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Simvastatin inhibits CD44 fragmentation in chondrocytes

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ABSTRACT

In human osteoarthritic chondrocytes, the hyaluronan receptor CD44 undergoes proteolytic cleavage at the cell surface. CD44 cleavage is thought to require transit of CD44 into cholesterol-rich lipid rafts. The purpose of this study was to investigate whether statins exert a protective effect on articular chondrocytes due to diminution of cholesterol. Three model systems of chondrocytes were examined including human HCS-2/8 chondrosarcoma cells, human osteoarthritic chondrocytes and normal bovine articular chondrocytes. Treatment with IL-1β + Oncostatin M resulted in a substantial increase in CD44 fragmentation in each of the three chondrocyte models. Pre-incubation with simvastatin prior to treatment with IL-1β + Oncostatin M decreased the level of CD44 fragmentation, decreased the proportion of CD44 that transits into the lipid raft fractions, decreased ADAM10 activity and diminished the interaction between CD44 and ADAM10. In HCS-2/8 cells and bovine articular chondrocytes, fragmentation of CD44 was blocked by the knockdown of ADAM10. Inhibition of CD44 fragmentation by simvastatin also resulted in improved retention of pericellular matrix. Addition of cholesterol and farnesyl-pyrophosphate reversed the protective effects of simvastatin. Thus, the addition of simvastatin exerts positive effects on chondrocytes including reduced CD44 fragmentation and enhanced the retention of pericellular matrix.

1. Introduction

CD44 is a single-pass transmembrane glycoprotein receptor and in many cell types, serves as a primary receptor for the glycosaminoglycan hyaluronan (HA). In articular chondrocytes, HA and proteoglycan-rich cell-associated matrices are anchored to the plasma membrane via the binding of HA to CD44 [1–3]. Previous reports have demonstrated proteolytic cleavage of CD44 from the cell surface and shedding in several tumor cell systems [4]. Our previous study demonstrated similar proteolytic cleavage of CD44 from the cell surface of articular chondrocytes, and moreover, that the shedding of CD44 is higher in chondrocytes derived from patients undergoing total knee replacement as well as *in vitro* models used to mimic osteoarthritis (OA) [5]. For example, in human OA chondrocytes, a substantial proportion of the CD44 undergoes degradation as compared to normal chondrocytes derived from human ankle cartilage [5]. CD44 cleavage also can be induced in normal articular chondrocytes by treatment with IL-1β, phorbol myristate acetate or HA oligosaccharides [5,6]. The signature pattern of CD44 degradation, present in both malignant cells and OA chondrocytes, includes the cleavage of the extracellular domain of CD44 by a metalloproteinase such as membrane type I (MT1-MMP, aka MMP14), ADAM17 or ADAM10 (Fig. 1A) [7]. The metalloproteinase action releases a 70 kD CD44 ecto-domain into the extracellular matrix, leaving a 18–20 kD C-terminal truncation fragment within the plasma membrane (termed CD44-EXT) [7]. The CD44-EXT fragment is then cleaved within the intramembranous domain by γ-secretase, releasing a 15 kD intracellular domain (CD44-ICD) into the cytoplasm [5]. The release of these CD44 domains can exert negative influences on chondrocyte function. A previous study reported that release of the CD44-ICD into the cytoplasm of chondrocytes competitively blocks interactions between full-length CD44 and cytoskeletal adaptor

Abbreviations: HA, hyaluronan; OA, osteoarthritis; OSM, Oncostatin M; BAC, bovine articular chondrocytes; HAC, human articular chondrocytes; MT1-MMP, membrane type I metalloproteinase; ICD, intracellular domain; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; FPP, farnesyl-pyrophosphate; GGPP, geranylgeranylpyrophosphate; Mev, mevalonic acid; GGTI, geranylgeranyltransferase 1 inhibitor; FTI, farnesyltransferase inhibitor; MβCD, methyl-β-cyclodextrin.

