SPANDIDOS PUBLICATIONS

Reovirus inhibits the peritoneal dissemination of pancreatic cancer cells in an immunocompetent animal model

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Abstract. The prognosis of pancreatic cancer with peritoneal dissemination has not improved. The aim of this study was to clarify whether oncolytic reovirus is effective against the peritoneal dissemination of pancreatic cancer in an immunocompetent animal model. The hamster pancreatic cancer cells HaP-T1 were inoculated into the peritoneal cavity of the hamster and reovirus ($1x10^8$ plaque-forming units) was administered into the peritoneal cavity on days 1, 3, 5 and 7 after HaP-T1 inoculations. The number and weight of the disseminated nodules in each group were recorded. Reovirus protein in the disseminated nodules was examined by immunohistochemical staining. The tumor volumes of peritoneal dissemination in the treatment group were significantly less than those in the control group ($p<0.05$). In addition, the amount of ascites was decreased in the treatment group in comparison to the control group. Immunohistochemical examination revealed that reovirus replication was seen only in the disseminated nodules but not in surrounding normal tissues. There were no serious side effects observed in this study. These data suggested that intraperitoneal administration of reovirus might be an effective form of oncolytic viral therapy for peritoneal dissemination of pancreatic cancer.

Introduction

Pancreatic cancer is a major cause of cancer death not only in Western countries but also increasingly in Japan. The prognosis of this disease is still poor with a 5-year survival rate of <5% (1,2). The principle reason of the poor prognosis is the high frequency of peritoneal dissemination and/or liver metastasis at the time of diagnosis or operation (3,4). In particular, patients with peritoneal dissemination suffer from abdominal distention or pain caused by peritonitis carcinomatosa. Unfortunately, the efficacy of systemic chemotherapy against peritoneal dissemination of pancreatic cancer is not sufficient. Therefore, the development of an effective modality of treatment is necessary in order to regulate the peritoneal dissemination of pancreatic cancer.

Human reovirus is a unique oncolytic, non-enveloped virus containing 10 segments of double-stranded RNA as its genome. Reovirus is a common isolate of the respiratory and gastrointestinal tract of humans, but it is not associated with any known human diseases and considered to be benign (5). Reovirus requires an activated Ras signaling pathway via direct Ras mutation or downstream of independent pathways of Ras such as epidermal growth factor receptor or Her-2 (Neu/ErbB-2) or sos in infected cultured cells (6). Activated Ras or an activated element of the Ras pathway inhibits double-stranded RNA-activated protein kinase activation thus allowing both viral protein synthesis and lytic infection to occur. The oncolytic efficacy of reovirus has been demonstrated in several cancer models *in vivo* such as models for malignant glioma, colon, ovarian and breast cancers and lymphoid malignancy (7-10). In addition, pancreatic cancer is susceptible to reovirus because the disease frequently activates the Ras signaling pathway with K-ras mutations (11). Beside, the antitumor efficacy of reovirus against liver metastasis of pancreatic cancer was confirmed in an immunocompetent animal model (12).

This study investigated the antitumor effect of intraperitoneal (i.p.) administration of reovirus against peritoneal dissemination of pancreatic cancer in an immunocompetent animal model.

Materials and methods

Cell line and virus. A Syrian golden hamster pancreatic cancer cell line HaP-T1, obtained from RIKEN cell bank (Ibaragi, Japan), was cultured in minimum essential medium containing 10% FBS, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and antibiotics. A previous study confirmed the HaP-T1 cell line to be susceptible to reovirus by cytopathic effect, viral protein synthesis and cell viability (12). Reovirus serotype 3, kindly provided by Dr K. Hirasawa (University of Calgary, Calgary, Canada), was prolonged in L929 cells grown in suspension in Joklik's modified Eagle's medium containing 5% FBS.

