Isolation and Characterization of Human Amniotic Mesenchymal Stem Cells and Their Chondrogenic Differentiation

INTRODUCTION Human amniotic mesenchymal (HAM) cells, sparsely located within the stromal layer of amniotic membrane, are known to be immune-privileged and suitable for allograft transplantation. No invasive procedures are needed to obtain amniotic membranes and there are fewer legal and ethical problems because it is excreted in the process of birth and discarded after parturition. If it is possible to obtain undifferentiated mesenchymal cells for chondrogenesis from HAM cells, it could become an excellent cell source for cartilage repair. However, freshly isolated HAM (fHAM) cells isolated from the third trimester undergo growth restriction within 4 passages under usual culture conditions.

To extend the application of HAM cells, we purified proliferative subpopulation from the fHAM cells and analyzed its mesenchymal stem cell characteristic. Also, suitable chondrogenic growth factor for the HAM-derived mesenchymal stem cells (HMSCs) was determined and their viability under xenogenic environment was examined.

MATERIALS AND METHODS

Isolation and cultivation of HAM cells

The human amniotic membrane was obtained from an uncomplicated Cesarean section with informed consent. The study and the use of the amniotic membrane were approved by the Research Ethics Committee of the University of Toyama. HAM cells were isolated by sequential trypsin and collagenase digestion and seeded at a density of 2 × 10^3 cells/cm^2. Within a week, before confluence, cells were passaged at the same density. Thereafter, frequent passaging in low cell density was continued. The population doubling levels (PDL) were calculated to draw proliferating curves at each passaging occasions.

Characterization of the proliferative subpopulation of fHAM cells

Cells cytometry analysis was carried out using antibodies against hematopoietic cell markers (CD14, CD34, CD45), MHC II antigen (HLA-DR) and MSCs markers (CD44, CD73, CD90, and CD105). Immunocytochemical staining was also performed to investigate stem cell marker expressions on cytospinned cell specimens using primary antibodies against, vimentin, CK5, CK18, Oct3/4, c-Myc, Sox2, Nanog, Klf4, CD44, SSEA-3 and SSEA-4. The cells were incubated with secondary antibodies followed by incubation with FITC-conjugated streptavidin. Nuclear staining was performed with Hoechst 33342.

Chondrogenic differentiation and suitable growth factor

Aliquots of 2 × 10^3 cells were centrifuged at 150 × g for 5 minutes to elaborate cell pellets and were cultured in 4 types of chondrogenic differentiation medium. Either one or both of 10 ng/mL transforming growth factor (TGF-β3) and 500ng/mL, bone morphogenetic protein (BMP-2) were supplemented to the basal medium (hMSC Differentiation BulletKit-Chondrogenic, Lonza). Pellets cultured for 4 weeks were embedded in paraffin and sectioned for hematoxylin and eosin (H-E) staining and toluidine blue staining. Reverse transcription polymerase chain reaction (RT-PCR) analysis was performed with extracted total RNA using primers for SOX9, COL2A1, Aggrecan, COL1A1, and GAPDH.

RESULTS

At the first period of primary culture, fHAM cells displayed spindle shape. The cells passaged within a week contained similar spindle-shaped proliferative cells, occasionally formed spotty colonies, and occupied whole cell population after 4-5 passages (Fig. 1). Over 50 PDL proliferation was observed with these colony forming cells designated as HAMα cells.

DISCUSSION

Our present results indicate that we have successfully isolated and established mesenchymal stem cell subpopulation through frequent passaging in low cell density. The ability of the HAMα cells to differentiate into chondrogenic lineage was also assessed and the chondrogenic medium supplemented with BMP-2 was optimal for the HAMα cells as judged by the metachromasia, aggerecan and type IIB isoform expression. In addition, survival of HAMα cells under xenogenic environment implied their low immunogenicity. Further study on the culture system to supply ready-made allo-transplantable HAMα cells or cell-matrix composist should be warranted for future clinical application.

SIGNIFICANCE

HAMα cells can be a useful cell source to produce allo-transplantable ready-made cell or cell-matrix compost for the regeneration therapy.

REFERENCES