Gene Expression Profiling and Differentiation of Immortalized Human Mesenchymal Stem Cells

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Abstract:Human bone marrow mesenchymal stem cells (hMSCs), tissue-derived stem cells, have the ability to differentiate into adipocytes, osteoblasts and chondrocytes, leading to attempt to apply these stem cells for regeneration of bone and cartilage. To address pluripotency of hMSCs, we analyzed gene expression profiles of several immortalized hMSCs with DNA chips that nearly covers the whole human genes. We isolated some factors that possibly relate to pluripotency of these hMSCs.

Introduction

Mesenchymal stem cells (MSCs) isolated from bone marrow have ability for multilineage differentiation, e.g. osteoblasts, chondrocytes, cardiomyocytes as well as adipocytes, which make human MSCs to attain increasing interest in recent years as a source of regenerating cells for therapeutic transplantation to the lost mesenchymal tissues[1)]. Reduced proliferative potential of hMSCs during long-term in vitro is associated with progressive telomere shortening[2)]. To overcome this disadvantage of hMSCs for basic and clinical research, human telomerase reverse transcriptase (hTERT), a catalytic subunit of telomerase ribonucleoprotein complex with an integral RNA component (hTR), has been genetically transduced into hMSCs, which successfully extended life span of the cells as well as elongated telomere lengths[3)].

DNA chip technology covering the whole genes has been expanding understanding of our knowledge about many cellular processes including cell cycle, cell differentiation and cell proliferation[4)]. Transcriptional analyses of pluripotent state of hMSCs with such DNA chips will help us to understand signaling pathways and molecular mechanisms involved in their differentiation, maintenance of the undifferentiated state and initial loss of pluripotency of hMSCs.

Here we reported the differentiation of immortalized human mesenchymal stem clones transduced with hTERT, and their gene profiling with whole-gene-type DNA chips.

Materials and Methods

Cell culture

Immortalized hMSCs were cultured in DMEM supplemented with 10% FBS. For osteoblastic differentiation, differentiation medium containing dexamethasone, ascorbate and β-glycerophosphate was used. For chondrocyte differentiation, differentiation medium containing dexamethasone, ascorbate and TGF-β3 was used.

DNA chip analysis

Total RNA was isolated from each hTERT-immortalized hMSC clones and parental hMSCs with ISOGEN (Nippon gene, Tokyo, Japan) and treated with RNase-free DNase I. Total RNA of each samples was amplified with T7 polymerase in the presence of aminoallyl-dUTP to produce antisense aminoallyl RNA. The aminoallyl RNA synthesized from hTERT-immortalized hMSC clones was coupled with amino-reactive Cy5. The aminoallyl RNA synthesized from parental hMSCs was coupled with amino-reactive Cy3 for reference probe. Following fragmentation to 150-300nt in alkaline solution, the labeled aRNA was hybridized to DNA chips (AceGene, Hitachi) and washed according to manufacture's protocol. Fluorescent signals on the DNA chip were scanned with ScanArray Lite (Packard BioScience) and analyzed with the QuantArray (Packard BioScience).

Results and Discussion

Differentiation and DNA chip analysis of hMSC clones

Twenty clones from GFP-positive immortalized hMSCs were established by a limited dilution method, and then examined their differentiation ability into adipocytes, osteoblasts and chondrocytes. Each hMSC had different ability of the differentiation. For example, clone #4 of hTERT-immortalized hMSC showed no ability to differentiate into multiple lineages. Clone #7 of hTERT-immortalized hMSC was able to differentiate into only adipocytes. Clone #12 of hTERT-immortalized hMSC had pluripotency into adipocytes, chondrocytes and osteoblasts. These results suggest that hMSCs from bone marrow have heterogeneous cell populations with different ability of differentiation.

DNA chip analysis

To understand molecular mechanism involved in the maintenance of the undifferentiated state and pluripotency of hMSC clones, we analyzed gene expression profiles in the hTERT-immortalized hMSC clones #4, #7 and #12, with different ability of differentiation by whole genome-type DNA chip. Cy-5 labeled probes made from clones of hTERT-immortalized hMSC were hybridized to oligonucleotide-type DNA chips with Cy-3 labeled probes made from parental hMSC as their reference. Among 31,104 gene probes on three DNA chips, 14,421 genes were confirmed as valid spots. Comparing with gene expression profiles of clone #4 and #7, 72 genes, including 17 transcription-related genes and 3 growth factor genes, were up- or down-regulated more than two-fold in hMSC clone #12. These genes are the candidates related to pluripotency of hMSCs. Particularly, seven genes (three were up-regulated and four were down-regulated more than three-fold) in clone #12 are likely the first targets to explain

Table 1 Genes expressed in the clone #12 more or less than 3-fold in other clones.

Position	Ref Seq #	Gene name	Expression ratio Clone#12 to clone #4	Clone #12to clone #7
AGhsA090922	-	-	0.14	0.33
AGhsA220313	NM_003735	protocadherin gamma subfamily a, 12; pcdhga12	0.24	0.24
AGhsA171003	NM_015987	heme-binding protein; hebp1	0.26	0.32
AGhsA030208	-	-	0.32	0.32
AGhsA040604	-	-	3.01	3.05
AGhsB091516	-	-	4.69	3.58
AGhsA210108	NM_001901	connective tissue growth factor; ctgf	4.73	4.46

pluripotency of hMSCs (Table1).

DNA methylation

We analyzed the global methylation patterns in three hMSC clones and compared their patterns with the pattern of ES cells or liver cells. As a result, clone #4 showed low level of methylation as ES cells showing that this clone belongs to the cells in phylogenetically more up-stream lineage, ie. mesodermal stem cells. Furthermore, considering that DNA methylation is involved in various biological phenomena, such as tissue-specific gene expression, cell differentiation, X-chromosome inactivation, genomic imprinting, changes in chromatin structure, and tumorigenesis, it is conceivable that the formation of specific DNA methylation patterns in MSCs is one of the important epigenetic events underlying mammalian development[5]. Epigenetic alternations in these hMSC clones are possibly related to the pattern of DNA methylation of specific genes that we isolated in the study.

References

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