EFFECTS OF GENE TRANSFER OF ANTIOXIDANT ENZYME PEROXINREDOXIN 5 ON HUMAN CHONDROCYTE FUNCTION

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Introduction: Osteoarthritis (OA) is the most common form of arthritis and is characterized by chronic pain and significant disability. There is some evidence that over-production of reactive oxygen species (ROS) is implicated in the pathogenesis of OA. However, little is known about the antioxidant defense system including ROS scavengers in articular cartilage. We have previously shown that a novel antioxidant enzyme peroxiredoxin 5 (PRDX5) is expressed in articular cartilage and its expression is up-regulated in osteoarthritis. In the current study, we investigated PRDX5’s role on chondrocyte function under oxidative stress by inducing over-expression of PRDX5 in human chondrocytes via gene transfer.

Methods: Under ethical approval from our local ethics committee, human osteoarthritic cartilage was removed from knee joints of patients undergoing total knee-replacement surgery. Human chondrocytes were isolated from cartilage by collagenase digestion and primary cultures were used for all experiments. A pcmv-EGFP expression vector was constructed that contained human PRDX5 cDNA with a mitochondrial targeting sequence. Human chondrocytes were transfected with the PRDX5 expression vector using Lipofectamine reagent (Invitrogen). Empty vector without PRDX5 gene was included in every experiment as a control. Transfection efficiency was determined by enhanced green fluorescence protein (EGFP) expression using flow cytometric analysis. Intracellular ROS production was assessed by flow cytometry using a cell permeable dye 2’,7’-dichloro-fluorescein-diacetate (DCF-DA). The PRDX5 protein expression was detected by Western blot using an anti-PRDX5 antibody. [35S]-sulphate incorporation assay was performed to assess proteoglycan synthesis by human chondrocytes.

Results: More than 20% transfection efficiency was achieved when human chondrocytes were subjected to a membrane permeable treatment with 0.00175% lysolecithin (Fig1). Successful PRDX5 gene transfer and protein expression in human chondrocytes were confirmed by Western blot analysis (Fig2). Inflammatory cytokine TNFα challenge to human chondrocytes resulted in an increase in intracellular ROS production, followed by an enhanced PRDX5 protein expression (Fig3). Under TNFα-induced oxidative stress, chondrocytes transfected with empty vector showed a significant decrease in proteoglycan synthesis compared to cells transfected with PRDX5 gene. This suggests a protective role of PRDX5 overexpression in chondrocyte function under oxidative stress.

Conclusions: The current study demonstrates that over-expression of PRDX5 in human chondrocytes can be achieved by cationic lipid-aided gene delivery. The inflammatory cytokine TNFα induced chondrocyte oxidative stress by increasing intracellular ROS production, and decreased chondrocyte proteoglycan synthesis. Overexpression of PRDX5 in human chondrocytes via gene transfer may protect human chondrocytes from oxidative stress and maintain the cellular function of proteoglycan synthesis in human chondrocytes.

References:

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