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Key words: reovirus, peritoneal dissemination, pancreatic cancer, immunocompetent model

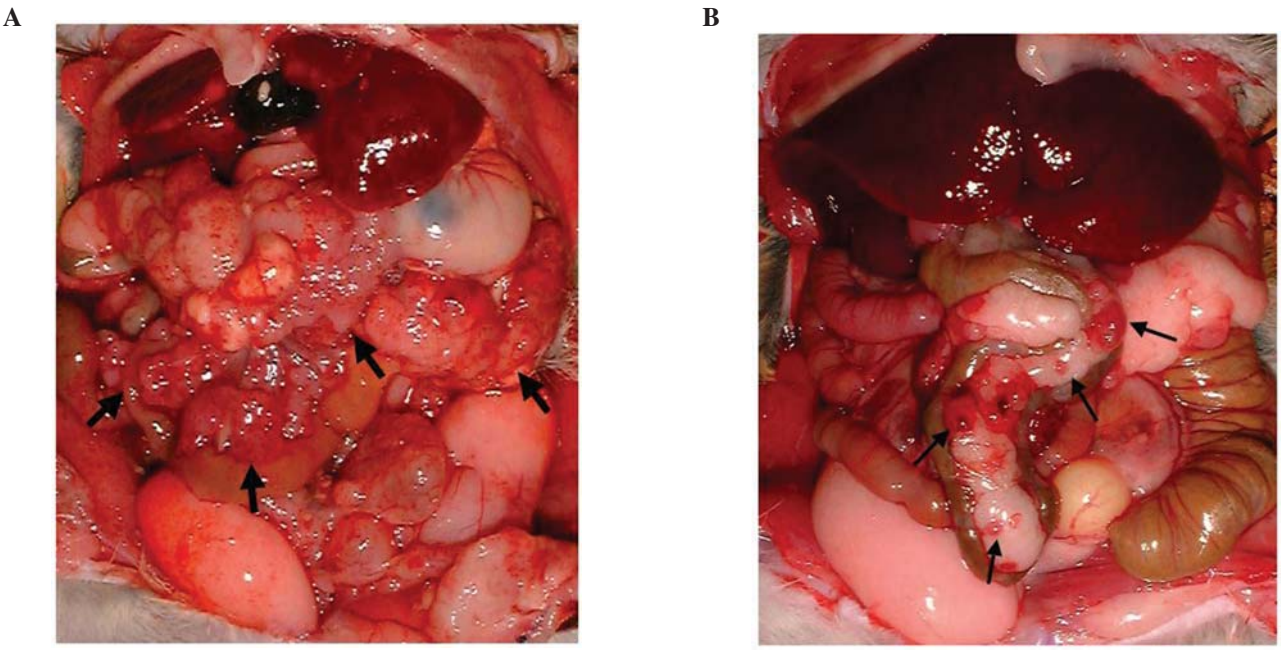

Figure 1. Macroscopic appearance of peritoneal dissemination on day 21 after the peritoneal implantation of HaP-T1 cells. (A) Control group. (B) Reovirus treatment group.

Administration (i.p.) of reovirus in the animal model. Twelve six-week-old male Syrian golden hamsters, obtained from Japan SLC, Inc. Hamamatsu Japan, were kept under specific pathogen-free conditions according to the Animal Center Guidelines. After hamsters were anesthetized using diethyl ether, HaP-T1 cells ($2.0x10^6$) suspended in 400 μl of PBS were injected into the peritoneal cavity of the hamster. Reovirus ($1x10^8$ plaque-forming units; PFU) in 200 μl of PBS was administered into the peritoneal cavity under general anesthesia on days 1, 3, 5 and 7 after HaP-T1 inoculations (n=6). Control injections were given with an equivalent amount of PBS (n=6). All animals were sacrificed on day 21 after the inoculation of HaP-T1 and the number and weight of the metastatic nodules on the peritoneal cavity were counted. The condition of the hamsters was checked twice a day during this study.

Immunohistochemical staining. The metastatic nodules on the peritoneal cavity and organs were fixed by 10% neutral-buffered formalin, embedded in paraffin and sectioned. The sections were incubated in a primary rabbit antireovirus polyclonal antibody (1:1000 in PBS with goat serum and 0.1% Triton X-100), which was also kindly provided by Dr K. Hirasawa. Reovirus protein was detected by immunohistochemistry, as described previously (11,12).

Statistical analysis. All values are expressed as the mean ±SD. Statistical analysis was performed with Student's t-test. A p-value <0.05 was considered to be significant.

