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Original article

Tumor growth suppression by adenovirus-mediated introduction of a cell growth suppressing gene *tob* in a pancreatic cancer model

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Abstract

TOB (transducer of ErbB-2) is a tumor suppressor that interacts with protein-tyrosine kinase receptors, including ErbB-2. Introduction of the *tob* gene into NIH3T3 cells results in cell growth suppression. In this study, we evaluated the effect of *tob* expression in pancreatic cell lines (AsPC-1, BxPC-3, SOJ) and discuss the tumor-suppressing effects of adenoviral vector expressing *tob* cDNA. We first measured the levels of endogenous *tob* mRNA being expressed in all pancreatic cancer cell lines. Then, we examined the effect of adenoviral vector containing *tob* cDNA (Ad-*tob* vector) on cancer cell lines. The viral vector was expanded with transfection in 293 cells. The titer of the vector was 350×10^6 pfu/ml. These cancer cells were able to be transfected with MOI 20 without adenoviral toxicity. The transfection of Ad-*tob* vector results in growth suppression of SOJ and AsPC-1 cell lines. The magnitude of the expression of the Ad-*tob* gene in cancer is correlated to tumor suppressive activity. We prepared pancreatic cancer peritonitis models using a peritoneal injection of AsPC-1 cells. In this model, bloody ascites and multiple tumor nodules were seen at the mesentery after 16 days. AdCA*tob* (50×10^6 pfu/day) was administered from day 5 to day 9 after 4 days of peritoneal injection of 2×10^6 AsPC-1 cells. Tumor growth suppression occurred 10 days after peritoneal injection of AdCA*tob* compared with the control group. There were no tumor nodules in the abdomen and no bloody ascites. These results suggest that the peritoneal injection of AdCA*tob* has potential to suppress the formation of pancreatic cancer peritonitis, and can be applied for chemotherapy-resistant cancer peritonitis.

Keywords: *Tob* gene; Tumor suppressor gene; Adenovirus vector; Gene therapy

1. Introduction

Pancreatic cancer is one of the leading causes of cancer deaths in the world. Diagnosis of pancreatic cancer is difficult,

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and once metastasis to the liver or peritoneal dissemination has occurred, current treatments, including surgery and chemotherapy, are difficult to induce complete remission [1].

Advances in science and technology for direct gene transfer into living animals have provided opportunities to develop treatment modalities of malignancies by somatic gene therapy [2–5].

TOB (transducer of ErbB-2) is a 45 kDa tumor suppressor that interacts with protein-tyrosine kinase receptors, including ErbB-2 [6,7]. ErbB-2 phosphorylates and interacts with Shc, which participates between active tyrosine protein kinases to the Ras signaling pathway. A point mutation or an elevated expression of ErbB-2 is commonly observed in pancreatic cancers and breast cancers. Matsuda et al. reported that the carboxy-terminal half of TOB is relevant to its interaction with ErbB-2 and the amino-terminal half is homologous to the growth suppressor protein BTG-1, and introduction of the *tob* gene into NIH3T3 cells results in cell growth suppression [6,8–10]. Expression of BTG-1 is high in quiescent cells and decreases when cells enter the growth cycle, suggesting that the gene product is inhibitory to G0/G1 progression. The *tob* is localized on chromosome 17q21, telomeric to the BRCA 1 locus.

Using the anti-proliferative function of TOB, here we evaluated *tob* expression in pancreatic cancer cell lines and discussed its potential as a useful candidate for genetic therapy of pancreatic cancer peritonitis with peritoneal (ip) injection of recombinant adenovirus vector containing the *tob* gene (AdCA*tob*) *in vitro* and *in vivo*.

2. Materials and methods

2.1. Target tumor cells, mice and antibodies

The human pancreatic carcinoma cell line SOJ and AsPC-1 producing carcinoembryonic antigen (CEA), were maintained in RPMI1640 medium (Hazleton Biologics, Inc., Kansas, USA) supplemented with 10% fetal calf serum (Cell Culture Laboratories, Ohio, USA) and 100 $\mu g\,ml^{-1}$ kanamycin. All cultures were incubated in high moisture air with 5% CO_2 at 37 °C. The medium was changed three times a week.

Male BALB/*cnu*/*nu* mice were obtained from Nihon SLC (Shizuoka, Japan) and used at 6–7 weeks of age. In each experiment, mice of similar age and weight were selected. Mice were housed in plastic cages and maintained in an air-conditioned room. The procedures for tumor implantation and sacrifice of the animals were in accordance with approved guidelines of the Institution's Animal Ethics Committee.

Mouse anti-human TOB monoclonal antibody (IgG 2a), 4B1, was obtained from Immuno-Biological Laboratories (Gunma, Japan).

2.2. Construction of plasmid

Expression plasmid pMIK-*tob* was constructed by inserting the 1.3 kbp *tob* cDNA fragment into pMIK vector (a derivative of pME18S, kindly provided by Dr. K. Maruyama, DNAX Res. Inst., CA, USA) [6].

2.3. Northern blot analysis

Total RNA of cancer cells was extracted by the guanidium isothiocyanate method. RNA samples (10 μg) were separated and blotted following the general protocol. One kbp *Hin*d III fragment of λ *tob* cDNA was used as a probe labeled with α-^{32}P-dCTP [6].

2.4. Recombinant adenovirus preparation

Adenovirus vector containing the *tob* driven by CAG promoter (AdCA*tob*) was prepared in this study following the method described previously [11–13]. Briefly, the 1.2 kb human *tob* fragment was blunt ended and subcloned into downstream of the CAG promoter of adenovirus vector. This expression cassette was subcloned into the *Swa*I site of the pAdex1cw cosmid, resulting in pAdex1*tob*. The pAdex1cw is a 42 kb cosmid containing a 31 kb adenovirus type 5 genome lacking *E1A*, *E1B*, and *E3* genes, as described previously. The expression cosmid cassette and adenovirus DNA-terminal protein complex were cotransfected into 293 cells by calcium phosphate precipitation. The recombinant viruses were propagated with 293 cells and viral solution was stored at −80 °C. The titers of viral stocks were determined by plaque assay on 293 cells. Adenovirus containing the *lacZ* gene coding for the bacterial enzyme β-galactosidase (AdCA*lacZ*) was used as a control to measure the efficiency of tumor cell infection.

2.5. Adenovirus-mediated lac Z expression in vitro

The pancreatic cancer cell lines were plated at a density of 50×10^3 cell/well in 24-well culture plates (Iwaki Glass, Tokyo, Japan) 12 h before AdCA*lacZ* infection. Then, culture medium was replaced with medium containing varying amounts of adenovirus per cell (MOI). After 48 h, the cells were stained with X-gal (Wako Ltd., Tokyo, Japan) and the number of β-galactosidase-positive cells was counted in order to demonstrate the transfection efficiency [13].

2.6. Cell growth assay

Human pancreatic cancer cell lines (50×10^3 cell) were cultured in 60 mm tissue culture dishes (Corning Glass Works, NY, U.S.A.) for 12 h. Then, the culture medium was replaced with suspensions of AdCA*lac*Z or AdCA*tob* at an MOI of 20. After transfection, the medium was changed every other day. Cell growth was assessed by counting the number of live cells on the indicated day after transfection. The results are the means ± SD from three independent experiments.

2.7. Protein immunoblotting

Six days after transfection of AdCA*lac*Z or AdCA*tob* into the pancreatic cancer cell lines, total protein was isolated by