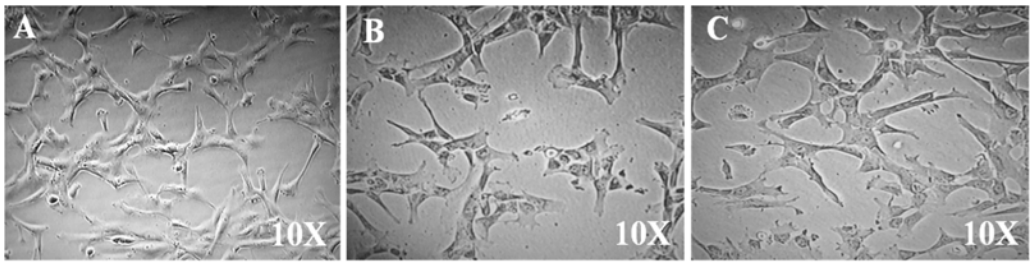

Figure 1 Morphological appearance of AF-MSCs (A), AM-MSCs (B) and WJ-MSCs (C) are showed at passage 4 with 10x magnifications. All types of MSCs presented fibroblastoid-type morphology.

Table 1 Comparison of phenotypic characteristics of AF-MSCs, AM-MSCs and WJ-MSCs

Markers	AF-MSCs	AM-MSCs	WJ-MSCs
CD29	92.88 ± 8.75	97.53 ± 5.21	98.30 ± 1.48
CD34	0.38 ± 0.60	0.43 ± 0.64	0.30 ± 0.35
CD45	0.81 ± 0.78	0.26 ± 0.17	0.31 ± 0.10
CD73	97.16 ± 3.75	98.94 ± 1.52	97.56 ± 4.12
CD90	80.70 ± 17.23	98.39 ± 2.94	99.68 ± 0.40
CD105	84.31 ± 13.40	99.89 ± 0.10	99.93 ± 0.08

2.5 Preparation of PBMC

Blood of healthy donor was collected in heparin tubes and diluted in 1:1 ratio with phosphate buffered saline (PBS). PBMC were isolated from diluted blood by Ficoll density-gradient centrifugation according to the manufacture's instruction. Cell viability was determined by trypan blue staining and cells were suspended in the culture medium. $1x10^6$ cells of PBMC were induced with PHA (5 μg/ml) (Roche, Indianapolis, IN) before co-culture with MSCs.

$$\%\text{inhibitory} = 1 - \frac{(\text{OD of A} - \text{OD of B})}{\text{OD of C}} \times 100$$

When A means the inactivated MSCs co-cultured with PHA-induced PBMC, B means the inactivated MSCs, and C means the PHA-induced PBMC.

2.7 Statistics Analysis

The Student's *t* test was used to analyze the inhibition rate of immunosuppressive potential of AF-MSCs, AM-MSCs and WJ-MSCs. Data were analyzed with the use of GraphPad Prism 5 software (GraphPad, San Diego, CA). A value of $P < 0.05$ was considered significant.

3. Results and discussion

The MSCs derived from 3 different sources (AF, AM and WJ) were obtained from Stem Cell Research and Development Unit, Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. The morphology of AF-MSCs, AM-MSCs and WJ-MSCs showed the fibroblast-like cells (Figure 1).

To investigate phenotypic characteristics, AF-MSCs, AM-MSCs and WJ-MSCs were stained with cluster of difference (CD) marker and analyzed by flow cytometer. These cells expressed positive signals of MSC markers, such as CD29, CD73, CD90 and CD105, whereas no signal was displayed for the CD34 and CD45 (Table 1).

For differentiation potential of AF-MSCs, AM-MSCs and WJ-MSCs, we found that these cells exhibited the ability to differentiate into adipocytes using Oil red O staining. They showed red color on endogenous lipid droplet. For osteogenic differentiation, AF-MSCs, AM-MSCs and WJ-MSCs displayed the dark brown color of alkaline phosphatase activity (Figure 2).

To determine immunological major histocompatibility complex (MHC) phenotypes, the AF-MSCs, AM-MSCs and WJ-MSCs at passage 5 were analyzed for *HLA-ABC* gene (MHC class I) and *HLA-DR* gene (MHC class II) with the use of RT-PCR analysis. We found that all MSC types showed positive expression of *HLA-ABC* and negative expression of *HLA-DR* (Figure 2).

