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New treatment strategy with nuclear factor-κB inhibitor for pancreatic cancer

Takashi Horiuchi, MD,[a,*] *Tadashi Uwagawa, MD, PhD,*[a] *Yoshihiro Shirai, MD, PhD,*[a] *Nobuhiro Saito, MD,*[a] *Ryota Iwase, MD, PhD,*[a] *Koichiro Haruki, MD, PhD,*[a] *Hiroaki Shiba, MD, PhD,*[a] *Toya Ohashi, MD, PhD,*[b] *and Katsuhiko Yanaga, MD, PhD*[a]

[a] *Department of Surgery, The Jikei University School of Medicine, Tokyo, Japan*
[b] *Division of Gene Therapy, Research Center for Medical Science, The Jikei University School of Medicine, Tokyo, Japan*

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ABSTRACT

Background: Because of difficulties with early diagnosis, most patients with pancreatic cancer receive chemotherapy. The National Comprehensive Cancer Network guidelines (version 2.2015) suggest therapy with gemcitabine (GEM) plus nab-paclitaxel (nPTX) as a category 1 recommendation for metastatic pancreatic ductal adenocarcinoma. According to the results of many studies, the activation of chemotherapeutic agents-induced nuclear factor-κB (NF-κB) causes chemoresistance. Hence, we hypothesized that the addition of nafamostat mesilate (NM), a potent NF-κB inhibitor, to GEM/nPTX therapy could enhance the antitumor effect in the treatment of pancreatic ductal adenocarcinoma.

Materials and methods: In vitro, we assessed NF-κB activity and apoptosis under treatment with NM alone (80 μg/mL), with GEM/nPTX, or with a combination of NM and GEM/nPTX in human pancreatic cancer cell lines (PANC-1, MIA PaCa-2, and AsPC-1). *In vivo*, orthotopic pancreatic cancer mice (BALBc nu/nu) were divided into four groups: control ($n = 13$), NM ($n = 13$), GEM/nPTX ($n = 13$), and triple combination ($n = 13$). NM (30 mg/kg) was delivered intraperitoneally three times a week, and GEM/nPTX was injected intravenously once a week to orthotopic pancreatic cancer model mice. In the triple combination group, mice received NM followed by GEM/nPTX on the first day to avoid GEM/nPTX-induced NF-κB activation.

Results: In vitro and *in vivo*, NM inhibited GEM/nPTX-induced NF-κB activation, and a synergistic effect of apoptosis was observed in the triple combination group. Furthermore, tumor growth was significantly suppressed in the triple combination group compared with the other groups.

Conclusions: NM enhances the antitumor effect of GEM/nPTX chemotherapy for orthotopic pancreatic cancer by inhibition of NF-κB activation.

* *Corresponding author*. Department of Surgery, The Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan. Tel.: +81 33 433 1111x3401; fax: +81 33 433 1230.
E-mail address: horiuchi@jikei.ac.jp (T. Horiuchi).

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Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States and the fifth in Japan.[1-3] Among patients with pancreatic ductal adenocarcinoma, the 5-y survival rate is worse compared with the rates for other malignant diseases.[2,4] Since 1997, the standard first-line treatment for patients with unresectable locally advanced or metastatic pancreatic ductal adenocarcinoma has been gemcitabine (GEM) therapy.[5] However, the therapeutic outcomes are not satisfactory, and several studies have reported 1-y survival rates of only 17%-23%.[5-7]

The Metastatic Pancreatic Adenocarcinoma Clinical Trial study showed that GEM plus nab-paclitaxel (nPTX) was tolerated and superior to GEM with statistical significance in overall survival, and this regimen is recommended in the National Comprehensive Cancer Network guidelines (version 2.2015) as a category 1 recommendation. However, the median overall survival is only 8.5 mo.[8]

Nuclear factor-κB (NF-κB) plays key oncogenic roles in angiogenesis, migration, invasion, proliferation, and chemoresistance in pancreatic cancer cells.[9-20] Most anticancer agents, including GEM and nPTX, induce the activation of NF-κB, which leads to chemoresistance. Therefore, inhibition of chemotherapy-induced NF-κB activation enhances the antitumor effect. We previously reported that nafamostat mesilate (NM), a synthetic serine protease inhibitor, inhibited NF-κB activation by inhibiting IκBα phosphorylation and induced apoptosis in pancreatic cancer cells *in vitro* and *in vivo*.[21-24] In Japan, NM has been used clinically for the treatment of disseminated intravascular coagulation and acute pancreatitis for more than two decades. We hypothesized that the addition of NM to GEM/nPTX chemotherapy for pancreatic ductal adenocarcinoma might enhance the antitumor effect in comparison with GEM/nPTX alone by inhibition of GEM/nPTX-induced NF-κB activation and induction of caspase-8-mediated apoptosis by NM.

