

GULREZ NADEEM : INDUCTION OF HUMAN WHARTON'S JELLY OF UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS TO BE CHONDROCYTES AND TRANSPLANTATION IN GUINEA PIG MODEL WITH SPONTANEOUS OSTEOARTHRITIS. THESIS ADVISOR : ASSOC. PROF. RANGSUN PARNPAI, Ph.D., 87 PP.

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Mesenchymal stem cells (MSCs) were successfully isolated from the umbilical cords of two donors. Two cell lines of the MSCs were obtained and characterized. The analyzed characteristics included cell surface markers, colony forming unit, population doubling time, surface protein expression, and differentiation potencies into three cell lineages (adipogenic, chondrogenic and osteogenic). The results revealed that only one cell line exhibited appropriate MSCs characteristics and it was recruited to do in the next experiments. In the determination of cell surface markers, flow cytometry was used instead of cell staining on culture dish because it can provide quantitative values of the positive cell proportion. MSCs were induced to be chondrocytes via the induction of chondrogenic differentiation for 28 days. The obtained chondrocytes were characterized by immunofluorescent staining, qPCR, and immunoblotting. In the project proposal, only type II collagen was proposed to be determined in the immunofluorescent staining and immunoblotting. However, Sox9, Aggrecan, and type X collagen were also detected in the immunofluorescent staining, and type X collagen was further determined in the immunoblotting to strongly confirm the results. Moreover, a human cartilage was included in the research for chondrocyte isolation and protein extraction. The human cartilage-derived chondrocytes were used to compare the gene expressions with the MSC-derived chondrocytes, whereas the human cartilage protein lysate was used to compare the proteins composition with guinea pig cartilage protein lysates. Early stage chondrocytes can be obtained by chondrogenic differentiation induction of MSCs for 14 days. For transplantation, MSCs-derived early stage chondrocytes were intra-articular injected into the knee joints of 7-month old guinea pigs which have symptom of early spontaneous

osteoarthritis. After transplantation for 5 weeks, the joints were collected. The results from the injection of MSCs-derived early stage chondrocytes were compared with those from the injection of undifferentiated MSCs and the injection of Hyaluronic acid. The results revealed that the transplanted cells were integrated into the guinea pig cartilage surfaces and restored the degenerated cartilages. The injection of MSCs-derived early stage chondrocytes recovered the degenerated cartilages better than the injection of undifferentiated MSCs and the injection of Hyaluronic acid. The tissues of the recovered cartilages after the injection of MSCs-derived early stage chondrocytes resemble the cartilages of 3-month old guinea pigs, which have no symptom of osteoarthritis, with no significant difference in the Mankin's scores from the histological assessment.

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