For immunosuppressive properties, MSCs were inactivated with mitomycin-C and co-cultured with PHA-induced PBMC in 1:10 ratio for 72 hr. The effect of MSCs was investigated on PBMC proliferation using MTT assay and showed into inhibition rate (%). AF-MSCs, AM-MSCs and WJ-MSCs suppressed the

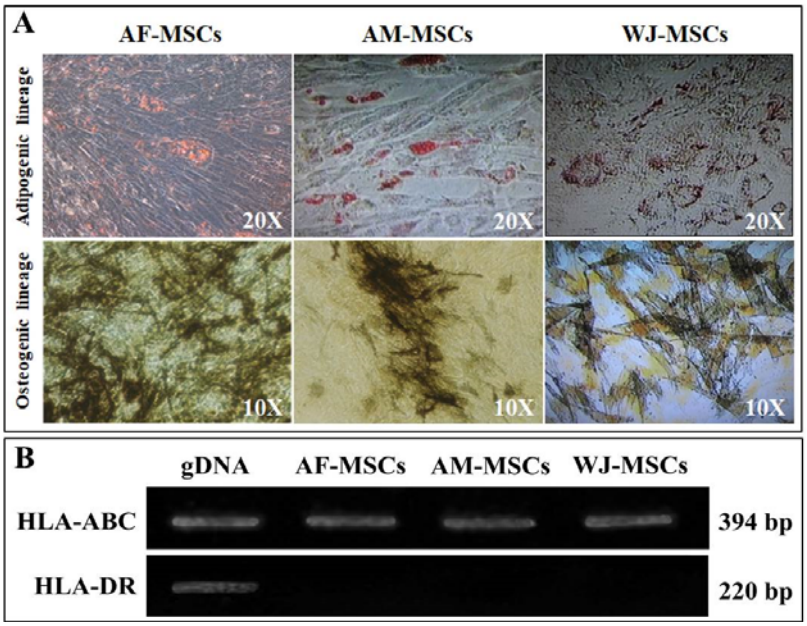

Figure 2 Differentiation ability and gene expression of AF-MSCs, AM-MSCs and WJ-MSCs. All types of MSCs showed to differentiate into adipocytes (red color) and osteoblasts (dark brown color) after culture under induction media (A). Gene expression analysis was performed with RT-PCR. AF-MSCs, AM-MSCs and WJ-MSCs showed expression of *HLA-ABC* but not *HLA-DR* (B).

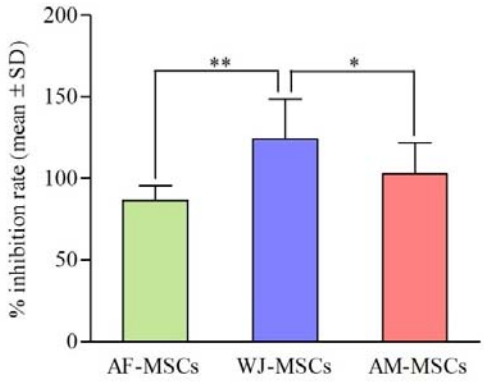

Figure 3 The immunosuppressive properties of AF-MSCs, AM-MSCs and WJ-MSCs after co-culture with PHA-induced PBMC for 72 hr using MTT assay. The data was showed the percentage of inhibition rate (mean ± SD, * *P* value < 0.05 and ** *P* value < 0.01).

proliferation of PHA-induced PBMC. WJ-MSCs showed the highest of the percentage of inhibition rate at the 124.06% ± 24.76%, whereas AF-MSCs showed at the 86.53% ± 8.81% (*P* value = 0.0033) and AM-MSCs showed 102.69% ± 19.2% (*P* value = 0.0448) (Figure 3).

MSCs derived from different fetal-stage tissues and fetal sources had affected immunosuppressive capacity [7-9, 11, 12]. Di Trapani M and *et al*. [12] demonstrated that first trimester of amniotic fluid stem (AFS) cells significantly inhibited T and NK cell proliferation, whereas second and third trimester of AFS cells were less efficient. Our work provided AF-MSCs from second trimester that showed lower inhibition rate than AM-MSCs and WJ-MSCs from full-term pregnancy. WJ-MSCs displayed the strongest immunomodulatory and immunosuppressive potential than placenta (PL)-MSCs [7]. Contrary to Talwadekar MD and *et al*. [9], PL-MSCs showed a higher immunosuppressive ability

than WJ-MSCs. Moreover, AF-MSCs from second trimester had slightly higher immunomodulatory effect on T cells than placenta MSCs [8]. Our study found that MSCs derived from various fetal-stage tissues and fetal sources had comparable characteristics but variation of immunosuppressive properties. The consideration of MSC sources should investigate the immune properties before their clinical use.

4. Conclusions

In this study, we found that MSCs derived from different sources showed differential immunosuppressive capacity. WJ-MSCs had the highest suppressive effect on PBMC proliferation among the 3 populations of MSCs in the co-culture experiments. Therefore, WJ-MSCs may be the best source of MSCs for use in regenerative medicine.

Acknowledgements

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