OVEREXPRESSION OF ANTIOXIDANT ENZYME PROXIREDOXIN 5 PREVENTS HUMAN TENDON FIBROBLAST APOPTOSIS AND LOSS OF CELLULAR FUNCTION DURING OXIDATIVE STRESS

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Introduction: Oxidative stress and apoptosis are implicated in tendon degeneration. Peroxiredoxin 5 (PRDX5) is a novel antioxidant enzyme recently identified in many living organisms. It participates directly in eliminating hydrogen peroxide (H2O2) and neutralizing other reactive oxygen species (ROS). We have previously reported that PRDX5 is upregulated in degenerative human tendon. However, effects of this upregulation on cellular function of tendon cells remain unknown.

The aims of this study were to evaluate the role of overexpression of PRDX5 on human tendon cell apoptosis and cellular function under oxidative stress.

Methods: Human tendon fibroblasts were isolated by collagenase digestion from rotator cuff tendon tissue collected from patients undergoing rotator cuff repair surgery. pcms-EGFP vector containing human PRDX5 cDNA with a peroxisomal targeting sequence and an empty vector without insert sequence were constructed. Cells were transfected with PRDX5 expression vector or empty vector using the Lipofectamine PLUS reagent (Invitrogen). Transfection efficiency was determined by enhanced green fluorescence protein (EGFP) expression using a flow cytometric technique. The PRDX5 mRNA and protein expressions were detected by Northern blotting and immunoblotting. Fluorescein isothiocyanate (FITC) or APC labelled Annexin-V was used to identify apoptotic cells. Propidium iodide (PI) was used to test necrotic cells. Both FITC/APC and PI staining were analysed by a FACSCalibur cytometer. Tendon fibroblast collagen synthesis was assessed by measuring the collagenase-sensitive fraction of protein incorporated [2-3-H]proline. Results were normalized by DNA concentrations (cpm/µg DNA).

Results: Enhanced PRDX5 expression under oxidative stress In vitro oxidative stress was created by exposing human tendon fibroblasts to H2O2. Addition of 50 µM H2O2 to cultured human tendon fibroblasts significantly increased apoptosis but not necrotic cells (Fig 1). Under the same experimental condition, enhanced PRDX5 mRNA and protein expression were observed (Fig 2).

Effective PRDX5 transfection in human tendon cells A successful PRDX5 gene transfer and protein expression were achieved by liposome added gene delivery detected by EGFP (Fig 3A). This was further confirmed by western blotting analysis of PRDX5 protein expression (Fig 3B).

Protective role of overexpression of PRDX5 in tendon cell under oxidative stress.

As shown in Fig 4A, PRDX5 transfection had no effect on tendon cell apoptosis compared with control (empty vector transfer). However, PRDX5 transfection prevented the increase in apoptosis when tendon cells were under H2O2 challenge. Furthermore, PRDX5 gene transfer prevented the decrease in tendon cell collagen synthesis under H2O2 challenge (Fig 4B).

Fig 1. Percentage of apoptotic and necrotic cells in human tendon cells 24 hrs after 50 µM H2O2 challenge detected by flow cytometry. Mean±SEM, n=3, *p<0.05 when compared with control (untreated cells).

Fig 2. PRDX5 expression was upregulated by exogenous H2O2, detected by Northern blotting (A) and Western blotting (B).

Fig 3 Representative flow cytometric profile of transfection efficiency (82%) detected by EGFP expression in tendon cells (A). Cells transfected with PRDX5 showed an increased PRDX5 protein expression compared to un-transfected or empty vector-transfected cells (B).

Fig 4. Effects of overexpression of PRDX5 via gene transfer on tendon cells apoptosis (A) and collagen synthesis (B). Mean±SEM, n=4, *p<0.05 when compared with that in cell transfected with empty vector after H2O2 challenge. ** p<0.05 when compared with empty vector transfection without H2O2 challenge.

Conclusion: Our data have demonstrated that H2O2 leads to the upregulation of antioxidant enzyme PRDX5 in tendon cells. Oxidative stress created by the addition of H2O2 resulted in an increase in tendon cell apoptosis and a decrease in tendon cell collagen synthesis. Apoptosis and decreased collagen synthesis were prevented by overexpression of PRDX5 via gene transfer to human tendon cells. The antioxidant enzyme PRDX5 may play a protective role against oxidative stress by reducing apoptosis and maintaining the cellular function of collagen synthesis in human tendon cells.

Reference: