Taiwanese Journal of Obstetrics & Gynecology 56 (2017) 664–671

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

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

Sen-Wen Teng [a, b], Yu-Shu Lo [c], Wei-Ting Liu [d], Yogi Hsuan [d], Willie Lin [d, *]

[a] Department of Obstetrics and Gynecology, Cardinal Tien Hospital-Hsintien, New Taipei City, Taiwan
[b] Department of Obstetrics and Gynecology, Fu Jen Catholic University, New Taipei City, Taiwan
[c] Research and Development Division, Meribank Biotech Co. Ltd., Taipei, Taiwan
[d] Meridigen Biotech Co. Ltd., Taipei, Taiwan

ARTICLE INFO

Article history:
Accepted 13 June 2017

Keywords:
Mesenchymal stem cells
Gene expression profiling
Gene expression pattern analysis
Protein interaction networks

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.

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

* Corresponding author. 7F., No.68, Sec. 1, Neihu Rd., Neihu Dist., Taipei City 11493, Taiwan. Fax: +886 2 2627 5275.
E-mail address: willie.lin@meridigen.com (W. Lin).

http://dx.doi.org/10.1016/j.tjog.2017.08.016

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

Table 1
17 normal healthy donors.

ID	Baby's gender	Umbilical cord		Placenta	
		Gene expression data	CD markers	Gene expression data	CD markers
Donor-01	F	Y	Pass	Y	Pass
Donor-02	M	Y	Pass	Y	Pass
Donor-03	M	Y	Pass	Y	Pass
Donor-04	F	Y	Pass	Y	Pass
Donor-05	F	Y	Pass	Y	Pass
Donor-06	M	Y	Pass	Y	Pass
Donor-07	M	N	N/A	Y	Pass
Donor-08	F	N	N/A	Y	Pass
Donor-09	M	N	N/A	Y	Pass
Donor-10	M	Y	Pass	Y	Pass
Donor-11	F	N	N/A	Y	Pass
Donor-12	M	Y	Pass	Y	Pass
Donor-13	M	Y	Pass	N	N/A
Donor-14	F	Y	Pass	Y	Pass
Donor-15	F	Y	Pass	Y	Pass
Donor-16	F	Y	Pass	Y	Pass
Donor-17	F	Y	Pass	Y	Pass

[23,24]). Finally, the constructed PPI network of *H. sapiens* have 16,596 proteins and 267,326 PPIs.

Result and discussion

Clustering the genome-wide gene expression data derived from four MSCs and fibroblast

As a first step in the genome-wide comparison of MSCs derived from placenta and Wharton's jelly within the umbilical cord (UC-MSC), we used two clustering method to evaluate the similarities of expression patterns within different MSCs. Here, the placenta-derived MSCs were isolated from amniotic membrane (AM-MSC), chorionic membrane (CM-MSC), chorionic disk (CD-MSC). In this study, we collected these MSCs from 17 different donors which are approval by the Ethics Committee of the Cardinal Tien Hospital as IRB No.: CTH-102-2-4-003. We also collected 6 samples of UC-MSC which are derived from public resource (i.e., Bioresource Collection and Research Centre). All of the MSCs were expanded to passages P3 by using the sample culture condition (i.e., using αMEM, 4 ng/ml bFGF, 10% FBS, 5% CO_2, and 20% O_2 in 37 °C). Table 2 summaries the number of analyzed MSC samples in genome-wide expression data.

Fig. 1A and B illustrate the hierarchical clustering and principal component analysis (PCA) results of the genome-wide gene expression data derived from four MSCs and fibroblast. We found that the genome-wide expression pattern of fibroblast, negative controls, have an obvious difference the all MSCs derived from four sources. Then, each MSC can be clustered into a specific sub-cluster by using the hierarchical clustering and PCA. We also found that AM-MSC and CM-MSC can be clustered into a larger sub-clusters based on both hierarchical clustering and PCA (Fig. 1). Because the amnion and chorion membranes are the neighbors on the anatomical structure of placenta, the MSCs derived from these two membranes may share some expression patterns. The CD-MSC derived from chorionic disk is located between membranes (i.e., amnion and chorion membranes) and umbilical cord. According to the result of hierarchical clustering, the expression pattern of CD-MSC is more similar to thus of UC-MSC than thus of AM-MSC and CM-MSC (Fig. 1A). Then, the components of CD-MSC derived from PCA are located between thus of AM-MSC and CM-MSC and thus of UC-MSC (Fig. 1B). Finally, the UC-MSC is the only MSC which is not derived from placenta and have the different expression pattern to that derived from amnion membrane and chorion membrane. These results implied that the MSCs derived from different sources would have their specific expression patterns. Then, the expression patterns of MSCs would be related to the relationship on the anatomical structure.

