190 OXIDIZED LOW-DENSITY LIPOPROTEIN SUPPRESSES TELOMERASE ACTIVITY IN CULTURED BOVINE ARTICULAR CHONDROCYTES THROUGH INACTIVATION OF PI3 KINASE/AKT

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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% confluence. 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 preincubated 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.

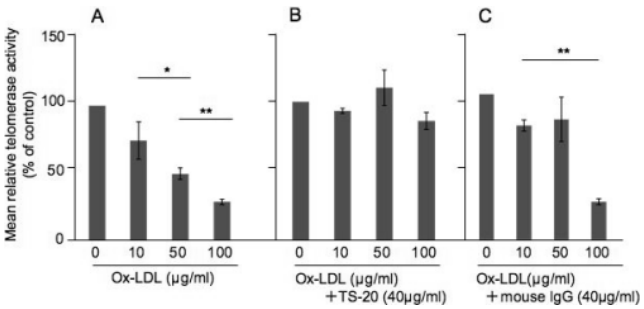

Figure 1. Dose response of inhibition of telomerase activity by ox-LDL in bovine articular chondrocytes. Cells were treated with various dose of ox-LDL (10 to 100 μg/ml) for 12 h (A). Some plates were pre-incubated with 40 μg/ml anti-LOX-1 antibody (TS-20) (B) or 40 μg/ml non-specific mouse IgG as for 30 min (C). *P < 0.05, **P < 0.01, n = 4.

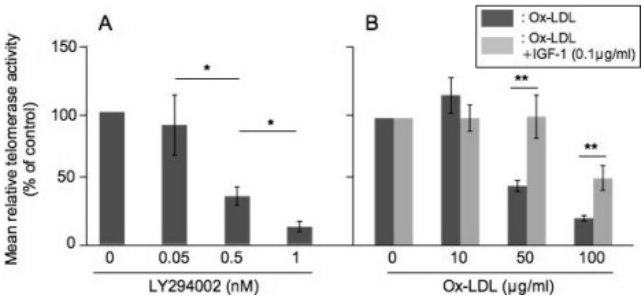

Figure 2. Effects of the PI3K/Akt pathway on telomerase activity. Telomerase activity was measured 12 h after addition of LY294002 at various dose (0, 0.05, 0.5 and 1 nM) (A) and 12 h after addition of ox-LDL at various dose (0, 10, 50 and 100 μg/ml) with IGF-1 (0.1 mu;g/ml) (B). *P < 0.05, **P < 0.01, n = 4.

Results: Telomerase activity was detectable in proliferating BACs cultured in DMEM with bovine fetal serum. Addition of ox-LDL suppressed the telomerase activity in a time- and dose-dependent manner (Fig 1). Pretreatments of cultured BACs with anti-LOX-1 antibody (TS-20) recovered the suppression of telomerase activity caused by ox-LDL addition. LY294002 suppressed the chondrocyte telomerase activity and IGF-1 reversed the effect of ox-LDL addition on the telomerase activity (Fig 2). 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.

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

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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 (20 ng/ml) for 7-days. To inhibit the activation of PKR, a pharmacological inhibitor of PKR (an imidazole-oxindole compound from Calbiochem; 1 μM) 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.