The role of PKCδ pathway in the intervertebral disc

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Introduction: Chronic back pain is a prevalent ailment among adults with a lifetime prevalence of about 70-85% in the United States [1]. One of the principal causes of back pain is the pathological degeneration of the intervertebral disc (IVD), which has been associated with collagen degradation and progressive loss of proteoglycans in the nucleus pulposus (NP) [2]. Studies have shown an upregulation of matrix metalloproteinases (MMP-13) in the degenerated IVD, suggesting a potential role in disc degeneration [3]. Previously, we demonstrated the critical role of PKCδ in the upregulation of MMP-13 after stimulation with catabolic growth factors such as basic FGF in human adult articular chondrocytes [4]. Moreover, activation of PKCδ may have opposite biological effects in cartilage homeostasis [5]. Phorbol-12-myristate-13-acetate (PMA) is a known activator of multiple PKC isoforms [6]. We hypothesize that the PMA-generated PKCδ pathway may be associated with disc degeneration in the IVD. On the other hand, other PKC isoforms (i.e., PKCα/β, ε) may exert different biological effects in the IVD. The aim of the present study is to determine the effect of the PKCδ-activated PKC pathways on the upregulation of MMP-13, proteoglycan (PG) production, and cell proliferation using bovine NP cells.

Materials and Methods: IVD tissue was harvested from bovine coccygeal tissue (15-18 months old) and chondrocytes were isolated from the nucleus pulposus (NP), digested, and captured in alginate, as previously described [7]. The beads were cultured in complete medium (DMEM/F12 supplemented with 10%FBS, 25μg/ml ascorbic acid, 50μg/ml gentamicin, 360μg/ml L-glutamine, antibiotics) for 21 days. PG production was assessed by Dimethylmethylene blue (DMMB) assay as previously described [8]. NP cells cultured in serum-free monolayer were treated with pathway-specific inhibitors, including inhibitors of PKCδ (rotterlin(4μM), PKCε/β (10μM), and PKCe (6μM)). Immunoblotting was performed by loading equal amount of total protein in the conditioned medium by protein assay (Pierce) on 10% SDS-PAGE gels, transferred, and blotted using anti-MMP-13 antibody (gift from Dr. Wu) as previously described [4]. BMP7 (100 ng/ml, Stryker) was used as control for PG production. Analysis of variance was performed using StatView 5.0 software. P values <0.05 were considered significant.

Results: MMP-13 Expression (Fig 1): Stimulation of bovine NP cells cultured in monolayer with PMA (5μM) augmented the production of MMP-13, and this PMA-induced augmentation was blocked by the addition of a pathway-specific inhibitor of PKCδ (PKCδI). Inhibitors of other PKC isoforms, such as PKCα/β and PKCe, fail to block PMA-induced MMP-13 expression.

PG Production (Fig 2): Incubation of cells cultured in alginate with PMA (0.5μM) for 21 days significantly decreased PG production compared to control. More importantly, the presence of PKCδI along with PMA completely ablated the catabolic effect mediated by PMA: when PKCδI was administered with PMA, PG production was increased by 40% compared to control, and 100% compared to PMA alone. Without PMA, incubation of cells with PKCδI maintained control level. The anabolic growth factor BMP7 (100ng/ml) was used as a positive control.

Cell Survival: The incubation of NP cells with PMA, PKCδI, or BMP7 had no effect on cell survival, as viability remained >90% for all treatment conditions.

Cell Proliferation (Fig 3): Stimulation of cells with PMA significantly enhanced IVD cellular proliferation as represented by DNA concentration. More importantly, this enhancement of proliferation was completely abrogated in the presence of PKCδ inhibitor.

Discussion: Previously, we reported that PKCδ activation is required for the IL-1β- and bFGF-mediated stimulation of MMP-13 in human articular chondrocytes [4]. Similarly, our inhibitor studies in bovine spine IVD tissues suggest that PMA, as a known activator of several PKC isoforms, accelerates MMP-13 expression, specifically via the PKCδ signaling pathway. Moreover, our results imply that the PKCδ pathway is associated with reduced PG production. These data suggest a potential beneficial role of blocking the PKCδ pathway in cartilage homeostasis. In summary, our findings are the first to demonstrate the specific catabolic role of the PKCδ pathway in the intervertebral disc. We have demonstrated the importance of the PKCδ pathway via its involvement in the upregulation of MMP-13, and the decrease in PG production without affecting cell viability in bovine IVD cells. These findings provide evidence for the possible therapeutic role of pathway-specific inhibitors of the PKCδ cascade in degenerative disc disease in the future.