Introduction: Osteosarcoma is a malignant tumor that most commonly affects adolescents and young adults. The development of effective chemotherapy and wide local excision has led to improved prognosis of patients with osteosarcoma. But pulmonary metastases occurred and are major reason for fatal outcomes. Only 20% of the patients were alive three years after developing pulmonary metastases in spite of aggressive chemotherapy and thoracotomy. In this study, we examined gene therapy for pulmonary metastases in murine osteosarcoma in order to improve the prognosis of patients with osteosarcoma.

Materials and Methods:

Tumor cells and animals: A murine osteosarcoma cell line, LM8, which preferentially metastasizes to lungs was used for evaluation of the efficacy of gene therapy. Male C3H/He mice aged 6 weeks were used to determine in vivo experiments.

Production of recombinant retrovirus vectors and retrovirus infection:

We produced recombinant retrovirus vectors (pLTRNL) bearing a herpes simplex virus thymidine kinase (HSV-tk) gene. The strategy is to infect osteosarcoma cells by virtue of HSV-tk gene expression, and to sensitize them to antiherpetic drug, ganciclovir (GCV). Ganciclovir is a guanosine analog, which is metabolized to a cytotoxic product by HSV-tk. In the other vector (LZRNL) the HSV-tk gene was replaced by Escherichia coli ß-galactosidase (lacZ) gene. To produce a transmissible virus, vector DNA was transfected with the calcium phosphate coprecipitation method into amphotropic producer cells (PA317). The murine osteosarcoma cells (LM8) were plated in 10 cm dishes and infected 24 h later by exposure for 48 h to virus from the PA317/LTRNL vector-producer line or the PA317/LZRNL in the presence of 8µg/ml polybrene. These were cultured with previous medium containing the G418 (neomycin analogue). The G418-resistant clones of LM8/LTRNL (LM8-tk) and LM8/LZRNL (LM8-Z) were selected randomly from the surviving colonies and used in the following experiments.

In vitro GCV sensitivity and bystander effect: The cytotoxicity of GCV was determined by using a tetrazolium-based colorimetric assay (MTT assay). LM8-tk and LM8-Z were cocultured in various ratios to examine bystander effect. The remarkable inhibition of tumor growth and suppression of tumor volume were confirmed in vivo experiments.

In vivo experiments: LM8-tk and LM8 cells were injected into C3H/He mice subcutaneously and GCV were administered, intraperitoneally. The tumor volume were examined for 28 days. We checked the weight of mice, the wet weight of removed lungs and the metastatic nodules on their surfaces on day 28.

Results: The morphology of the murine osteosarcoma cell line was unchanged after retroviral vector-mediated transduction of lacZ or HSV-tk genes.

In vitro GCV sensitivity: The cytotoxic activity of GCV was dose-dependent in the HSV-tk gene-transduced clones of the murine osteosarcoma cell line. However, no effect was show in cells without gene transduction. 47% of the gene transfected cells were killed at the concentration of 1 µM of GCV, and 86% of the cells at the concentration of 10µM(Fig 1.).

In vitro bystander tumoricidal effect: We observed the bystander effect at various cell ratio in cocultures of LM8-tk and LM8-Z. At a ratio of LM8-tk : LM8-Z cells of 1 : 0 to 1 : 2, 65% of cells were killed (Fig 2.).

In vivo experiments: The mean weight of mice after injected with LM8-tk cells was 25g and the weight of mice after injected with LM8 was 20g. The mean wet weight of lungs treated with LM8-tk was 162 mg and the mean wet weight of lungs treated with LM8 was 216 mg. On day 28 the mean tumor size of LM8 was 1854 mm3 and the mean number of nodules were 12. The mean tumor size of LM8-tk was 53 mm3 and suppressed significantly. Pulmonary metastases were not observed macroscopically(Fig 3.).

Conclusion and discussion: Gene therapy have been applied to many incurable cancers. The transfer of HSV-tk gene seems to be an important tool for gene therapy for malignancy. When HSV-tk transduced cells was present, nontransduced tumor cells were also destroyed, showing bystander effect. The remarkable inhibition of tumor growth and suppression of tumor volume. (Tumor volume = 1/2xaxbxh a: long diameter, b : short diameter)

References


**Department of Orthopaedic Surgery, Osaka Hospital for Railroad Employees, Osaka-City, Osaka Prefecture, Japan.

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