Evaluation of the human telomerase reverse transcriptase (hTERT) expression in mesenchymal stem cells from human fetal membranes at different age groups

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Introduction

Mesenchymal stem cells (MSCs) are multipotent regional stem cells contained in all mesenchymal tissues (mainly in the bone marrow), fetal membranes (placenta, umbilical cord tissues(Wharton's jelly) and umbilical cord blood) adding to adult tissues, and have capable to differentiation into various types of mesenchymal tissues, as well as into cells of other germ layers [1]. But in-vitro , MSCs which derive from bone marrow " not immortal " where make a limited number of divisions in the culture and come to a state of irreversible stopping which is called (senescence)[2] It has been suggested that the shortening of telomeres associated with the terminal replication problem serves as a mitotic clock, whose work ultimately leads to aging in these cells [3].The telomere is the region of the chromosome localized at its end and play important role in the stability of chromosomes. In humans telomere contains special DNA sequences that ensure accurate replication of chromosomes" TTAGGG " where maintained it by Telomerase enzyme (hTERT) which has an important role in maintaining telomeric end by adding telomere repeat TTAGGG[4] . Overexpression of hTERT is associated with increase in telomere length and its repression cause telomere shortening[5]. Telomere length and expression levels of hTERT in mesenchymal stem cells from humanFetal membranes (hFM-MSCs) are still not clearly established. As such we in the present study evaluated effect the mothers age from three different groups on telomerase expression in MSCs from human fetal membranes ,to understand if there exists any difference between the.

Methods & Materials

- Derive and establish hMSCs in-vitro after collecting a 48 full-term freshly delivery human placenta during routine normal deliveries at King Abdulaziz University Hospital (KAUH)), following informed consent mothers and ethical approval from Biomedical Research Ethics Committee,Faculty of Medicine at King Abdulaziz University, Jeddah, Saudi Arabia (Ref-No: 21-71, KAU)
- Divide the samples(Placenta and Umbilical cord Wharton's jelly) into categories according to mothers age as follows ; Group I: young (20-29 years); Group_middle (30-39 years) and Group III_ old (40-50 years)
- hFM-MSCs were derived, established in primary culture (90%DMEM-LG; 10%FBS;1%L-glutamine; 2%P/S and 5 ng/ml b-FGF
- Characterized for their stemness properties by CD marker expression (positive markers: CD29, CD44, CD73 and CD90, negative markers: for CD34and CD45)
- In addition, Total RNA was extracted from fresh hFM-MSCs and cDNA prepared. Real-time PCR (qRT-PCR) was performed to analyze hTERT expression levels.GAPDH was used as the internal control. Were analyzed using ΔΔCT method.
- Normal skin fibroblast cells used as a control to compare hTERT expression level in the different age groups.