Differential expression genes and involved functions

To further investigate the specific expression patterns of four different MSCs, we identified the DEGs of MSC by using fibroblast samples as negative controls. Here, the DEGs of MSCs are identified by using following criteria: 1) adjusted p-values derived from student *t*-test and Bonferroni adjustment are less than 0.01; 2) fold-changes between MSCs and fibroblast are greater than 2; 3) adjusted p-values derived from one-way ANOVA are less than 0.01. Finally, we identified 833, 1,067, 953, and 1202 DEGs of AM-MSC, CM-MSC, CD-MSC, and UC-MSC, respectively (Fig. 2A). Then, we used the Venn diagram to evaluate these DEGs (Fig. 2B). We found that there are four regions on the Venn diagram having more than 200 genes (>10% in Fig. 2B). The UC-MSC specific region (named UC-specific genes) has most DEGs (390 genes). AM-MSC and CM-MSC share 324 common genes (named AM-CM-specific genes) which are not the DEGs of UC-MSC and CD-MSC. These AM-CM-specific genes can be considered as the placenta-specific genes. Otherwise, UC-MSC and CD-MSC share 333 common genes (named UC-CD-specific genes) which are not the DEGs of AM-MSC and CM-MSC. Here, we also defined the UC-CD-specific genes as the UC-like genes. Finally, there are 239 common genes which are the umbilical cord-placenta consistence DEGs. These results implied that the AM-MSC and CM-MSC share more similar expression pattern with each other than thus of UC-MSC and CD-MSC. Then, UC-MSC is different to other MSCs derived from placenta. The DEGs of CD-MSC are more

Table 2
Number of analyzed MSC samples in the genome-wide expression data.

Source	Amnion membrane (AM-MSC)	Chorion membrane (CM-MSC)	Chorion plate (CD-MSC)	Umbilical cord (UC-MSC)	Fibroblast (FB)
No. of samples	5	5	14	23	12

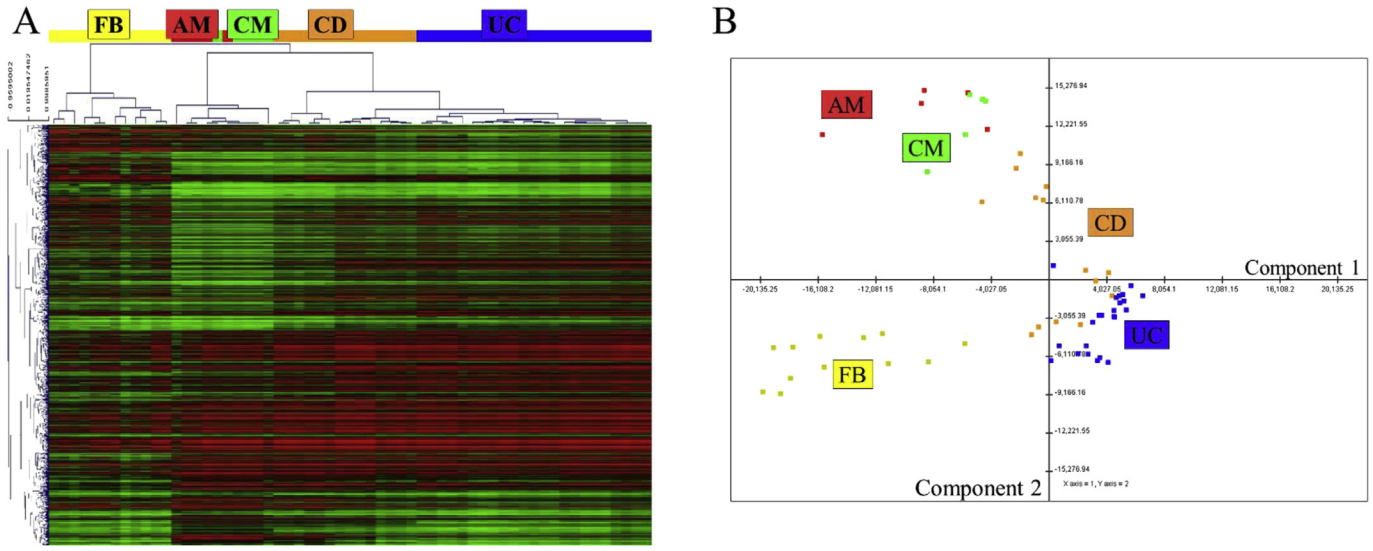

Fig. 1. (A)Hierarchical cluster and (B) principal components analysis of genome-wide gene expression data of MSCs derived from four sources and fibroblast. Although our MSCs are derived from four different source, the genome-wide expression pattern of fibroblasts (i.e., negative controls) are different to all of MSCs. Each MSC can be clustered into a specific sub-cluster based on both hierarchical clustering and principal component analysis. These results imply that different MSCs may have their specific DEGs and their corresponding functions.

