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AB0065

N-CADHERIN IS DOWN-REGULATED BY DECOY RECEPTOR 3 IN SPECIFICALLY RHEUMATOID SYNOVIAL FIBROBLASTS

K. Fukuda¹, Y. Miura², S. Hayashi¹, T. Maeda³, R. Kuroda¹. ¹Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan; ²Division of Orthopedic Science, Department of Rehabilitation Science, Kobe University Graduate School of Health Sciences, Kobe, Japan; ³Department of Immunology and Rheumatology, Stanford University, Stanford, USA

Background: Decoy receptor 3 (DcR3) is a secreted decoy tumour necrosis factor receptor and competitively binds and inhibits the TNF family including Fas ligand, LIGHT, and TL1A. We previously reported that DcR3 overexpressed in rheumatoid synovial fibroblasts (RA-FLS) stimulated by TNF α protects the cells from Fas-induced apoptosis.¹ We recently reported that DcR3 binds to TL1A expressed on RA-FLS resulting in the negative regulation of cell proliferation induced by inflammatory cytokines.² Further, we newly revealed the gene expression profiles in RA-FLS regulated by DcR3 by using microarray data analysis.³ Among the genes in the profile, we demonstrated the possible involvement of tryptophan hydroxylase 1 down-regulated,⁴ interleukin 12B up-regulated by DcR3⁵ and centrosomal protein 70 kDa⁶ in the pathogenesis of RA. The profiles also indicated that Cadherin 2/type 1 N-cadherin (CDH2) was up-regulated by DcR3 (fold change 1.93).³ CDH2 has been reported to be associated with cell attachment and migration,⁷ osteoblast differentiation,⁸ and the proliferation of RA-FLS.⁹ The hemophilic interaction of CDH2 suppresses the proliferation of RA-FLS through increasing the P27^{Kip1} that inhibit cell-cycle progression.⁹

Objectives: In this study, we analysed CDH2 expression in RA-FLS stimulated with DcR3 in detail to reveal the involvement of CDH2 and DcR3 in the pathogenesis of RA.

Methods: Real-time polymerase chain reaction (real-time PCR). RA-FLS were stimulated with various concentration of DcR3-Fc or 1,000 ng/ml of IgG1, or left untreated in serum-free Opti-MEM for 12 hour. The relative expression levels of CDH2 mRNA were quantified by real-time PCR.

Immunohistochemistry.

Anti-CDH2 antibody was applied to frozen sections of synovial tissues from patients with RA or OA for overnight. After that, the expression of CDH2 protein was evaluated by immunohistochemical analysis.

Results: Real-time PCR demonstrated that DcR3-Fc significantly increased the expression of CDH2 mRNA in RA-FLS (104% with 10 ng/ml, 112% with 100 ng/ml, and 200% with 1000 ng/ml). Immunohistochemistry revealed that CDH2 was expressed more in the sublining layer of rheumatoid synovium than OA synovium.

Conclusions: In the gene expression profiles, we focused on CDH2 as the gene was highly up-regulated and belonged to major functional clustering categories; protein complex assembly, cell motility, regulation of transcription, cell membrane and glycosylation. In this study, we showed that the expression of CDH2 mRNA in RA-FLS was induced by DcR3 and that CDH2 was increased in the sublining layer of rheumatoid synovium. In this study, we demonstrated that DcR3 significantly induced CDH2 expression in RA-FLS. Considering the fact that CDH2 inhibits RA-FLS proliferation, DcR3 signalling may control the hyperplasia of RA synovium.

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AB0066

COMBINATION OF IL-10 AND IL-18 BUT NOT IL-6 AND IL-18 INDUCES IFN-GAMMA PRODUCTION AND SURFACE EXPRESSION OF TRAIL ON NK CELLS

K. Sato, Y. Aizaki, H. Yazawa, T. Mimura. Department of Rheumatology and Applied Immunology, Saitama Medical University, Saitama, Japan

Background: Adult-onset Still's disease (AOSD) is a systemic inflammatory disease, the cause of which is largely unknown. AOSD has been recently classified as one of the autoinflammatory diseases, in which innate rather than acquired immunity plays an important role. Serum IL-18 has been shown to be significantly high in AOSD patients.

Objectives: The aim of this study was to quantify the levels of multiple cytokines in the serum of AOSD patients, and compare the serum cytokine profile with that of healthy controls. We also attempted to evaluate the effects of the cytokines that were upregulated in the AOSD serum on natural killer (NK) cells, since NK cells are cells of innate immunity and IL-18 has been shown to enhance their cytotoxicity.¹

Methods: We quantified the serum levels of 10 cytokines (IFN- α , IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A and TNF- α) in patients with AOSD and healthy controls using multiplex bead array assays and IL-18 using ELISA. We next sorted NK cells from peripheral blood mononuclear cells (PBMCs) of healthy controls, stimulated them *in vitro*, and quantified the level of IFN- γ in the culture supernatant by ELISA and also assessed the surface expression level of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) on the cells.

Results: Compared to samples from healthy controls, the mean serum levels of IL-6 and IL-18 from AOSD patients were significantly higher. IL-10 was detectable in some of the patients. Originally, IL-18 was identified as a stimulator of IFN- γ production², however, serum IFN- γ was below the detection limit. When NK cells were stimulated *in vitro* with IL-18 alone, the protein level of IFN- γ in the culture supernatant was still below the detection limit. When we further added IL-6 and/or IL-10 to the culture, the combination of IL-10 and IL-18, but not IL-6 and IL-18, induced IFN- γ . As IL-6 is a classic pro-inflammatory cytokine and IL-10 is considered to be an anti-inflammatory cytokine, this result was rather unexpected. Thus, we evaluated the expression of IL-6 and IL-10 receptors on NK cells and found that IL-10 receptors (IL-10R and IL-10R β) were present, but IL-6 receptors (IL-6R and gp130) were absent. We also assessed TRAIL expression on NK cells. Here, too, the combination of IL-10 and IL-18 induced TRAIL expression the most potently. Although a previous research showed that the combination of TLR3 and IL-18 signalling synergistically induced TRAIL on NK cells in an IFN- γ dependent manner³, the addition of an anti-IFN- γ antibody did not diminish the TRAIL expression.

Abstract AB0066 – Figure 1

Conclusions: A combination IL-10 and IL-18, not IL-6 and IL-18, induced the production of IFN- γ and surface expression of TRAIL in NK cells. This TRAIL expression did not evidently depend on IFN- γ . TRAIL was expected to be useful for the treatment of malignancy, but it turned out to be toxic to hepatocytes⁴. Since NK cells are rich in the liver and the abnormality of liver function is among the major symptoms in AOSD, we suggest that the combination of IL-10 and IL-18 may cause liver dysfunction by inducing TRAIL on NK cells in the liver.

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