Results

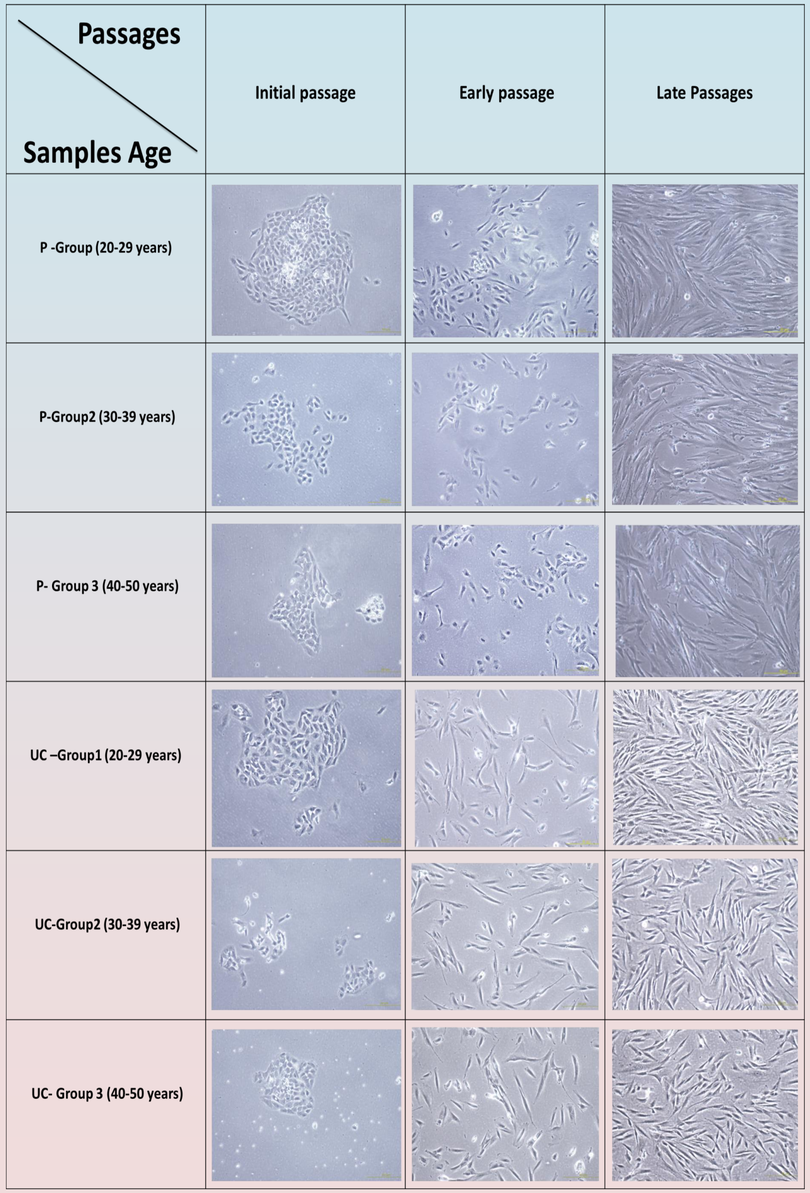

Figure 1: Cellular morphology of *hFM-MSCs

Representative images of hFM-MSCs (placenta (P) and Umbilical cord wharton's jelly (UC)) from three different age groups during cell culture at various passages ; intial passage (P0), elarly passage (P2) and late passage (P4). The cells showed short to long like fibroblast cells . Magnification 10x.

***hFM-MSCs: Mesenchymal Stem Cells from Fetal Membranes**

Figure 2: CD marker analysis of hFM-MSCs by Flow cytometry.

Representative histogram of the flow cytometry analysis (FACS) of MSCs related. CD surface-marker expression on hFM-MSCs from early passages in three different age groups . The hFM-MSCs werepositive for CD73, CD90, CD105, CD44, CD29 and negative for CD34 and CD45. All postive CD surface markers demonstrated more than 80% expression.

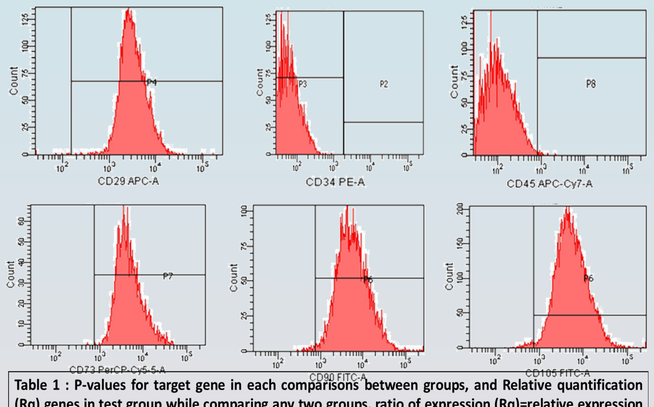

Table 1 : P-values for target gene in each comparisons between groups, and Relative quantification (Rq) genes in test group while comparing any two groups, ratio of expression (Rq)=relative expression of test/relative expression of control.

Sample	Control	Test	P-Value*	Rq value #
Placenta	Normal Skin Fibroblast cell	Young Age	0.003	2.82
		Meddle Age	0.004	2.07
		Old Age	0.009	1.18
Umbilical cord	Normal Skin Fibroblast cell	Young Age	0.002	0.1
		Meddle Age	0.001	0.03
		Old Age	0.007	0.04

* P-value <0.05 = significant

Rq value above 1 indicate upregulation and below 1 indicates downregulation

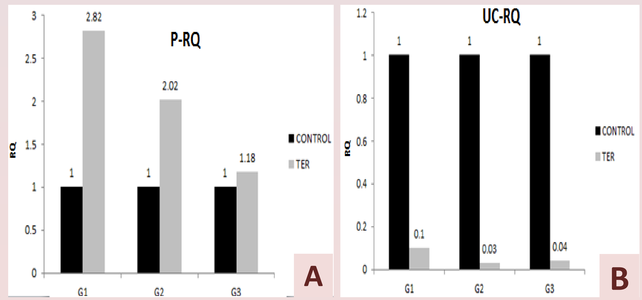

Figure 3: Expression pattern of hTERT gene in *G1 control, G2 and G3.

Description: Rq graphs of P and UC groups represents the comparative expression quantification of gene (test vs control) where control is normalized to 1; Rq value above 1 indicate upregulation and below 1 indicates downregulation

***G1: Young age (20-29 years) ; G2: Middle age (30-39 years);G3: Old age (40-50 years)**

Conclusions

The present study demonstrated that hFM-MSCs in each comparisons groups were spindle shaped plastic-adherent cells and showed the expression for hMSCs markers addition to different proliferation rates through different passages. as well as considerable differences were determined within the hTERT expression levels, where showed a significant highly expressed genes in placenta groups compared to to the control and umbilical cord which showed a lower expressed genes in each age groups. Identification of the expression levels of hTERT in stem cells from Fetal membranes will directly serve to indicate its life-span and replicative capacity, which will help to the selection of the best donors age and cells sources to more accurate use in cell therapy and regenerative medicine.

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