DcR3 expresses in bone and soft tissue tumors

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 reported in lung cancers, colon adenocarcinomas, lymphomas, gastrointestinal tumor, hepatocellular carcinoma, and gliomas. In contrast, no data are available regarding the expression and amplification of DcR3 gene in bone and soft tissue tumors. We investigate DcR3 expression in bone and soft tissue tumors and analysed the functions of DcR3 to Fas-induced apoptosis.

MATERIALS AND METHODS

Tumor samples: Twenty tumor samples were obtained by open biopsy at Kobe University Hospital, Japan. Informed consents were obtained from all patients. Tumor tissues were one osteosarcomas, 4 MFH, 6 liposarcomas, 2 synovial sarcomas, one chondrosarcomas, 5 schwannomas, one giant cell tumor.

Cell Lines: Seven cell lines were 3 osteosarcomas (KHOS, KTHOS and MG63) and 4 MFH (Nara H, Nara F, TNMY-1 and GBS-1). KTHOS and TNMY-1were established in our laboratory. GBS-1 was kindly provided by Dr. H. Kanda (Department of Pathology, The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Japan). Nara-F and Nara-H were purchased from ScienStuf Co., Nara, Japan. The cell lines were cultured in MEM medium containing 10% FBS and antibiotics.

RT-PCR: Total RNAs were isolated using an RNeasy Mini Kit® from tumor samples and cell lines. RNA was converted to cDNA by reverse transcription and amplified for 35 cycles by PCR. GAPDH was used as an internal control for RNA integrity. RT-PCR products were run on 2% agarose gel, stained with ethidium bromide, and visualized by UV illumination.

Real time PCR: The reaction was carried out in 7 cell lines. PCR products were measured by ABI PRISM 7700 Sequence Detection System. Relative expression levels of DcR3 were compared with expression of GAPDH. We used SW480 (colon adenocarcinoma cell line) as positive control.

Western blotting: To analyze DcR3 proteins, we used 7 cell lines. After isolating proteins in cytoplasm, proteins were separated under reducing condition by electrophoresis on 12% polyacrylamide gel and transblotted electrically onto PVDF membrane. DcR3 proteins were detected using mouse anti-human DcR3 monoclonal antibody.

Down-regulation of DcR3: DcR3 siRNA was transfected into 7 cell lines by lipofection method. Inhibition levels of DcR3 mRNA were compared between 7 cell lines transfected with DcR3 siRNA and non specific control siRNA by real time PCR.

RESULTS

DcR3 mRNA was highly expressed in 12 of 14 (85%) malignant bone and soft tissue samples. DcR3 mRNA was expressed in 6 of 12 (50%) benign bone and soft tissue tumor samples (Fig.1). However, there was no significant difference between malignant tumor samples and benign samples (p=0.910). By result of real time PCR, expression of DcR3 in osteosarcoma was about 34% of expression in SW480. Expression of DcR3 in MFH was about 23% (Fig.2). In all (100%) malignant bone and soft tissue tumor cell lines, both mRNA and protein of DcR3 expressed (Fig.3). Down regulation of DcR3 by siRNA was detected in KHOS, KTHOS, MG63, and GBS1, in which DcR3 were relatively overexpressed among 7 bone and soft tissue cell lines (Fig. 2 and 4).

DISCUSSIONS AND CONCLUSION

Shen et al. has reported that 29 of 48 (60%) hepatocellular carcinomas were positive for mRNA expression. Tsuji et al. has reported that DcR3 highly expressed in 5 of 7 (71%) pancreatic cancer cell lines and 10 of 15 (67%) pancreatic cancer tissues. In comparison with those studies, our study showed that DcR3 highly expressed in malignant bone and soft tissue tumors. Down regulation of DcR3 gene expression by siRNA may depend on the potential extent of DcR3 gene expression. Therefore, inhibition of DcR3 overexpression may lead to apoptosis and result in reduction of tumor growth.