**INTRODUCTION**

DcR3 is recently identified soluble decoy receptor that competes with Fas in binding to FasL and inhibits Fas mediated apoptosis. Decoy receptor 3 (DcR3)/ TR6 is a member of the tumor necrosis factor receptor superfamily. DcR3 binds to at least three different ligands : Fas ligand (FasL), LIGHT, and TL1A. Overexpression of DcR3 has been reported in lung cancers, colon adenocarcinomas, lymphomas, gastrointestinal tumor, hepatocellular carcinoma, and gliomas.

We reported that mRNA of DcR3 was expressed in bone and soft tissue tumors. We showed that DcR3/Fas ratio in high-grade malignant tumors was higher than that in benign tumor.

In this study, we demonstrated that DcR3 siRNA enhanced Fas mediated apoptosis in osteosarcoma cells.

**MATERIALS AND METHODS**

**Tumor samples**

We used 3 osteosarcoma cell lines (KHOS, KTHOS, MG63). The cell lines were cultured in MEM medium containing 10% FBS and antibiotics.

Expression of DcR3 was measured by RT-PCR, real time PCR, and western blotting.

**Down-regulation of DcR3**

DcR3 siRNA and non specific siRNA control (Dharmacon Inc) were transfected into 3 cell lines using Lipofectamin 2000 according to the manufacturer’s protocol. DcR3 siRNA and control was applied to 3×10^5 cell with Opti MEM. After incubation for 6 h, the mixture was replaced with MEM supplemented with 10% FBS and incubated for 24 h.

**Real time PCR**

DcR3 mRNA was amplified for 45 cycles. PCR products were measured by ABI PRISM 7700 Sequence Detection System (Applied Biosystem). Inhibition levels of DcR3 mRNA were compared between 3 cell lines transfected with DcR3 siRNA and non specific control siRNA by real time PCR.

**Fas-induced apoptosis in osteosarcoma cell lines**

Osteosarcoma cell lines transfected DcR3 siRNA were incubated with recombinant human Fas-ligand (hFas-L) (R&D Systems) and adriamycin for 12h.

**TUNEL staining**

2×10^5 osteosarcoma cell line were cultured in 8-well chamber slides. After various stimulations, apoptotic cells were determined using TUNEL assay kit (Wako) according to the manufacturer’s protocol.

**Statistical analysis**

We used t-test for comparisons between groups. P-values less than 0.05 were considered significant.

**RESULTS AND DISCUSSIONS**

Expression of DcR3 mRNA was inhibited by siRNA. Extent of DcR3 mRNA was 44% in KHOS, 39% in KTHOS and 27% in MG63, compared to the controls (Fig.1).

The number of TUNEL positive cells was not significantly different between in cells treated with DcR3 siRNA and FasL, and in cells treated with control DcR3 siRNA and FasL (Fig.2A). The number of TUNEL positive cells was significantly higher in the cells treated with DcR3 siRNA, FasL and adriamycin than that in the cells treated with control DcR3 siRNA, FasL and adriamycin (Fig.2B).

Wei et al. has reported that DcR3 mRNA expression showed significant relationship with the apoptosis index in HCC. He reported that there was the relationship with DcR3 mRNA expression and drug induced cell death.

In our study, expression of DcR3 mRNA could decrease by siRNA. Down regulation of DcR3 expression by siRNA enhanced adriamycin induced cell death.

**CONCLUSIONS**

Down regulation of DcR3 enhanced adriamycin -induced cell death in vitro. Therefore, adriamycin treatment coupled with down regulation of DcR3 may be one of new therapies for osteosarcoma.

**REFERENCES**