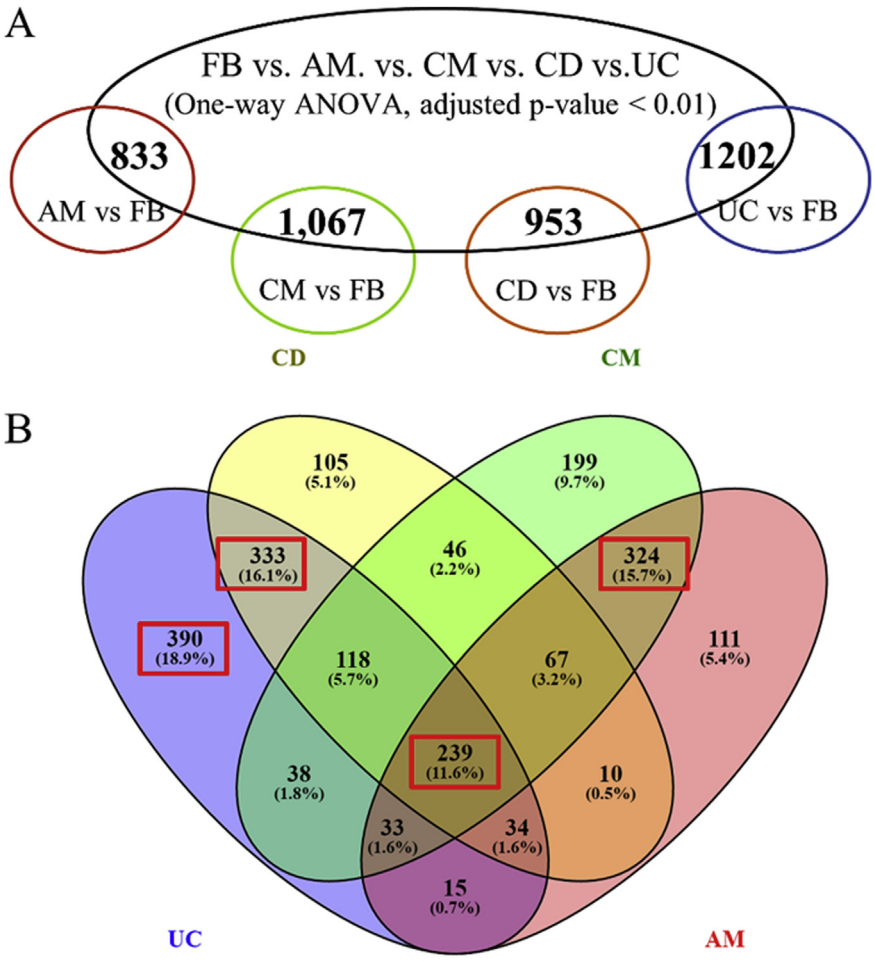

Fig. 2. (A) Differential expression genes of MSCs derived from amniotic membrane (AM), chorionic membrane (CM), chorionic disk (CD), and umbilical cord (UC), respectively. (B) The Venn diagram of differential expression genes of MSCs derived from four sources. (A) Differential expression genes of MSC are identified by using Student t-test, Bonferroni adjustment, and one-way ANOVA. Finally, we identified 833, 1,067, 953, and 1202 DEGs of AM-MSC, CM-MSC, CD-MSC, and UC-MSC, respectively. (B) We found that there are four types of DEGs on the Venn diagram having more than 200 genes (>10%).

similar to thus of UC-MSC than thus of AM-MSC and CM-MSC. These results are consistent with hierarchical clustering and PCA.

According to Venn diagram (Fig. 2B), we identified four types of DEGs (i.e., UC-specific, placenta-specific, UC-like, UC-placenta consistence). To further understand these genes, we illustrated the expression patterns and annotated the functions by considering the cellular processes, signaling pathway, cellular component, and metabolic process (Fig. 3). The UC-specific genes include 260 up-regulated and 130 down-regulated genes. According to 333 UC-CD-specific genes, most of these genes (278 genes) are up-regulated. Otherwise, most (247 genes) of AM-CM-specific genes are down-regulated. Finally, UC-placenta consistence genes include 64 up-regulated and 175 down-regulated genes. Fig. 3A and B illustrates the heat map and box-plots of 260 up-regulated UC-specific genes, 278 up-regulated UC-like genes, 64 up-regulated UC-placenta consistence genes, 130 down-regulated UC-specific genes, 247 down-regulated placenta-specific genes, and 175 down-regulated UC-placenta consistence genes. These results indicated that most of UC-placenta consistence genes are down-regulated (compare to fibroblast). AM-MSC and CM-MSC which are neighbors on the anatomical structure share several down-regulated DEGs.

