INTRODUCTION: The poliovirus is a nonenveloped plus-strand RNA virus belonging to Picornaviridae, and is the causative pathogen of paralytic poliomyelitis. The selective targeting of motor neurons by poliovirus is most likely determined by the distribution of its cellular receptor, the Ig superfamily molecule CD155 (also known as poliovirus receptor: PVR). Recently, a number of reports have indicated that the poliovirus induces cell death in some types of cancer originating from the neural crest, including malignant glioma [1] and neuroblastoma [2]. We herein investigated whether a soft tissue sarcoma cell line, HT1080, shows upregulated expression of CD155. We also examined whether live-attenuated polioviruses have oncolytic effects on soft tissue sarcomas in vitro and in vivo.

METHODS: Cell Lines: HT1080 human fibrosarcoma cells were maintained in MEM supplemented with fetal bovine serum. Virus: A live-attenuated poliovirus vaccine containing the Sabin 1 strain (Japan Poliomyelitis Research Institute, Japan) was used as an oncolytic virus. The virus titer was determined by measuring the 50% tissue culture infective dose (TCID50) in HeLa cells. Viability assay: HT1080 cells were seeded at 1.0x10^4 per well on 96 well plates and treated with live-attenuated poliovirus at a multiplicity of infection of 2, 0.2, or 2.0x10^-2 TCID50/cell, respectively. At different intervals, the cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay (Promega, USA). Real-time reverse transcription-polymerase chain reaction (RT-PCR): Total RNA was extracted from the cells using ISOGEN (Nippon Gene, Japan). RNA was then reverse-transcribed into cDNA using the 1st Strand cDNA Synthesis Kit for RT-PCR (Roche Applied Science, Germany). The real-time quantitative PCR analysis was performed on an ABI PRISM® 7000 instrument (Applied Biosystem, USA) using TaqMan® Gene Expression Master Mix (Applied Biosystem). The endogenous 'house-keeping' gene, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), was used to evaluate the efficiency of reverse transcription. The primers and probe for real time PCR were purchased from Applied Biosystems. Western blotting analysis: The primary antibody was a goat anti-CD155 antibody, sc-27754 (1:200, Santa Cruz Biotechnology, USA) [3], and the secondary antibody reaction was performed using a peroxidase-conjugated secondary antibody (Dako, Carpinteria, USA) and visualized using the ECL substrates (GE Healthcare Bio-Sciences, USA). In vivo xenograft model: For xenografting, 1x10^5 HT1080 cells in 0.1 ml of PBS were subcutaneously injected with a 26-gauge needle into the right flanks of 4-week-old BALB/c nu/nu mice. The tumor size was measured with calipers two a week, and the tumor volume was calculated using the ellipsoid formula: length x width^2 x 0.52. The live-attenuated poliovirus (1x10^5 TCID50, n=13) was injected into the right flank tumor only once (or vehicle alone for control animals, n=10) when the tumor volume reached 0.20-0.25 cm^3, and the tumor size was subsequently monitored every 3-4 days.

RESULTS: The expression of CD155 in HT1080 cells. First, we examined the expression of CD155 in HT1080 cells. Quantitative real-time PCR revealed that the expression levels of CD155 mRNA in HT1080 was 2.0-fold higher than that in HeLa cells. (p<0.05, Student’s t-test)(Fig. 1A). A similar level of CD155 protein expression was observed in HT1080 cells and HeLa cells (molecular weight ~80 kDa)(Fig. 1B). These results demonstrate that CD155 is highly expressed in HT1080 cells.

DISCUSSION: We clearly showed that live-attenuated polioviruses possess an inherent capacity to increase the viability of HT1080 sarcoma cells in vitro and suppress their growth in vivo as xenograft tumors. A poliovirus receptor, CD155 is essential for the development of an infection in the tumor cells. Although CD155 has been shown to be selectively expressed on a wide variety of tumor cells [4], the expression pattern of CD155 in soft tissue sarcoma has not been examined. Therefore, a further evaluation of the CD155 expression in soft tissue sarcomas is necessary before this oncolytic therapy can be applied for clinical studies. In this experiment, the evaluation of neural cytotoxicity was impossible, because mouse CD155 is not able to bind to Sabin 1 strains. Thus, further confirmation of the safety of this treatment is warranted.

We demonstrated the expression of both CD155 mRNA and protein in the HT1080 fibrosarcoma cell line, and showed the therapeutic potential of a novel oncolytic virotherapy using live-attenuated poliovirus for bone and soft tissue sarcomas.

SIGNIFICANCE: Our novel oncolytic virotherapy using live-attenuated poliovirus has a potential to be used for bone and soft tissue sarcoma treatment without any complications.