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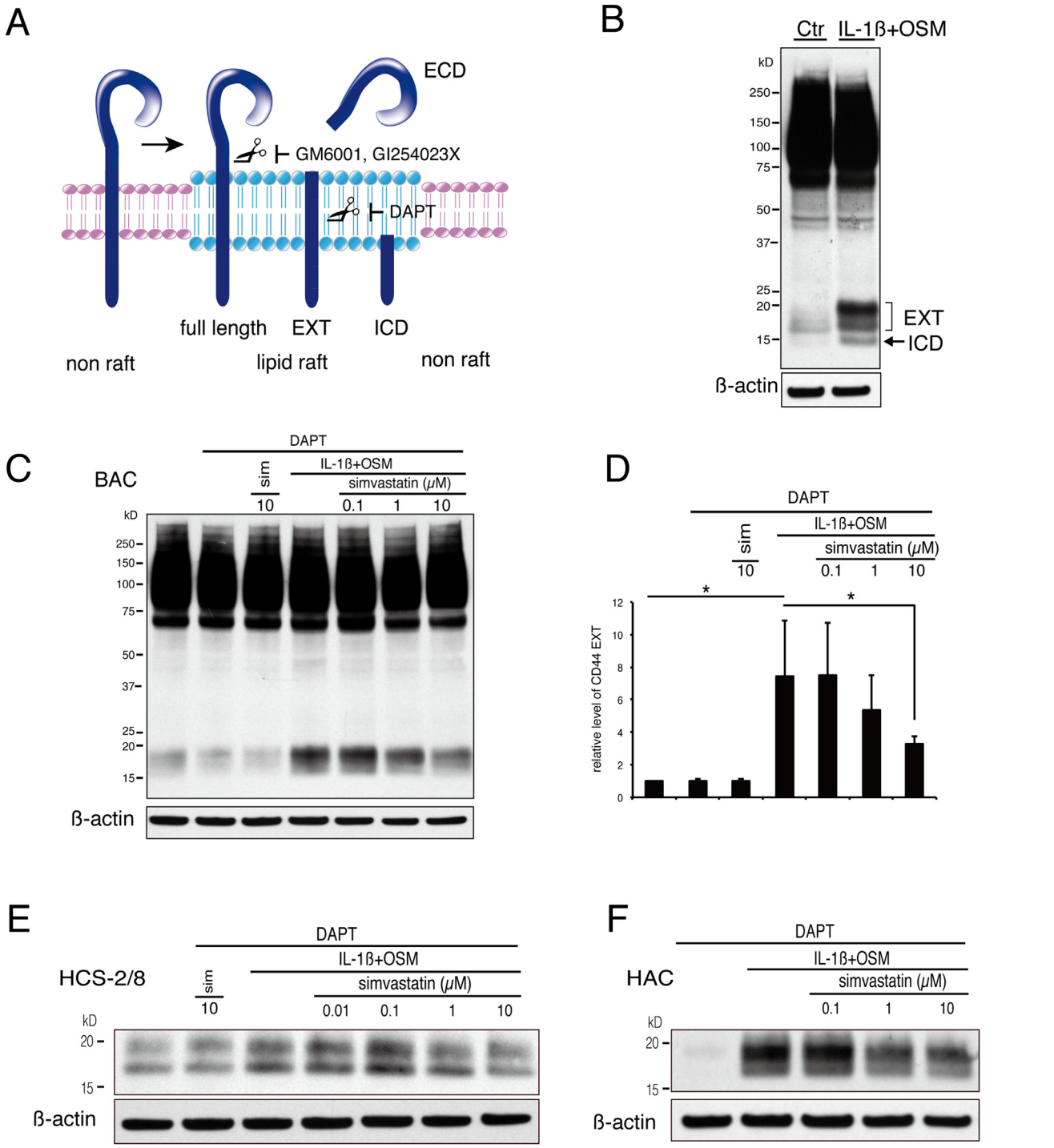

Fig. 1. Simvastatin inhibits CD44 fragmentation induced by IL-1β + Oncostatin M stimulation. (A) CD44 cleavage requires that CD44 transit into lipid raft (horizontal arrow). CD44 can be cleaved by 2 steps. The first is a metalloproteinase mediated cleavage that generates an extracellular domain fragment (ECD) and a C-terminal fragment (CD44-EXT). Next, cleavage of CD44-EXT by γ-secretase generates CD44-ICD. These cleavages can be blocked by inhibitors (GM6001; general MMP inhibitor, GI254023X; ADAM10 inhibitor, DAPT; γ-secretase inhibitor). (B–F) Cell lysates were analyzed by Western blotting using anti-CD44 cytotail antibody. (B) Upon treatment of bovine articular chondrocytes (BAC) with 0.5 ng/ml IL-1β and 10 ng/ml Oncostatin M (OSM) for 48 h, CD44 fragmentation was enhanced. The enhanced fragmentation included the 18–20 kD doublet CD44-EXT bands and the 15 kD CD44-ICD bands. (C) In the presence of 5 μM γ-secretase inhibitor DAPT, pre-incubation with indicated dose of simvastatin for 48 h inhibited the IL-1β + OSM induced CD44 fragmentation in bovine articular chondrocytes (BAC). (D) The histogram depicts mean values of relative level of CD44 EXT band ± S.D. from three independent experiments; $^{*}p < 0.05$. (E) (F) Pre-incubation with indicated dose of simvastatin and 5 μM DAPT for 48 h inhibited the IL-1β + OSM induced CD44 fragmentation in (E) HCS-2/8 cells and (F) human articular chondrocytes (HAC). Panel F is representative of experiments from using chondrocytes from three OA patients.

proteins—interactions that are required to stabilize retention of a pericellular matrix [6]. In other cell systems, release of the shed CD44 ecto-domain acts as a decoy receptor for HA, preventing HA binding to the cell surface [8]. Thus, proteolytic cleavage of CD44 not only results in a loss of full-length CD44 but also the generation of two potential dominant negative domains, leading to continued,