Fig. 3C and D illustrate the annotations of cellular processes, signaling pathway, cellular component, and metabolic process based on the Gene Ontology [15]. Here, the p-values of functional enrichments are based on the hypergeometric test. According to the up-regulated genes, UC-specific DEGs are related to movement of sub-cellular component. Then, we found the UC-like genes are involved in the cell cycle, cell division, and DNA repair process and located on the intracellular (Fig. 3C). Finally, the UC-placenta consistence genes are involved in the actin filament-based process and related to the movement of cell or sub-cellular component (Fig. 3C). According to the down-regulated genes, we found that placenta-specific genes are located on the cell projection, plasma membrane, and neuron part (Fig. 3D). The significant functions of down-regulated placenta-specific genes are involved in movement of cell or sub-cellular component, cell communication, and cell projection organization (Fig. 3D). Then, the down-regulated umbilical cord-placenta consistence genes are involved in cell death, cell growth, cell developmental processes.

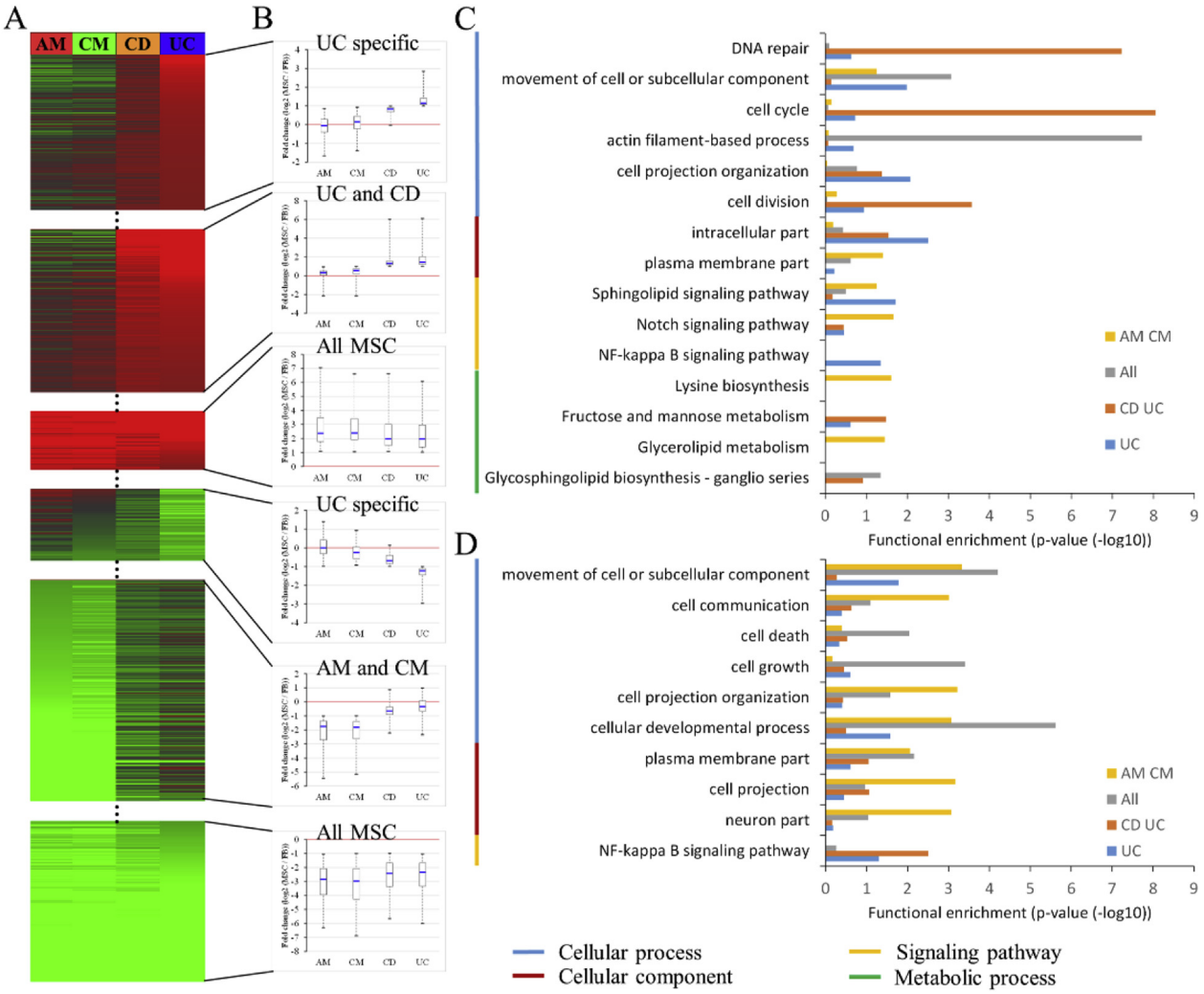

Fig. 3. The (A) heat map and (B) box-plots of UC-specific, UC-CD-specific, AM-CM-specific, all-MSC-specific genes. The cellular processes, signaling pathway, cellular component, and metabolic process annotations of (C) up-regulated and (D) down-regulated specific genes. According to the Venn diagram (Fig. 2B) of DEGs, we studied four types of DEGs which have more than 200 genes (>10%). We evaluated the functions based on Gene Ontology and hypergeometric test.

UC-like genes and cell cycle pathway

Most of UC-like genes are up-regulated and are involved in the cell cycle, cell division, and DNA repair process. To further investigating the relationship between the UC-like genes and cell cycle, we used the KEGG pathway database [16] to study the UC-like genes. Fig. 4 illustrates the cell-cycle pathway derived from KEGG and the UC-CD-specific genes. There are seven up-regulated genes (i.e., MCM7, ORC1, DBF4, CHEK1, ESPL1, CDC20, and CDC45) involved in cell-cycle pathway. There are four genes (i.e., MCM7, ORC1, CDC45, and DBF4) are the components of the origin recognition complex (ORC) and minichromosome maintenance (MCM) complex. These two complexes play important roles during the initiation step of DNA replication [25,26]. The Serine/threonine-protein kinase Chk1 is required for checkpoint-mediated cell cycle arrest and activation of DNA repair in response to the presence of DNA damage or unreplicated DNA [27]. Previous study suggests that CHEK1 would involve in stem cell aging and regenerative potential [28]. Then, CDC20 (Cell division cycle protein 20 homolog) which is required for full ubiquitin ligase activity of the anaphase promoting complex/cyclosome (APC/C) is a regulatory protein in the cell cycle [29,30]. The up-regulated CHEK1 and CDC20 may be associated with the immortalization of MSCs [31]. Our results indicated that the up-regulated UC-like genes which play an important role in cell cycle regulation and DNA replication are related to immortalization of MSCs.

Protein–protein interaction networks of up-regulated UC-like genes

According to 278 up-regulated UC-like genes, we also illustrated the protein interaction network to study these genes. The protein interaction network is constructed by considering five public protein–protein interaction databases [17–21]. We also used several predicted protein–protein interaction based on published methods [22–24]. We have identified a maxima sub-network including 47 up-regulated UC-CD-specific genes. Seven up-regulated genes (i.e., MCM7, ORC1, DBF4, CHEK1, ESPL1, CDC20, and CDC45) involved in KEGG cell-cycle pathway are also located on the maxima sub-network. ORC1, CDC20, CDC45, CHEK1, and MCM7 are the hubs which have more than 15 interactions on the complete protein-interaction network. We also found that there are several histone proteins (e.g., HIST1H4A, HIST1H3A, and HIST1H2AC) are the hubs of the sub-network. The histones play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability

