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Dendritic cells adenovirally-transduced with full-length mesothelin cDNA elicit mesothelin-specific cytotoxicity against pancreatic cancer cell lines *in vitro*

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ABSTRACT

Mesothelin (MSLN) is an attractive candidate as a molecular target for pancreatic cancer immunotherapy. The purpose of this study was to demonstrate that cytotoxic T lymphocytes (CTLs) generated from peripheral blood mononuclear cells (PBMCs) by stimulation with genetically-modified dendritic cells (DCs) expressing MSLN could produce specific anti-tumor immunity against pancreatic cancer cells endogenously expressing MSLN. MSLN-specific CTLs were generated from PBMCs of healthy donors by *in vitro* stimulation with DCs adenovirally-transduced with the full-length MSLN gene (DC-AxCAMSLN). The cytotoxic activity was tested using a 4-h ^{51}Cr-release assay. The pancreatic cancer cell lines (PK1, CfPAC1, AsPC1), a lymphoblastoid cell lines (LCL) transduced with the MSLN gene, and LCL pulsed with MSLN-epitope peptides were used as target cells. MSLN-specific CTLs induced by *in vitro* stimulation with DC-AxCAMSLN killed pancreatic cancer cell lines expressing MSLN in an HLA-restricted fashion. These CTLs also showed cytotoxic activity against autologous LCL pulsed with multiple MSLN-derived epitope peptides. In addition, $CD8^+$ T cells, as well as $CD4^+$ T cells, sorted from these CTLs showed significant production of interferon-γ when stimulated with DC-AxCAMSLN. The *in vitro* stimulation of PBMCs with DCs transduced with the full-length MSLN gene elicited a potent MSLN-specific cytotoxic activity against pancreatic cancer cell lines endogenously expressing MSLN by recognizing multiple MSLN epitopes and activating both $CD8^+$ T cells and $CD4^+$ helper T cells. These results therefore suggest the potential of developing future clinical applications of the vaccines using genetically-modified DCs expressing MSLN.

1. Introduction

Pancreatic cancer has an extremely poor prognosis, with an overall 5-year survival of 5% [1]. Curative surgery for patients with pancreatic cancer significantly improves their prognosis; however the majority of patients with pancreatic cancer are diagnosed at an advanced stage that makes curative resection very difficult [2]. Chemotherapy using gemcitabine is the standard treatment for unresectable pancreatic cancer at present, although its effects are relatively limited [3]. The development of more effective treatment strategies is therefore urgently needed.

Immunotherapy is a novel approach to the management of pancreatic cancer [4]. The clinical potential of various types of vaccines, such as peptide-based vaccines, dendritic cell vaccines, whole tumor cell vaccines, and recombinant viral- or bacterial-vector based vaccines has been demonstrated in early phase clinical trials [5–10]. The immunological and clinical responses in these studies have been promising, however, they are still insufficient for generating significant clinical benefits. Mesothelin

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(MSLN), a glycosylphosphatidylinositol-linked cell surface glycoprotein, is overexpressed in pancreatic ductal adenocarcinomas, however, is not expressed in normal tissues except mesothelial cells, which makes it an attractive candidate as a molecular target for pancreatic cancer immunotherapy [11–14]. In fact, several early phase clinical trials targeting MSLN have recently been reported, including a peptide vaccine, a DNA vaccine, a recombinant immunotoxin, and a chimeric anti-MSLN monoclonal antibody, and immunological responses and some minor clinical responses have been reported [15–20].

Dendritic cells (DCs) are potent antigen-presenting cells that play a critical role in the initiation of anti-tumor immune responses [21]. We have previously shown that DCs genetically transduced with the full-length tumor-associated antigen (TAA) are promising for cancer vaccine development [22,23]. This genetically-modified DC vaccine therapy has several advantages, including the fact that delivery of a broad repertoire of both major histocompatibility complex (MHC) class I and class II restricted epitopes offers the possibility for polyvalent immunization and synergistic $CD4^+$ and $CD8^+$ T-cell responses. Our previous studies have demonstrated that DCs adenovirally-transduced with natural TAA such as gp70 and carcinoembryonic antigen (CEA) were effective for inducing TAA-specific cytotoxic T lymphocytes (CTLs) and that they elicited potent anti-tumor responses in mouse models [22,23].

