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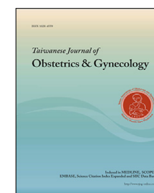

Original Article

A genome-wide comparison of mesenchymal stem cells derived from human placenta and umbilical cord

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ABSTRACT

Objective: The human umbilical cord and placenta have been considered as attractive alternative sources for noninvasive isolation of human mesenchymal stem cells (hMSCs). Different sources of MSC may have individual differentiation potential and phenotype. In this study, we compared the genome-wide expression data of umbilical cord and placenta derived hMSCs to identify specific differential expression genes (DEGs) and corresponding functions.
Materials and methods: We collected human placental tissues and umbilical cord from healthy full-term placenta (n = 17). The genome-wide gene expression data of hMSCs were used to analyze and compare with that of fibroblasts. We identified the differential expression genes (DEGs) based on the Student's t-test and one-way ANOVA.
Results: According to the DEGs of umbilical cord and placenta, we used the Venn diagram to evaluate the consistence and specific genes. There are 390 umbilical cord specific DEGs which functions are related to movement of sub-cellular component. Then, the DEGs derived from placenta have two major clusters (i.e., placenta-specific (AM-CM-specific) and UC-like (UC-CD-specific)). 247 placenta-specific DEGs are down-regulated and involved in cell communication. 278 UC-like genes are up-regulated and are involved in the cell cycle, cell division, and DNA repair process. Finally, we also identified 239 umbilical cord-placenta consistence DEGs. According to the umbilical cord-placenta consistence DEGs, 175 genes are down-regulated and involved in cell death, cell growth, cell developmental processes.
Conclusion: We identified the consistence and specific DEGs of human placenta and umbilical cord based on the genome-wide comparison. Our results indicated that hMSCs derived from umbilical cord and placenta have different gene expression patterns, and most of specific genes are involved in the cell cycle, cell division, cell death, and cell developmental processes.
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Introduction

Mesenchymal stem or stromal cells (MSCs), which have a fibroblast-like morphology, are self-renewing multipotent cells holding great potential in regenerative medicine and tissue engineering. Strong immunomodulatory capacity and extensive differentiation ability, several studies have shown that treatment with MSC is beneficial to patients suffering from a plethora of diseases (e.g., autoimmune diseases [1,2] and cardiovascular [3]). Depending on the stimuli and culture conditions, MSC can be differentiated *in vitro* into cells of the mesodermal lineage such as osteocytes, adipocytes and chondrocytes [4,5]. The human MSC (hMSC) have been isolated from a variety of tissues such as bone marrow, adipose tissue, umbilical cord, placenta, with each population displaying individual differentiation potential and phenotype [6–8]. However, previous studies have indicated that adult tissue derived MSCs (e.g., bone marrow and adipose tissue) would gradually lose proliferation, differentiation, and immunomodulation potential during *in vitro* expansion [9,10]. The human umbilical cord and placenta, which are normally discarded after labor, have been considered as attractive alternative sources for noninvasive isolation of hMSCs. In addition, the efficient isolation of hMSCs from

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umbilical cord or placenta have been reported by previous studies [11,12]. Therefore, genome-wide evaluation of umbilical cord and placenta derived hMSCs would provide an opportunity for understanding the individual phenotypes and characteristics of these hMSCs.

Isolation of MSCs from placenta-derived tissues is complicated by the fact that non-MSCs, such as fibroblasts, placenta-derived epithelial cells, and placenta-derived reticular cells, often coexist with MSCs [12]. More important, Fibroblasts, in particular, are usually the main source of contamination. Fibroblasts are considered mature mesenchymal cells that are particularly abundant in the connective tissue. Fibroblasts and MSCs have a similar morphological appearance; they both proliferate well and have many identical cell-surface markers [13,14]. The major difference between MSCs and fibroblasts are functional properties. MSCs retain multipotent stemness and immunomodulation capacity, but fibroblasts seem more limited in both of these functional areas. Therefore, the genome-wide evaluation of umbilical cord and placenta derived hMSCs should consider the fibroblasts as the negative control to identify the MSC-specific genes.

To address issue, we collected genome-wide gene expression data to evaluate the specific genes of hMSCs derived from umbilical cord (UC) and placenta (i.e., amniotic membrane (AM), chorionic membrane (CM), chorionic disk (CD)). Firstly, we used hierarchical clustering and principal component analysis (PCA) to compare the genome-wide expression patterns of four hMSCs. We found that each hMSC can be clustered into a specific sub-cluster. The hMSC derived from umbilical cord has the different expression pattern to that derived from placenta. The amnion and chorion membranes are the neighbors on the anatomical structure of placenta, and MSCs derived from two membranes share some expression patterns. Then, we identified the differential expression genes (DEGs) based on the Student's t-test and one-way ANOVA. We used the Venn diagram to evaluate DEGs and found four regions (i.e., UC-specific, AM-CM-specific (i.e., placenta-specific), UC-CD-specific (i.e., UC-like), UC-placenta consistence) having more than 200 genes. We evaluated the significant pathways and cellular components of these genes based on Gene Ontology and KEGG annotations. We found that UC-CD-specific genes are involved in the cell cycle, cell division, and DNA repair process and located on the intracellular. The placenta-specific genes are involved in movement of cell or sub-cellular component, cell communication, and cell projection organization. Then, all-MSC-specific genes are involved in cell death, cell growth, cell developmental processes.

