Isolation and characterization of mesenchymal stem cells from human amnion and decidua

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Objectives: The purpose of this study is to isolate a population of multipotent cells from human amnion and decidua, respectively.

Methods: Human placentas (gestational age, 30~42 weeks) were obtained after vaginal or cesarean deliveries. Amnions and deciduas were divided mechanically. The collected cells from the amnion and decidua were cultured. Cultured cells were immunophenotypically characterized. The adipogenic, osteogenic and neurogenic differentiation capacities were tested, and their growth kinetics were analyzed.

Results: We successfully isolated MSCs from both the amnion and decidua. The phenotype of MSCs cultured from different fetal and maternal parts of the placenta was comparable. The growth kinetics of MSCs derived from amnions and deciduas were similar. Isolated MSCs were differentiated into various cell lines such as adipogenic, osteogenic, myogenic and neurogenic cells.

Conclusions: The human amnion and decidua could be an excellent source of MSC because they are easily obtainable after delivery and showed a higher expansion capacity than that of MSCs from adult bone marrow.

Key Words: Amnion, decidua, mesenchymal stem cells

Introduction

Mesenchymal stem cells (MSCs) have great therapeutic potentials because they are capable of self-renewal, multilineage differentiation such as adipose cell, connective tissue, bone, and cartilage. MSCs can be obtained mainly in bone marrow (BM) and other tissues like peripheral blood, periosteum, muscle, adi-
pose tissue, and connective tissue of human adults.\textsuperscript{1,3}  
In human BM, MSCs constitute the stromal trabecular scaffold which tightly interacts with hematopoietic stem cells (HSCs), and so they provide an appropriate microenvironment for control of the maturation, differentiation and survival of blood-born cells.\textsuperscript{2,4} Because the marrow stromal cells are important sources of cytokines and microenvironmental structural supports for hematopoesis, they have unique immunoregulatory functions, which support hematopoesis and enhance the engraftment of hematopoietic stem cells after co-transplantation, and they contribute to the reduction of the incidence of graft-versus-host disease following hematopoietic stem cell transplantation.\textsuperscript{5-8}

Currently, BM represents the main source of MSCs for both experimental and clinical studies. However, acquiring MSCs from BM is limited because MSCs in BM are decreased with age; it needs painful procedure, and has a risk of viral contamination.\textsuperscript{9} So most attention have been paid to tissues containing cells with higher proliferative potency, capability of differentiation, and lower risk of viral contamination and many clinical trials have been performed to acquire MSCs from other than BM such as periosteum, trabecular bone, adipose tissue, synovium, skeletal muscle, fetal pancreas, lung, liver, amniotic fluid, umbilical cord blood (UCB), umbilical cord tissue, and placenta. Among them, UCB, umbilical cord tissue, and placenta are thought as appropriately alternative sources of MSCs because they are discarded after delivery, so they have no restriction such as ethical problem or viral contamination.

Many groups succeeded in isolating MSCs from umbilical cord blood, but controversial results were obtained by others who suggested that cord blood is not the adequate source for MSCs.\textsuperscript{9-13} It was showed that MSCs are present in UCB in a low frequency,\textsuperscript{14} or are even undetectable.\textsuperscript{12}  
Zhang et al. described the presence of MSCs in term human placenta but they did not analyze the origin of the placenta villi-derived cells.\textsuperscript{15}  
In this study, we tried to isolate a population of multipotent stem cells from human term amnion and decidua, if so, and sought to culture and characterize them.

**Materials and Methods**

1. **Sample Collection**

Placentas were derived from term third-trimester pregnancies after informed consent. Placenta tissues were harvested from 123 deliveries at a mean gestational age (GA) of 38+1 (standard deviation [SD], ±2.7) weeks. All women underwent elective cesarean section or vaginal delivery. The institutional review board of Korea University Medical Center Guro Hospital approved the protocol.

2. **Cell isolation and Culture of Mesenchymal Stem Cells**

We peeled off amnion and decidua mechanically from placenta, and washed them with phosphate-buffered saline (PBS) several times to remove the blood from them. The tissues were incubated with 1 mg/mL collagenase for 30 min after chopped into small pieces (approximately 1 cm\textsuperscript{3}) by scissors. The mononuclear cells in collagenase were collected. After centrifugation, cells washed with PBS, centrifuged, cultured with α-minimum essential medium (MEM; GIBCO, NY), supplemented with 10% fetal bovine serum, and seeded the cells to T75 flask (Greiner Bio One GmbH, Frickenhausen, Germany). Cultures were maintained at 37°C in a humidified atmosphere with 5% CO\textsubscript{2}, 3 to 5 days after initiating incubation, the small digested residues were removed and the culture was continued.