Results

The macroscopic appearance of peritoneal dissemination with or without reovirus treatment are shown in Fig. 1. Multiple disseminated nodules with bloody ascites were observed in the whole peritoneal cavity in the control group, in contrast, only a few peritoneal disseminated nodules without ascites were observed in the reovirus treatment group. The total weight of peritoneal disseminated nodules was 4.9±1.1 g in the control group and 2.3±0.8 g in the reovirus treatment group and the difference was statistically significant ($p<0.05$; Fig. 2A). The total number of peritoneal disseminated nodules was 47.0±6.5 in the control group and 13.0±6.1 in the reovirus treatment group. The difference was also statistically significant ($p<0.05$; Fig. 2B). In this experiment, reovirus was repeatedly administered in the treatment group of hamsters, but there were no serious side effects, such as gangrene and/or necrosis induced by reovirus injection. This indicates that multiple i.p. administration of reovirus can safely and effectively suppress the growth of peritoneal dissemination.

Immunohistochemical examinations revealed that reovirus protein was detected only in the disseminated nodules, not in the surrounding normal tissues and those nodules in the control group (Fig. 3). In the other organs (brain, heart, lung, liver, intestine and kidney), reovirus protein was not detected by immunohistochemistry.

Discussion

Cancer remission induced by viral infection has been reported previously including varicella-induced remission of acute lymphoblastic leukemia (13) and measles-induced remissions of Burkitt's lymphoma (14) and Hodgkin's lymphoma (15). From the view point of anticancer agents, competent virus ideally replicates in cancer cells but not in normal cells. Reovirus is a replication-competent, naturally existing virus that preferentially kills cancer cells with an active Ras signaling pathway, a common characteristic of cancer cells (6).

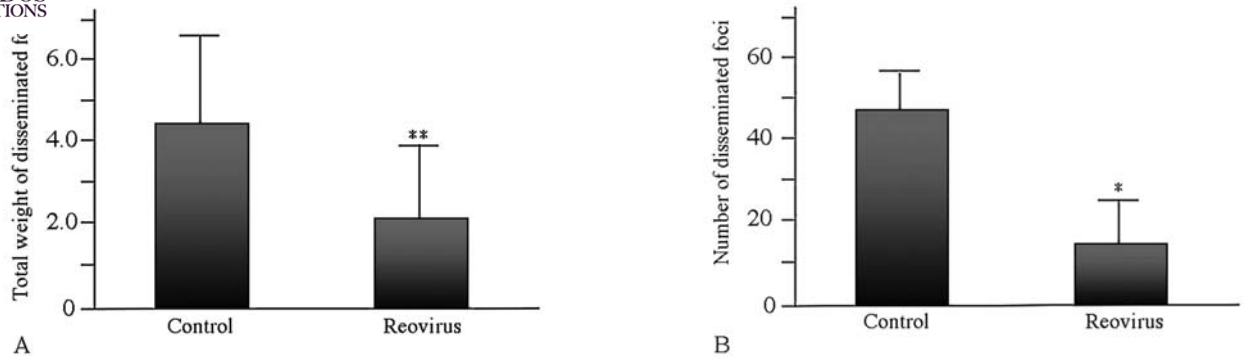

Figure 2. (A) Total weight of peritoneal disseminated nodules. (B) Number of peritoneal disseminated nodules. The difference was statistically significant ($^{*}p<0.05$; $^{**}p<0.01$). Data represents mean ± SD.

