Lipophilic Statins Inhibit CD44 Fragmentation In Chondrocytes

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Disclosures:

Introduction: The hyaluronan (HA)/proteoglycan-rich cell-associated matrix is anchored to chondrocytes via the binding of HA to CD44, the principal cell surface receptor for HA. In human osteoarthritic (OA) chondrocytes, a substantial proportion of the CD44 undergoes degradation similar to cleavage observed in several tumor cell systems [1]. CD44 cleavage also can be induced in normal articular chondrocytes by treatment with IL-1β. This signature degradation pattern includes the cleavage of the extracellular domain of CD44 by a metalloproteinase (e.g., ADAM17, MT1-MMP) releasing into the extracellular matrix a shed CD44 ecto-domain, leaving the 18-20 kD C-terminal fragment within the membrane (CD44-EXT) [2]. The CD44-EXT fragment is then cleaved within the intramembranous domain releasing a 15 kD intracellular domain (CD44-ICD) into the cytoplasm. The potential metalloproteases and γ-secretase that participate in CD44 cleavage are localized in cholesterol rich lipid rafts. Cleavage of CD44 results in decreased functional CD44 and also CD44 ecto-domain generation which could act as a decoy receptor for HA, leading to decreased chondrocyte-associated matrix and cartilage degeneration. Statins are a family of inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol biosynthetic pathway. Statins are also known to have non-lipid related properties including a broad range of immunomodulatory and anti-inflammatory effects. A previous study reported that statins suppress the cartilage degeneration induced by proinflammatory cytokines [3]. The present study was designed to examine whether simvastatin could inhibit IL-1β + Oncostatin M (OSM)-induced CD44 fragmentation in chondrocytes.

Methods: Cell culture: HCS-2/8 cells, a chondrocyte cell line established from a human chondrosarcoma, were grown in DMEM (Gibco-BRL) supplemented with 10% FBS and 1% antibiotics. HCS-2/8 cells were pretreated with the indicated dose of simvastatin for 48h prior to stimulation with 0.1 ng/ml IL-1β and 10 ng/ml Oncostatin M (Cell Signaling Technology, USA) for 24h. The final concentration of DMSO was set to 0.1% in all culture conditions.

Western Blotting: Total protein was exacted using Cell Lysis Buffer (Cell Signaling Technology, USA) containing protease inhibitor cocktail. Total protein was loaded and separated on Novex 4-12% gradient sodium dodecylsulfate polyacrylamide gel electrophoresis gels (Invitrogen). Antibodies specific for the CD44 cytotail [1], β-actin (Cell Signaling Technology, USA), and flotillin-1, MT1-MMP, ADAM10, ADAM17 (all from Abcam, USA) were used for analysis. Sucrose gradient ultracentrifugation for isolation of lipid rafts: Cell lysates were prepared in TNE buffer containing 1% Triton X-100 and a protease inhibitor cocktail. Lysate was mixed with 80% sucrose and this was layered at the bottom of the centrifuge tube and overlaid with 35% and 5% sucrose solutions respectively. Gradients were centrifuged at 39000 xg for 20h in Optima L-60 (SW41 T1 rotor) and fractionated into 10 equal-volume fractions. Flotillin-1 within each fraction was characterized by Western blotting; fractions 2 and 3 which exhibited high expression of the lipid raft marker flotillin-1 were defined as lipid raft fractions.

Results: Inhibition of CD44 fragmentation by simvastatin treatment: We confirmed that IL-1+OSM stimulation induced the CD44 fragmentation in HCS-2/8 cells. The enhanced fragmentation included the 18-20 kD doublet CD44-EXT bands and the 15 kD CD44-ICD bands (Figure 1A). Pre-incubation with indicated dose of simvastatin inhibited the IL-1β+OSM induced CD44 fragmentation in a dose dependent manner (Figure 1B).
Supplementation with mevalonic acid (MA) or the isoprenoids, farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP) and squalene, partially counteracted the inhibitory effect of simvastatin on CD44 fragmentation (Figure 2A). In addition to these results, inhibitors of geranylgeranyl transferase (GGTi) and that of farnesyl transferase (FTi), inhibited CD44 fragmentation in a dose dependent manner (Figure 2B).

Simvastatin disrupts cholesterol rich lipid rafts: Methyl-β-cyclodextrin (MβCD) inhibited the IL-1β+OSM induced CD44 fragmentation (Figure 3A) suggesting that lipid rafts are necessary for cleavage CD44. Sucrose density gradient analysis showed that simvastatin and MβCD significantly lowered the band of the lipid raft maker of flotillin-1 (Figure 3B). From these results, the cholesterol lowering effect with simvastatin to inhibit of CD44 fragmentation appears similar to MβCD. Next, sucrose density gradient analysis was used to detect the localization of MT1-MMP ADAM10 and ADAM17; all three were prominent in lipid raft fractions following IL-1β and OSM stimulation (Figure 3C). Nevertheless, ADAM10 was localized in lipid raft fractions in both normal and stimulated conditions. The raft localization of all three MMPs was disrupted with pre-incubation with MβCD and simvastatin.
Discussion: In the present study, we demonstrated that simvastatin treatment clearly suppressed the CD44 fragmentation induced by IL-1β+OSM. Takahashi et al reported that CD44 fragmentation could have an important role in chondrocytes de-differentiation [1]. Statins inhibit cholesterol synthesis at the level of MA formation or the various intermediate productions including pyrophosphate. Since the inhibitory effect of simvastatin was counteracted by MA supplementation in this study, CD44 fragmentation was inhibited thorough the prevention of MA synthesis, especially GGPP and cholesterol synthesis by simvastatin. These data suggest that simvastatin exerts an inhibitory effect on CD44 fragmentation (induced by IL-1β+OSM) through the suppression of isoprenylation and also cholesterol synthesis. Other studies have reported that activity of membrane type MMPs such as MT1-MMP, ADAM10 and ADAM17 result in CD44 shedding and that CD44 present in lipid rafts is a substrate for these MMPs. In this study, simvastatin disrupted lipid rafts and inhibited membrane type MMPs cleavage of CD44. Our results are consistent with the fact that CD44 is first cleaved at extracellular domain by membrane type MMPs within the cholesterol rich lipid raft. The results of the present study suggest that statins may provide new chondroprotective effects by preventing CD44 fragmentation.

Significance: This study is the first to demonstrate that simvastatin inhibit the CD44 fragmentation induced by IL-1β+OSM. Acknowledgments: We thank Warren Knudson and Cheryl Knudson for their important suggestions to the experiments and providing the anti CD44 cytotail antibodies.


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