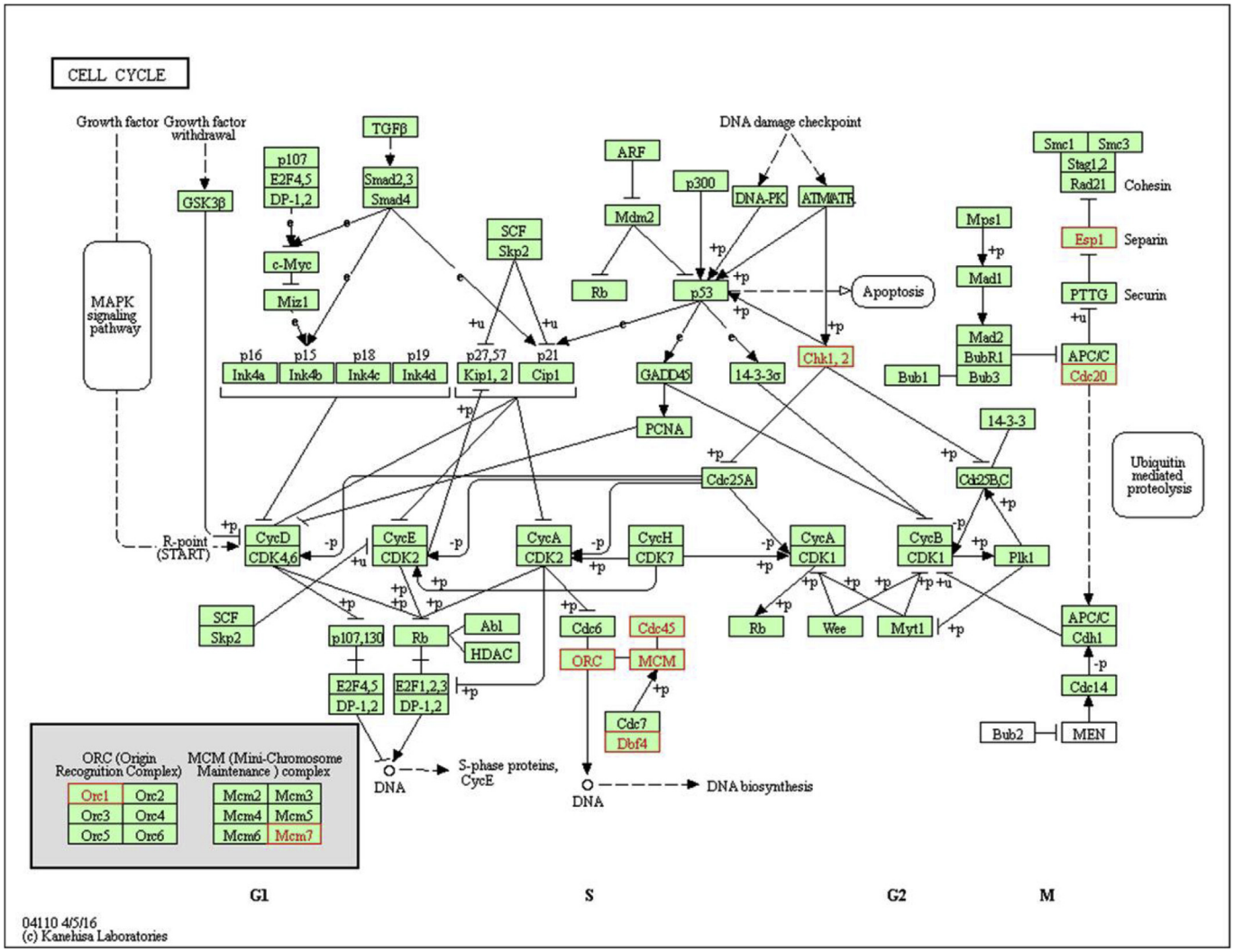

Fig. 4. The KEGG cell-cycle pathway and involving up-regulated UC-CD-specific genes. According to KEGG database, there are seven up-regulated UC-CD-specific genes (i.e., MCM7, ORC1, DBF4, CHEK1, ESPL1, CDC20, and CDC45) involved in cell-cycle pathway. We found that there are four genes (i.e., MCM7, ORC1, CDC45, and DBF4) are related to the origin recognition complex (ORC) and minichromosome maintenance (MCM) complex. These two complexes play important roles during the initiation step of DNA replication.