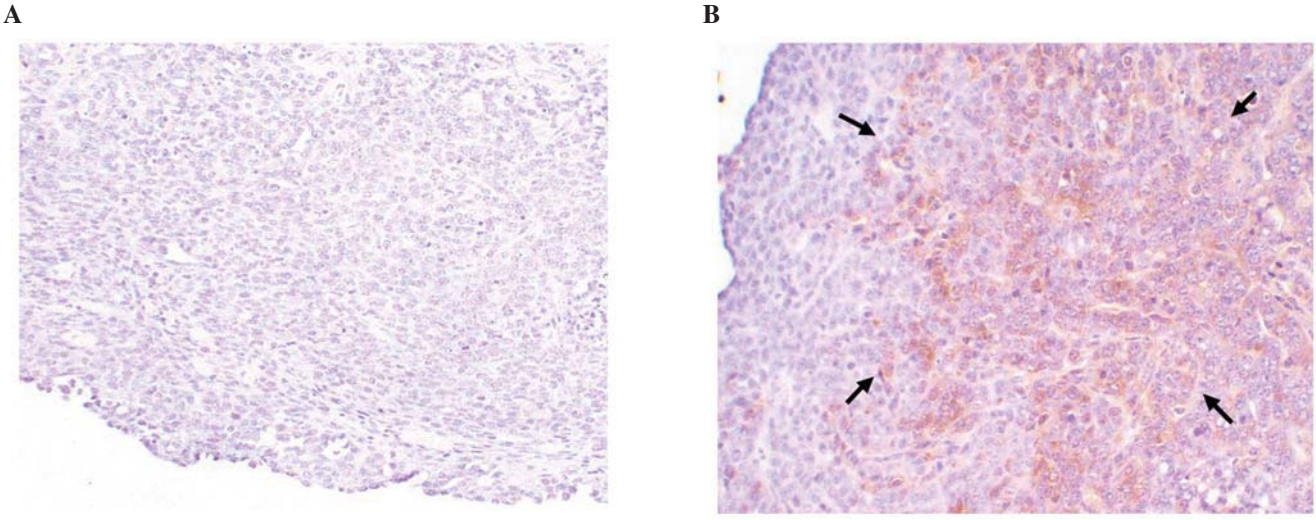

Figure 3. Immunohistochemical examination of the peritoneal disseminated nodules in the i.p. reovirus experiment. (A) Reovirus protein was not detected in the disseminated nodules in the control group. (B) Reovirus protein was only detected in the disseminated nodules but not in the surrounding normal tissue. Original magnification, x100.

Consideration of the host immune system is particularly important when reovirus is introduced by systemic delivery for the targeting of cancers that have metastasized. The immune system could have a variety of effects that could be theoretically either beneficial or harmful. Beneficial effects would include enhancing the oncolytic effect by an immune-related inflammatory response within the tumor. Some studies demonstrated that to induce cytokines into oncolytic viruses such as herpes simplex virus could improve tumor cell killing (16,17). In contrast, immune responses may reduce the antitumor effects of an oncolytic virus by reducing the amount of virus that reaches the tumor or by causing serious side effects.

The direct injection of reovirus into tumors was effective in both naive and immune-competent animals (11,12). A previous study demonstrated that the intratumoral (i.t.) administration of reovirus could be effective in targeting not only primary tumors but also remote tumors, thereby eliciting a significant tumor regression without any evident side effects in an immunocompetent hamster model. In addition, the intravenous (i.v.) administration of reovirus was also shown to be effective in metastatic liver tumors in the same model. Therefore, at least in this immunocompetent animal model, i.t. and i.v. reovirus treatments demonstrate evidence of efficacy and safety, even in the face of a competent immune system. In the present study, i.p. reovirus treatment was also found to be effective against the intraperitoneal dissemination of pancreatic cancer, without evident side effects. Reovirus replication was detected in disseminated tumor nodules but not in normal tissues of the peritoneal cavity by immunohistochemical examination. These results therefore suggest that the use of reovirus may thus be effective in patients with a common spread of pancreatic cancer in the peritoneum and at later stage of cancer development.

Oncolytic viruses including genetically engineered vaccinia, herpes simplex virus, adenovirus and measles and non-engineered viruses such as Newcastle disease virus and reovirus have been studied in clinical trials (18-21). Most of these trials have shown systemic efficacy via i.t. or i.v. administration of viruses. A phase I i.v. dose escalation trial of reovirus was reported in patients with cutaneous metastases from systemic cancers (n=29) (22). Antitumoral

efficacy was suggested in 5 patients with stable disease for 4 to 6 months, >50% decrease of blood tumor marker and/or tumor necrosis was also observed. Therefore, reovirus i.v. administration is considered to be effective in treatment-refractory cancer patients. In a clinical setting, repeated i.p. reovirus treatment may be beneficial because i.p. administration of virus in the patients with peritonitis carcinomatosa is more feasible than i.t. administration against visceral cancers. Since i.p. reovirus therapy has not yet been clinically reported, further clinical trials are eagerly anticipated in the near future.

In conclusion, the peritoneal dissemination of pancreatic cancer is a critical problem because of the limited number of available therapeutic modalities. The present data indicate that the i.p. administration of reovirus could potentially be useful as a new treatment modality against the peritoneal dissemination of pancreatic cancer.

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