INTRODUCTION

Oxidative stress occurs when the metabolic balance of a cell is disrupted through exposure to excess pro-oxidant. Whilst it is known that oxidative stress can cause early degenerative changes observed in experimental osteoarthritis and that a major drawback of current cartilage and intervertebral disc tissue engineering is that human mesenchymal stem cells (MSCs) from osteoarthritis (OA) patients express type X collagen [1], a marker of late-stage chondrocyte hypertrophy (associated with endochondral ossification) little is known whether the expression of type X collagen in MSCs from OA patients can be related to oxidative stress or inflammatory reactions that occur during this disease.

MATERIALS AND METHODS

Gene expression - Cells were lysed and proteins were separated on 10% acrylamide gels (SDS-PAGE) and transferred to nitrocellulose membranes. Protein expression was detected by Western blot using specific antibodies directed against type X collagen (COL10; Sigma-Aldrich), as well as the anti-oxidant enzymes Mn-superoxide dismutase (MnSOD; Upstate), catalase (CAT; Abcam) and glutathione peroxidase-1 (GPx-1; Abcam) and inflammation related proteins cyclooxygenase-1 (COX-1; Cayman) and intercellular adhesion molecule-1 (ICAM-1; Santa Cruz). GAPDH was used as a housekeeping gene and served to normalize the results. Protein levels were analyzed using a Bio-Rad VersaDoc equipped with a cooled CCD 12 bit camera. Correlations between the expressions of the different genes were realized using the correlation Z test with StatView (SAS Institute, Cary, NC).

RESULTS

Results confirmed that type X collagen was over-expressed in MSCs from OA patients when compared to expression in cells of normal donors (Figure 1). MnSOD, CAT, and COX-1 were also over-expressed.

DISCUSSION

Oxidative stress is a known risk factor for OA: it leads to chondrocyte and cartilage aging and may be a possible cause of osteoarthritis development. In this study, we showed that the level of anti-oxidant enzymes correlates with type X collagen expression in MSCs from OA patients. This suggests that oxidative stress may lead to the up-regulation of stem cell hypertrophy. Results also suggest that prostaglandin production through COX-1 activity is associated with anti-oxidant enzyme expression (MnSOD) and hypertrophy (type X collagen expression). Further studies are however necessary to better understand whether the increased expression of these proteins is the cause or the effect of type X collagen over-expression in MSCs from OA patients.

REFERENCES


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