Material and methods

Cell culture

Human pancreatic cancer cell lines PANC-1, MIA PaCa-2, and AsPC-1 were obtained from American Type Culture Collection (Rockville, MD). PANC-1 and MIA PaCa-2 were cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum (Gibco BRL, NY) and 1% penicillin and streptomycin (Gibco BRL). AsPC-1 was cultured in Roswell Park Memorial Institute 1640 medium (RPMI 1640) containing 10% fetal bovine serum (Gibco BRL) and 1% penicillin and streptomycin. The cells were incubated at 37°C in a humidified 5% CO_2 atmosphere.

Reagents

NM was donated by Torii Pharmaceutical Co, Ltd (Tokyo, Japan) and was stored in a stock solution (5 mg/mL) in sterile water at −20°C. GEM was purchased from Eli Lilly Japan (Kobe, Japan). nPTX was purchased from Taiho Pharmaceutical Co, Ltd (Tokyo, Japan). Protease and phosphatase inhibitor cocktail tablets were obtained from Roche Diagnostics (Indianapolis, IN).

Antibodies

Monoclonal antibodies specific to cleaved caspase-8 and cleaved caspase-3 were obtained from Cell Signaling Technology (Beverly, MA). Anti-β-actin antibody was purchased from Sigma–Aldrich (St. Louis, MO).

In vitro *experiment treatment groups*

Based on a previous study, the concentrations of anticancer agents were determined.[25] PANC-1, MIA PaCa-2, and AsPC-1 cells were treated with NM (80 μg/mL; NM group), GEM (1000 nM, 494 nM, and 23.9 μM, respectively) and nPTX (500 nM, 683 nM, and 4.9 μM, respectively; GEM/nPTX group), GEM plus nPTX with NM (triple combination group), or vehicle-only (control group) for the appropriate time in each analysis. In triple combination group, the cells were treated with NM for 3 h before GEM/nPTX treatment.

Animals

Five-wk-old male nude mice (BALBc nu/nu) purchased from CLEA Japan Inc (Tokyo, Japan) were housed under specific pathogen-free conditions in a biologic cabinet at the Laboratory Animal Facility of the Jikei University School of Medicine. The animals were maintained in a 12-h light-dark cycle at a temperature of 22°C ± 2°C and humidity of 55± 5% in a room with filtered air supply. CLEA Rodent Diet CE-2, which is a good laboratory practice-compliant, standard rodent diet consisting mainly of vegetable protein with a proper balance of animal protein, was obtained from CLEA Japan, Inc (Tokyo, Japan) and given to the animals. The protocol of animal experiments was reviewed and approved by the Institutional Animal Care and Use Committee of the Jikei University (no. 26-005) and conformed to the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan (2006).

In vivo *experimental protocol*

The mice were anesthetized with isoflurane. The site of incision was chosen at left flank on the splenic silhouette. After making a 5-mm incision to enter the abdominal cavity without injury to the underlying organs, the spleen was gently mobilized, and the pancreas was delivered together with the spleen through the incision. A suspension of 5.0×10^6 PANC-1 cells in 50 μL of phosphate buffered saline was injected into the tail of the pancreas using a 29-ga needle. Once hemostasis was confirmed, the tail of the pancreas was placed in the abdomen, and the wound was closed in two layers. At 6 wk after injection, the animals were treated with intraperitoneal injection of NM (30 mg/kg) three times a week, or with intravenous (i.v.) injection of GEM (50 mg/kg) and/or nPTX (0.5 mg/kg) once a week. For control group, the equal amount of distilled water was injected intraperitoneal