DCR3 EXPRESSED IN RHEUMATOID SYNOVIAL FIBROBLASTS PROTECTS THE CELLS FROM FAS-INDUCED APOPTOSIS.

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Introduction
Decoy receptor 3 (DcR3)/TR6/M68 is a member of the tumor necrosis factor receptor (TNFR) superfamily. DcR3 lacks the transmembrane domain of conventional TNFRs to be a secreted protein. DcR3 is overexpressed in a variety of tumors, including lung and colon cancers1, gliomas, gastrointestinal tract tumors, autoimmune tissues, and virus-associated leukemias. Over expression of DcR3 might benefit tumors by helping them avoid cytotoxic and regulatory effects of FasL, LIGHT, and TL1A. Meanwhile, rheumatoid arthritis (RA) is the inflammatory joint disease characterized by hyperplasia of synovial tissue and pannus formation growing invasively into the cartilage, followed by cartilage and bone destruction. Analyses of hyperplastic synovial tissues of patients with RA reveal features of transformed long-living cells such as somatic mutations, expression of oncoproteins, and resistance to apoptosis. The resistance to apoptosis further contributes to synovial hyperplasia and is closely linked to the invasive phenotype of rheumatoid synovial fibroblasts (RA-SFs). We hypothesize that DcR3 may contribute to the pathogenesis of RA to be a noble therapeutic intervention for RA. In this study, we investigated DcR3 expression in RA-SFs and analyzed the function of DcR3 to cell apoptosis induced by Fas.

Materials and Methods
Expression of DcR3 in RA-SFs
RA-SFs were prepared from synovial tissues obtained during joint surgery from patients with RA. Expression of DcR3 in RA-SFs was measured by RT-PCR and immunoblotting.

Down-regulation of DcR3 and induction of apoptosis by Fas-L
DcR3 siRNA was transfected into RA-SF by lipofection method. Relative expression levels of DcR3 mRNA were compared between the RA-SFs transfected with DcR3 siRNA and non-specific control siRNA by means of TaqMan® real time PCR. Apoptosis in RA-SFs was induced by Fas-L (100ng/ml) for 24h in the presence of cycloheximide (100µg/ml).

Detection of apoptosis
Apoptosis was detected with TUNEL method and immunoblotting of cleaved caspase 8 and cleaved PARP (Poly(ADP) ribose polymerase).

Results
DcR3 mRNA was expressed in RA-SFs, and DcR3 protein was expressed in the cytoplasm of RA-SFs
DcR3 mRNA was expressed in all the RA-SF samples investigated (Fig. 1a). DcR3 protein was also expressed in the cytoplasm of all the samples (Fig. 1b).

DcR3 siRNA introduced to knock-down for DcR3 expression
DcR3 expression in RA-SFs was significantly down-regulated by the transfection with DcR3 siRNA compared with non control specific siRNA (Fig. 2).

Down-regulation of endogenous DcR3 induced Fas-induced apoptosis in RA-SFs
Down-regulation of endogenous DcR3 by the transfection of DcR3 siRNA increased Fas-induced apoptosis in RA-SFs. TUNEL positive cells were significantly increased in RA-SFs transfected with DcR3 siRNA compared with control (Fig. 3a,b). The cleavage of caspase 8 and PARP were increased in RA-SFs transfected with DcR3 siRNA compared with non specific control siRNA (Fig. 4a,b).

Conclusion
DcR3 was expressed in RA-SFs. Down-regulation of endogenous DcR3 increased Fas-induced apoptosis in RA-SFs. Expression of DcR3 may contribute to the hyperplasia of synovial tissues in RA. The inhibition of DcR3 expression might be a noble therapeutic intervention for RA.

Paper No: 1568
52nd Annual Meeting of the Orthopaedic Research Society