Centrosomal Protein 70kda Is Down-regulated By Decoy Receptor 3 In Specifically Rheumatoid Synovial Fibroblasts

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Introduction: Decoy receptor 3 (DcR3) is a secreted decoy tumor necrosis factor receptor (TNFR) and competitively binds and inhibits the TNF family including Fas-ligand (FasL), LIGHT, and TL1A. DcR3 is overexpressed in tumor cells and might benefit tumors by helping them to avoid cytotoxic and regulatory effects of the ligands. We previously reported that DcR3 overexpressed in rheumatoid synovial fibroblasts (RA-FLS) stimulated by TNF-alpha protects the cells from Fas-induced apoptosis [1]. We recently reported that DcR3 induces VLA-4 expression in THP-1 macrophages to inhibit cycloheximide-induced apoptosis [2], and that DcR3 binds to TL1A expressed on RA-FLS resulting in the negative regulation of cell proliferation induced by inflammatory cytokines [3]. Therefore, DcR3 may regulate gene expressions in RA-FLS by binding to TL1A on RA-FLS as a ligand. Further, we newly revealed the gene expression profiles in RA-FLS regulated by DcR3 by using microarray data analysis. The profiles indicated centrosomal protein 70kDa (CEP70) was down-regulated by DcR3 (fold change 1.87) [4]. Centrosome forms the backbone of cell cycle progression mechanism. Further, CEP family protein is the active component of centrosome and plays a vital role in centriole biogenesis and cell cycle progression control [5]. In this study, we studied CEP70 as one of the key molecules in DcR3-TL1A signaling in RA-FLS based on the genes expression profiles regulated by DcR3.

Methods: Isolation and culture of synovial fibroblasts. Synovial tissues were obtained from patients with RA fulfilling the 1987 criteria of American College of Rheumatology who underwent total knee arthroplasty but had never been treated with biologics, under a research protocol approved by the ethics committee. Tissue specimens were minced and digested in Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Grand Island, NY) containing 0.2% collagenase (Sigma, St. Louis, MO) for 2 hours at 37°C. Dissociated cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD) and 100 units/ml of penicillin/streptomycin. After overnight culture, non-adherent cells were removed, and adherent cells were further incubated in fresh medium. All experiments were conducted using cells from passages 3-4.

Real-time polymerase chain reaction (real-time PCR). RA and osteroarthrititis (OA) -FLS were stimulated with 1ng/ml of recombinant human TNFα or IgG1 for 24 hours, or with various concentration of DcR3-Fc or control IgG1 for 12 hours. After the incubation, RNA was extracted with QIAshredder and RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacture’s protocol and reverse-transcribed to cDNA. The relative expression levels of CEP70 mRNA were compared using TaqMan® real-time PCR on StepOne™ real-time PCR system (Applied Biosystems, Foster City, CA). Pre-designed primers and probes
for CEP70 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control were obtained from Applied Biosystems.

Immunohistochemistry. Rabbit anti-human CEP70 polyclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) diluted 1:200 was applied to frozen sections of synovial tissues from patients with RA or OA for over night. Sections were stained with HistoFine simple stain Kit and DAB chromogen (Nichirei, Tokyo, Japan), followed by counterstaining with hematoxylin.

**Results:** Real-time PCR revealed that the expression of CEP70 mRNA in RA-FLS was higher than that in OA-FLS (Figure 1) and that TNFα significantly decreased the expression of CEP70 mRNA in RA and OA-FLS (Figure 2A and 2B; RA, 51%; OA, 59%). DcR3-Fc also significantly decreased the expression of CEP70 mRNA in RA-FLS in a dose dependent manner (Figure 2C; 81% with 10ng/ml, 73% with 100ng/ml, and 57% with 1000ng/ml). In contrast, DcR3-Fc did not decrease CEP70 mRNA in OA-FLS (Figure 2D). Immunohistochemical analysis revealed that CEP70 protein was expressed more in superficial lining layer of rheumatoid synovium than that of OA synovium (Figure 3).

**Discussion:** In this study, we revealed that CEP70 was increased in RA-FLS and that the expression of CEP70 in RA-FLS was decreased by DcR3 in a disease-specific fashion. DcR3 may affect the pathogenesis of RA through CEP70.

**Significance:** This study newly revealed DcR3 may contribute to cell cycle progression control through CEP70 in RA-FLS.

2. Tateishi K. et al., Biochem Biophys Res Commun. 389, 593-598, 2009

Figure legends:

Figure 1. Expression of CEP70 mRNA in RA or OA-FLS. (A) Relative expression levels of CEP70 (black bar) mRNA in RA-FLS. n=10. (B) Relative expression levels of CEP70 (black bar) mRNA in OA-FLS. n=10. The expression levels of GAPDH mRNA were assigned a value of 1 (white bar).

Figure 2. Expression of CEP70 mRNA in RA or OA-FLS incubated with various stimulants. (A) Relative expression levels of CEP70 mRNA in RA-FLS after 24 hours of incubation with 1000 ng/ml (black bar) DcR3-Fc, or serum-free Opti-MEM medium only (white bar). n=12. (B) Relative expression levels of CEP70 mRNA in OA-FLS after 24 hours of incubation with 1000 ng/ml (black bar) DcR3-Fc, or serum-free Opti-MEM medium only (white bar). n=10. (C) Relative expression levels of CEP70 mRNA in RA-FLS after 12 hours of incubation with 10 (light gray bar), 100 (dark gray bar), or 1000 ng/ml (black bar) DcR3-Fc, 1000 ng/ml IgG1 (oblique lined bar), or serum-free Opti-MEM medium only (white bar). n=10. (D) Relative expression levels of CEP70 mRNA in OA-FLS after 12 hours of incubation with 1000 ng/ml (black bar) DcR3-Fc, 1000 ng/ml IgG1 (oblique lined bar), or serum-free Opti-MEM medium only (white bar). n=10. Untreated cells were assigned a value of 1. *P<0.05, **P<0.01.

Figure 3. Immunohistochemical analysis. Photographs illustrate synovial tissue sections from patients with RA or OA. Scale bars; 50 µm.
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