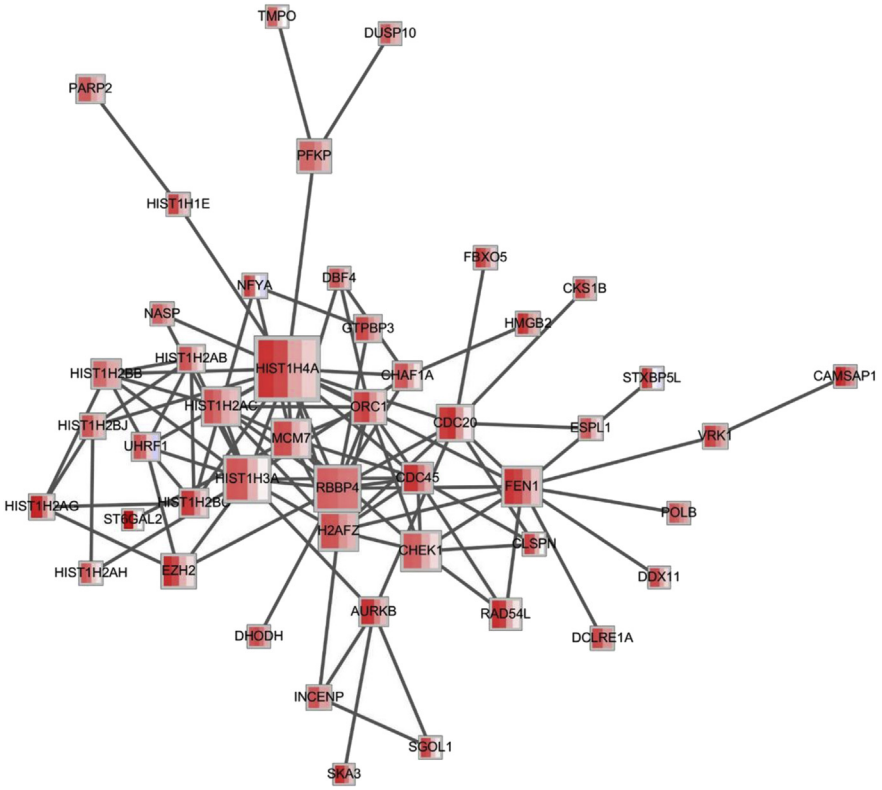

Fig. 5. The protein-interaction network of up-regulated UC-CD-specific genes. The protein interaction network is constructed by considering five public protein–protein interaction databases (i.e. Intact, BioGRID, MINT, MIPS, and DIP). We also used several predicted protein–protein interaction based on published methods (i.e., PPISerach and 3D-interologs). There are 47 up-regulated UC-CD-specific genes involving in the maxima sub-network. Seven up-regulated genes (i.e., MCM7, ORC1, DBF4, CHEK1, ESPL1, CDC20, and CDC45) which are involved in KEGG cell-cycle pathway are also located on the maxima sub-network. ORC1, CDC20, CDC45, CHEK1, and MCM7 are the hubs which have more than 15 interactions on the complete protein-interaction network.

[32]. Our results indicated that the up-regulated UC-like genes are related to ORC, MCM complex, and histones. All of these complexes play an important role in cell cycle regulation and DNA replication.

Conclusion

In this study, we collected genome-wide gene expression data to evaluate the specific genes of hMSCs derived from umbilical cord and placenta including the amniotic membrane, chorionic membrane, chorionic disk. The placental tissues and umbilical cord were collected from healthy full-term placenta. According to the hierarchical clustering and principal component analysis results, each hMSC can be clustered into a specific sub-cluster. In addition, AM-MSC and CM-MSC, derived from amnion and chorion membranes which are the neighbors on the anatomical structure of placenta, share similar expression patterns (Fig. 1). UC-MSC is not derived from placenta and have the different expression pattern to that derived from placenta. The CD-MSC derived from chorionic disk is located between membranes (i.e., amnion and chorion membranes) and umbilical cord, and the expression pattern of CD-MSC is more similar to thus of UC-MSC than thus of AM-MSC and CM-MSC (Fig. 1). According to the DEGs, we identified four types of DEGs (i.e., UC-specific, placenta-specific, UC-like, and UC-placenta consistence genes). Most of UC-like gene are up-regulated and involved in the cell cycle, cell division, and DNA repair process (Fig. 3C). Most of placenta-specific genes are down-regulated, located on the plasma membrane, and involved cell communication (Fig. 3D). Then, the UC-placenta consistence DEGs are involved in cell death, cell growth, and cell developmental processes and down-regulated. Finally, we used the KEGG database and PPI network to investigate the UC-CD-specific genes (Figs. 4 and 5). We found that the up-regulated UC-like genes are related to ORC, MCM complex, and histones which play important roles in cell cycle regulation and DNA replication. More important, the up-regulated CHEK1 and CDC20 may be associated with the immortalization of MSCs. We believe that identified DEGs and their corresponding functions could provide us opportunity to infer the further applications of hMSCs derived from human placenta and umbilical cord.

Ethics approval and consent to participate

The study was approval by the Ethics Committee of the Cardinal Tien Hospital as IRB No.: CTH-102-2-4-003. The MSCs were collected from placenta and umbilical cord after neonatal delivery with informed consent from pregnant mothers who aged from 20 to 45.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References

[1] Tyndall A, van Laar JM. Stem cells in the treatment of inflammatory arthritis. Best Pract Res Clin Rheumatol 2010;24(4):565–74.
[2] Wang D, Li J, Zhang Y, Zhang M, Chen J, Li X, et al. Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. Arthritis Res Ther 2014;16(2):R79.
[3] Hare JM, Fishman JE, Gerstenblith G, DiFede Velazquez DL, Zambrano JP, Suncion VY, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA 2012;308(22):2369–79.

