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Lentivirus-mediated RNAi on aggrecanases of chondrocytes for cartilage tissue engineering: *In vitro* and *in vivo*

Zhenghui Wang

Xi'an Jiaotong University, China

Aggrecanases plays important role in degrading aggrecan. To further explore the potential of Lentivirus in cartilage tissue engineering, the aggrecanases inhibited chondrocytes by Lentivector-mediated RNAi were seeded into scaffolds for tissue engineered cartilage and observe the effects of RNA interference (RNAi) on chondrocyte proliferation, function and immunological rejection after allogenic tissue-engineered cartilage transplantation. In vitro engineered cartilage-like tissue grown on the scaffolds was characterized by histology, scanning electron microscopy, biochemical assays and analysis of gene expression at different time points of the in vitro culture. The allograft and immunological response were examined at 1, 2, 4, 8 and 12 months postoperatively with hematoxylin and eosin histo-chemical staining, immune-histochemical staining (aggrecan, type II collagen, class I and II major histocompatibility complex) and flow cytometry for peripheral blood cluster of differentiation 4+ (CD4+) and CD8+ T-cells. The transduced constructs showed more cell proliferation and extracellular matrix of chondrocytes than that of un-transduced constructs. The glycosaminoglycan production and hydroxyproline content of tissue grown on the transduced constructs were higher than that of the un-transduced group. The expression levels of aggrecan and collagen II were evidently heightened in the transduced constructs compared with the un-transduced construct. Compared to the control group, the RNAi group had fewer eukomonocytes infiltrated, which were only distributed around the graft. The ratio of CD4+/CD8+ T-cells in the RNAi group was significantly lower than the normal one. The aggrecanases RNAi for chondrocytes promoted the formation of engineered cartilage and decreased the immunological rejection effect.

wangzhenghui77@xjtu.edu.cn

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