The purpose of this study was to determine the usefulness of DCs adenovirally-transduced with the whole human MSLN gene as a novel vaccine for patients with pancreatic cancer. We investigated whether these genetically-modified DCs expressing MSLN can induce cytotoxic T lymphocytes (CTLs) that show MSLN-specific cytotoxic activity against pancreatic cancer cells endogenously expressing MSLN, while also trying to clarify whether they can simultaneously induce MSLN-specific $CD4^+$ helper T cells *in vitro*.

2. Materials and methods

2.1. Cell lines

The human pancreatic cancer cell lines PK1 (HLA-A24/24), CfPAC1 (HLA-A2/3), and AsPC1 (HLA-A1/26) were purchased from the American Type Culture Collection (Manassas, VA, USA). Autologous Epstein-Barr virus (EBV)-transfected B-lymphoblastoid cells (LCL) were generated from healthy donor peripheral blood mononuclear cells (PBMCs) transformed by EBV, as described previously [24]. The cells were cultured in RPMI-1640 (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Co., Carlsbad, CA), 2 mM L-glutamine (Invitrogen), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen).

2.2. Immunohistochemistry for MSLN

MSLN protein expression was examined by immunohistochemical staining to evaluate the expression pattern of MSLN in 34 consecutive specimens of pancreatic tumors (invasive ductal adenocarcinoma: 10, intraductal papillary mucinous neoplasms (IPMNs) adenoma: 7, carcinoma in situ: 7, invasive adenocarcinoma: 10) that were resected at Wakayama Medical University Hospital. Invasive ductal adenocarcinoma is the most common neoplasm of the pancreas, consisting more than 85% of pancreatic tumors. IPMNs are defined as grossly visible, mucin-producing, predominantly papillary epithelial neoplasms arising from the main pancreatic duct or branch ducts. The intraductal components of IPMNs display broad spectrum of dysplasia ranging from adenoma to carcinoma in situ, and one third of IPMNs have an associated invasive adenocarcinoma and some patients with a non-invasive IPMN subsequently develop invasive ductal adenocarcinoma. Formalin-fixed, paraffin-embedded tissue sections (5 μm) were deparaffinized and rehydrated. Antigen retrieval was performed in 10 mM of sodium citrate buffer (pH 6.0) heated at 121 °C in a steamer for 7 min. The endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 5 min. After being rinsed in Tris-buffered saline (TBS), the sections were incubated with a blocking reagent: Protein block (Dako, Kyoto, Japan) for 20 min at room temperature. The sections were incubated overnight at 4 °C with the primary antibody, a 1:20 dilution of a mouse monoclonal antibody to MSLN (Clone 5B2; LAB VISION, Fremont CA, USA). After rinsing in TBS, the primary antibody was visualized using the Histofine Simple Stain PO kit (Nichirei, Tokyo, Japan) according to the manufacturer's instruction manual. The sections were developed in DAB at room temperature, and counterstained with Mayer's hematoxylin. The immunolabeling of >10% of the neoplastic cells was defined as positive.

2.3. Generation of human DCs

Monocyte-derived DCs were used as antigen-presenting cells to induce CTL responses against MSLN. DCs were generated *in vitro* from the peripheral blood of healthy volunteers. PBMCs isolated from a healthy volunteer's buffy coats using Ficoll-Paque™ PLUS (GE Healthcare, Piscataway, NJ, USA) were separated by adherence to a PRIMARIA™ tissue culture dish (Becton Dickinson) in order to enrich the monocyte fraction. The monocyte-enriched population was cultured for 5 days in AIM-V medium (Invitrogen) containing 2% heat-inactivated autologous serum (AS) supplemented with 1000 U/mL recombinant human granulocyte–macrophage colony-stimulating factor (GM-CSF; R&D Systems, Inc., Minneapolis, MN, USA) and 500 U/mL recombinant human interleukin (rhIL)-4 (kindly provided by Ono Pharmaceutical co., Tokyo, Japan), and then was cultured for additional 24–48 h in the presence of 1000 U/mL rhIL-6 (R&D Systems), 10 ng/mL recombinant human tumor necrosis factor-α (rhTNF-α; R&D Systems), 10 ng/mL rhIL-1β (R&D Systems), and 1 μg/mL prostaglandin E_2 (Sigma–Aldrich) to induce final maturation [25]. The mature DCs were harvested, and the expression of cell surface molecules was analyzed by flow cytometry. Approximately 95% of the cells showed the expression of CD11c, CD80, CD83, and CD86 (data not shown).

2.4. Recombinant adenoviral vector construction

MSLN cDNA, cloned into the pBluescript SK(−) plasmid (provided by Chugai Pharmaceutical co., Ltd., Tokyo, Japan)