[4] Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 1968;6(2):230–47.
[5] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284(5411):143–7.
[6] Kern S, Eichler H, Stoeve J, Kluter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24(5):1294–301.
[7] Pevsner-Fischer M, Levin S, Zipori D. The origins of mesenchymal stromal cell heterogeneity. Stem Cell Rev 2011;7(3):560–8.
[8] Davies OG, Cooper PR, Shelton RM, Smith AJ, Scheven BA. A comparison of the in vitro mineralisation and dentinogenic potential of mesenchymal stem cells derived from adipose tissue, bone marrow and dental pulp. J Bone Min Metab 2015;33(4):371–82.
[9] Wagner W, Bork S, Horn P, Krunic D, Walenda T, Diehlmann A, et al. Aging and replicative senescence have related effects on human stem and progenitor cells. PLoS One 2009;4(6):e5846.
[10] Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. Cell Stem Cell 2008;2(4):313–9.
[11] Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K. Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells 2004;22(5):649–58.
[12] Shen SP, Liu WT, Lin Y, Li YT, Chang CH, Chang FW, et al. EphA2 is a biomarker of hMSCs derived from human placenta and umbilical cord. Taiwan J Obstet Gynecol 2015;54(6):749–56.
[13] Linge C, Green MR, Brooks RF. A method for removal of fibroblasts from human tissue culture systems. Exp Cell Res 1989;185(2):519–28.
[14] Lorenz K, Sicker M, Schmelzer E, Rupf T, Salvetter J, Schulz-Siegmund M, et al. Multilineage differentiation potential of human dermal skin-derived fibroblasts. Exp Dermatol 2008;17(11):925–32.
[15] Gene Ontology C. Gene Ontology consortium: going forward. Nucleic Acids Res 2015;43(Database issue):D1049–56.
[16] Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017;45(D1):D353–61.
[17] Kerrien S, Aranda B, Breuza L, Bridge A, Broackes-Carter F, Chen C, et al. The IntAct molecular interaction database in 2012. Nucleic Acids Res 2012;40(Database issue):D841–6.
[18] Chatr-Aryamontri A, Breitkreutz BJ, Oughtred R, Boucher L, Heinicke S, Chen D, et al. The BioGRID interaction database: 2015 update. Nucleic Acids Res 2015;43(Database issue):D470–8.
[19] Licata L, Briganti L, Peluso D, Perfetto L, Iannuccelli M, Galeota E, et al. MINT, the molecular interaction database: 2012 update. Nucleic Acids Res 2012;40(Database issue):D857–61.
[20] Mewes HW, Dietmann S, Frishman D, Gregory R, Mannhaupt G, Mayer KF, et al. MIPS: analysis and annotation of genome information in 2007. Nucleic Acids Res 2008;36(Database issue):D196–201.
[21] Xenarios I, Salwinski L, Duan XJ, Higney P, Kim SM, Eisenberg D. DIP, the Database of Interacting Proteins: a research tool for studying cellular networks of protein interactions. Nucleic Acids Res 2002;30(1):303–5.
[22] Chen CC, Lin CY, Lo YS, Yang JM. PPISearch: a web server for searching homologous protein-protein interactions across multiple species. Nucleic Acids Res 2009;37(Web Server issue):W369–75.
[23] Lo YS, Huang SH, Luo YC, Lin CY, Yang JM. Reconstructing genome-wide protein-protein interaction networks using multiple strategies with homologous mapping. PLoS One 2015;10(1):e0116347.
[24] Lo YS, Chen YC, Yang JM. 3D-interologs: an evolution database of physical protein- protein interactions across multiple genomes. BMC Genom 2010;11(Suppl. 3):S7.
[25] Dutta A, Bell SP. Initiation of DNA replication in eukaryotic cells. Annu Rev Cell Dev Biol 1997;13:293–332.
[26] Brewster AS, Chen XS. Insights into the MCM functional mechanism: lessons learned from the archaeal MCM complex. Crit Rev Biochem Mol Biol 2010;45(3):243–56.
[27] McNeely S, Beckmann R, Bence Lin AK. CHEK again: revisiting the development of CHK1 inhibitors for cancer therapy. Pharmacol Ther 2014;142(1):1–10.
[28] Alt EU, Senst C, Murthy SN, Slakey DP, Dupin CL, Chaffin AE, et al. Aging alters tissue resident mesenchymal stem cell properties. Stem Cell Res 2012;8(2):215–25.
[29] Fang G, Yu H, Kirschner MW. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. Mol Cell 1998;2(2):163–71.
[30] Yu H. Cdc20: a WD40 activator for a cell cycle degradation machine. Mol Cell 2007;27(1):3–16.
[31] Liu TM, Ng WM, Tan HS, Vinitha D, Yang Z, Fan JB, et al. Molecular basis of immortalization of human mesenchymal stem cells by combination of p53 knockdown and human telomerase reverse transcriptase overexpression. Stem Cells Dev 2013;22(2):268–78.
[32] Albig W, Kioschis P, Poustka A, Meergans K, Doenecke D. Human histone gene organization: nonregular arrangement within a large cluster. Genomics 1997;40(2):314–22.