Oxidized low-density lipoprotein suppresses telomerase activity in cultured bovine articular chondrocytes through inactivation of PI3 kinase/Akt

S. Zushi, M. Akagi, H. Kishimoto, Y. Miki, T. Teramura, C. Hamanishi.
Kinki University School of Medicine, Osaka-Sayama City, JAPAN

Purpose: Oxidized low-density lipoprotein (ox-LDL) is one of most important molecules for pathogenesis of atherosclerosis. Recently, it has been demonstrated in vitro and vivo studies that lectin-like ox-LDL receptor-1 (LOX-1) expresses on articular chondrocytes and suggested that its ligand ox-LDL plays some roles in degeneration of articular cartilage and pathogenesis of osteoarthritis. The purpose of this study is to show that ox-LDL binding to LOX-1 suppresses telomerase activity in cultured bovine articular chondrocytes (BACs) through inactivation of the PI3 kinase/Akt pathway.

Methods: Time course and dose response of telomerase activity following ox-LDL addition were investigated in primary cultured bovine articular chondrocytes (BACs, 10 month old) in monolayer with 70% confluency. The telomerase activity was measured by the stretch PCR (Telochaser, TOYOBO Co., Tokyo, Japan). To ascertain whether suppression of telomerase activity is attributable to ox-LDL binding to LOX-1, BACs were pre-incubated with anti-bovine LOX-1 monoclonal antibody (TS-20) or non-specific mouse IgG. Furthermore, telomerase activity was measured after adding LY294002 (a specific inhibitor of PI3K) and IGF-1 (an activator of PI3K) in culture medium to clarify involvement of the PI3K/Akt pathway to the suppression by ox-LDL. Finally, we investigated using immunoblot analysis whether ox-LDL addition affects phosphorylation of Akt.

Results: Ox-LDL addition rapidly decreased the amount of phosphorylated Akt (pAkt) in BACs, whereas native LDL did not. Furthermore, pretreatment with TS-20 significantly recovered the reduction of pAkt caused by ox-LDL addition.

Conclusions: Increase in mitotic and anabolic activity of chondrocytes has been indicated in early phase osteoarthritis, where telomerase activity of chondrocytes is supposed to be up-regulated by growth factors. In this study, ox-LDL suppressed the telomerase activity in chondrocytes through inactivation of the PI3K/Akt, which may play an important role in failure of repairing responses of chondrocytes and in progression of cartilage degeneration through cell senescence. Osteoarthritis and atherosclerosis may have a common pathogenetic molecule, ox-LDL.

A PIVOTAL ROLE FOR PROTEIN KINASE R IN PRO-INFLAMMATORY CYTOKINE-INDUCED UPREGULATION OF MMP-9 IN BOVINE ARTICULAR CARTILAGE CHONDROCYTES.

S. J. Gilbert, V.C. Duance, D. J. Mason. Cardiff University, Cardiff, UNITED KINGDOM

Purpose: Our studies analysing genes regulated in cartilage at the onset of osteoarthritis (OA) revealed up-regulation of the gene encoding the protein kinase R (PKR)-activating protein, PACT. We subsequently showed that PKR is involved in tumour necrosis factor-alpha (TNF-α) signalling in articular cartilage. The aims of the current study were to investigate whether treatment of articular cartilage chondrocytes with pro-inflammatory cytokines could induce a degradative phenotype that was mediated through the PKR signalling pathway.

Methods: Full depth, bovine articular cartilage chondrocytes were harvested from the metacarpophalangeal joint of 7-day old calves, plated at 1 × 10^6 cells/well of a 24 well plate and cultured in serum free media supplemented with ITS and ascorbate-2-phosphate (50 μg/ml). Chondrocytes were treated with either an oncostatin-M (OSM; 10 ng/ml) and interleukin-1 (Il-1; 5 ng/ml) combination or TNF-α alone (20 ng/ml) for 7-days. To inhibit the activation of PKR, a pharmacological inhibitor of PKR (an imidazole-oxindole compound from Calbiochem; 1 mM) was added to duplicate cultures 30 minutes prior to the addition of cytokine treatments. At the end of the 7-day culture period, media was collected for analysis of cell death (LDH release) and MMP-9 release (gelatin zymography). Cells were lysed in 0.9% Triton-X100 and extracts and associated matrix were analysed for type II collagen (Western blot). Cell number was calculated by measuring the LDH content of the cell lysate.

Results: A combined treatment of OSM and Il-1 resulted in an increase in cell number over the 7-days of culture compared to untreated controls (p<0.05). The addition of the PKR inhibitor alone to chondrocytes had no affect on cell number. However, when added prior to the addition of OSM+Il-1, a significant increase in cell number was observed (p<0.001 vs control; p<0.05 vs OSM+Il-1 alone). OSM+Il-1 increased the extent of necrotic cell death occurring in chondrocyte cultures (p<0.05) which was unaffected by the addition of the PKR inhibitor. TNF-α had no affect on the level of necrotic cell death over the 7-days. An increase in proMMP-9 was observed in the media of TNF-α treated chondrocytes which was completely abolished following PKR inhibition. The production of both pro and active MMP-9 was upregulated by OSM+Il-1 treatment (p<0.05) and this was significantly reduced following PKR inhibition (p<0.05). TNF-α reduced the amount of type II collagen detected in the cell associated material (p<0.05). The addition of the PKR inhibitor prior to treatment with TNF-α resulted in the restoration of the level of type II collagen (p<0.001 vs TNF-α alone). Whilst treatment of chondrocytes with OSM+Il-1 resulted in a marked reduction in the amount of type II collagen detected, pre-treatment with the PKR inhibitor had no affect.

Conclusions: The pro-inflammatory cytokines, OSM and Il-1 in combination increased proliferation of articular chondrocytes which was further enhanced when PKR was inhibited. This implicates a role for the activation of the PKR pathway in the control of pro-inflammatory cytokine-mediated cell proliferation. OSM in combination with Il-1 also increased the extent of cell death over the culture period but this was not regulated via PKR. The expected upregulation of MMP-9 was significantly decreased by inhibition of PKR. Type II collagen was reduced in all cultures treated with the pro-inflammatory cytokines but only TNF-α signalled via PKR to achieve this. This study provides a mechanism of how pro-inflammatory cytokines may mediate some of the well-established degradative effects that occur in articular cartilage and implicates PKR as a critical signalling molecule in arthritis.