Finally, we used the KEGG pathway and protein–protein interaction (PPI) network to study the UC-like genes. Four genes (i.e., MCM7, ORC1, CDC45, and DBF4) are the components of origin recognition complex (ORC) and minichromosome maintenance (MCM) complex which play important roles during the initiation step of DNA replication. According the PPI network of UC-CD-specific genes, several histone proteins (e.g., HIST1H4A, HIST1H3A, and HIST1H2AC) are the hubs of network and play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. Our study provides the genome-wide comparison of mesenchymal stem cells derived from human placenta and umbilical cord. We believe that the identified specific DEGs are able to provide clues to study the biomarkers and their corresponding functions.

Materials and methods

Overview

In this study, we were provided the genome-wide comparison of placenta-derived MSCs, which were isolated from amniotic membrane (AM-MSC), chorionic membrane (CM-MSC), chorionic disk (CD-MSC), and Wharton's jelly within the umbilical cord (UC-MSC). All MSCs derived from different origins have been amplified, collected (5×10^6–10^7 cells/experiment), and submitted to Welgene Biotech company (http://www.welgene.com.tw/) for microarray. We used the mRNA expression level derived from fibroblasts as the negative control. The differential expression genes (DEGs) of four MSCs were identified by using following criteria: 1) adjusted p-values derived from *t*-test and Bonferroni correction are less than 0.01; 2) fold-changes are greater than 2; 3) adjusted p-values derived from one-way ANOVA are less than 0.01. Then, we compared the DEGs and identified the source-specific genes. According to the source-specific genes, we inferred the significant pathways and cellular components based on Gene Ontology [15] and KEGG [16] annotations. We also described the protein–protein interaction networks of the source-specific genes.

Isolation of hMSCs from placenta and umbilical cord

Placental tissues and umbilical cord were collected from healthy full-term samples (n = 17, Table 1). Written informed consent was obtained from individual mothers before the study, which was approved by the Ethics Committee of the Cardinal Tien Hospital. The age range of the maternal donors was 20–45 years old. The placentas and umbilical cords were kept at 4 °C until placement in a biological safety cabinet. According to our previous study [12], we have constructed a protocol for collecting the hMSC from placentas and umbilical cords. The placental tissues and umbilical cord were cut into small pieces (i.e., 1–2 mm^3) and digested with 10 U/mL collagenase, 2.5 U/mL dispase, and 0.05% Trypsin-EDTA for 90 min at 37 °C. All samples were thoroughly washed three times with sterile phosphate-buffered saline (PBS). Then, samples were collected in 15 mL tubes and centrifuged at 250 g for 5 min. The cell pellet fraction was re-suspended in α minimal essential medium (αMEM; Invitrogen, Waltham, NA, USA) with 10–15% fetal bovine serum (FBS; Invitrogen), 2 mM L-glutamine, 1 ng/mL basic fibroblast growth factor (FGF; Peprotech, Rocky Hill, NJ, USA), and PSF (100 U/mL penicillin, 100 mg/mL streptomycin, 0.25 mg/mL Fungizone; Invitrogen), then plated in T75 flasks. Cultures were washed from three to five times with PBS after 7 days to remove non-adherent cells from plastic-adherent colonies. The culture was maintained in αMEM supplemented with 10–15% FBS, 2 mM L-glutamine, 1 ng/mL basic FGF, and PSF at 37 °C with saturated humidity and 5% CO2. Finally, the hMSCs were maintained in αMEM supplemented with 10% FBS, 2 mM L-glutamine, 1 ng/mL basic FGF at 37 °C in saturating humidity and 5% CO2.

Genome-wide expression data

The genome-wide expression data of four MSCs and fibroblast (i.e. negative control) were provided by Welgene Biotech company (http://www.welgene.com.tw/). The MSCs have been amplified, collected (5×10^6~10^7 cells/experiment), and submitted to Welgene Biotech company for microarray.

Protein–protein interaction network

To further investigate the source-specific genes, we constructed the protein–protein interaction (PPI) network of *Homo sapiens* to study involving pathways of source-specific genes. Here, the PPI network of *H. sapiens* were constructed by annotated and predicted PPIs. The annotated PPIs are collected from five public protein–-protein interaction databases (i.e. Intact [17], BioGRID [18], MINT [19], MIPS [20], and DIP [21]). The predicted PPIs are identified base on published methods (i.e., PPISerach [22] and 3D-interologs

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