DecR3 induces cell proliferation through MAPK signaling in chondrocytes of osteoarthritis

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Introduction: Decoy receptor 3 (DcR3), a soluble receptor belonging to the TNF receptor (TNFR) superfamily, competitively binds and inhibits the TNF family including Fas-ligand (Fas-L), LIGHT, and TL1A [1][2]. DcR3 is expressed and inhibits the Fas-induced apoptosis in synoviocytes with osteoarthritis (OA) and rheumatoid arthritis (RA) in addition to various tumour cells [3][4]. In this study, we investigated the functions of DcR3 in chondrocytes.

Materials and Methods: Expression of DcR3 in cartilage tissues were detected by immunohistochemistry. Expressions of DcR3 in chondrocytes were measured by RT-PCR and western blotting. OA chondrocytes were incubated with DcR3-Fc chimera protein (DcR3-Fc) before induction of apoptosis by Fas and apoptosis was detected with TUNEL staining and western blotting of caspase 8 and PARP. OA chondrocytes were incubated with DcR3-Fc and the proliferation was analysed by WST assay. Activation of ERK, P38MAPK and JNK in OA chondrocytes were measured by western blotting after incubation with DcR3-Fc, MEK1/2 inhibitor PD098059, or P38 MAPK inhibitor SB203580. Results: DcR3 was expressed in chondrocytes. DcR3-Fc protects OA chondrocytes from Fas-induced apoptosis. DcR3-Fc protein increased cell proliferation and induced the phosphorylation of ERK in OA chondrocytes. DcR3 induced proliferation of OA chondrocytes was inhibited by PD098059.

Results: Immunohistochemical staining of OA cartilage tissues revealed that DcR3 was expressed ubiquitously at both chondrocytes and matrix in all layers of cartilage tissues (Fig 1a).

RT-PCR and real-time PCR revealed that DcR3 mRNA was expressed in all the individual samples of OA chondrocytes (Fig 1b). Western blotting confirmed that DcR3 protein was expressed in OA chondrocytes (Fig 1c).

TUNEL positive apoptotic cells induced by Fas-L in chondrocytes were significantly decreased in a dose dependent manner when chondrocytes were pre-incubated with DcR3-Fc protein (Fig 2a). Cleavage of caspase 8 and PARP was also significantly decreased (Fig 2b). The cleavage of caspase 8 was inhibited to 12 % of control and the cleavage of PARP was inhibited to 1 % of control by pretreatment with 100 ng/ml of DcR3-Fc.

Proliferation assay revealed that the growth rates of chondrocytes were not significantly increased by the treatments with 5000 ng/ml of IgG1 in control medium. However, the growth rates of chondrocytes were significantly increased by the treatments with least 1000 ng/ml of DcR3-Fc in control medium (Fig 2c).

Western blotting revealed that ERK signaling was activated by the treatment with 5000 ng/ml of DcR3-Fc for 10 min (Fig 3a). However, neither P38 MAPK nor JNK signaling was not activated by the treatment with 5000 ng/ml of DcR3-Fc for up to 30 min (Fig 3a).

Proliferation assay revealed that chondrocytes proliferation was significantly increased by the treatment with 5000 ng/ml of DcR3-Fc. However, chondrocytes proliferation increased by DcR3-Fc was inhibited by PD098059 (Fig 3b). Chondrocytes proliferation was increased by the treatment with 50μM of SB203580, and proliferation was further increased by the treatment with SB203580 and DcR3-Fc (Fig 3b).

Western blotting confirmed that the activation of ERK signaling by 5000 ng/ml of DcR3-Fc was inhibited by PD 98059 but not by SB203580 (Fig 3c).

Discussion: DcR3 regulates the proliferation of chondrocytes by protecting the cells from Fas-induced apoptosis and activation of ERK signaling, but not of p38 MAPK or JNK. DcR3 is a possible key molecule contributing to the regeneration of cartilage in OA.


Figure 1
(a) Immunohistochemistry
(b) RT-PCR
(c) Western blotting

Figure 2
(a) Proliferation assay
(b) Cleaved Caspase 8 and PARP

Figure 3
(a) Western blotting
(b) ERK signaling

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