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Different roles of sphingosine kinase 1 and 2 in pancreatic cancer progression

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ABSTRACT

Background: Pancreatic cancer is a disease with poor prognosis, and development of new treatments is necessary. Sphingosine-1-phosphate (S1P), a bioactive lipid mediator produced by sphingosine kinases (SphK1 and SphK2), plays a critical role in progression of many types of cancer. However, little is known about the role of sphingosine kinases in pancreatic cancer. This study investigated the roles of sphingosine kinases in pancreatic cancer progression.

Materials and methods: S1P levels in pancreatic cancer and noncancerous pancreatic tissue were measured in 10 patients. We generated PAN02 murine pancreatic cancer cell lines with a clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system genes 9 (Cas9)-mediated deletion of *SphK1* or *SphK2* and assessed cell growth and migration. In an animal model, we assessed the survival of mice injected with PAN02 cells intraperitoneally.

Results: S1P levels in the pancreatic cancer tissue were significantly higher than those in noncancerous tissue. SphK1 knockout (KO) cells showed greater proliferation and migration than wild type (WT) cells, and SphK2 KO cells showed less proliferation and migration than WT cells. Animal experiments showed that the survival of mice injected with SphK1 KO cells was significantly shorter than those injected with WT cells, and the survival of mice injected with SphK2 KO cells was longer than those injected with WT cells.

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Surprisingly, cytotoxic assay using gemcitabine showed that SphK1 KO cells survived less than WT cells, and SphK2 KO cells survived more than WT cells.
Conclusions: S1P produced by SphK1 and SphK2 may have different functions in pancreatic cancer cells. Targeting both SphK1 and SphK2 may be a potential strategy for pancreatic cancer treatment.

Introduction

Pancreatic cancer is one of the deadliest cancers in the United States with more than 55,000 estimated new cases and more than 44,000 estimated deaths in 2018. This makes pancreatic cancer the third most lethal cancer in both sexes.[1] It is characterized by aggressive biology and early metastasis, with 1-y survival rates of 12% to 28%.[2] The only treatment that meaningfully prolongs life is complete surgical resection of the tumor; however, about 80% of patients have advanced unresectable disease at the time of diagnosis.[3] Therefore, developing promising drug therapies, including molecular targeted drugs, is an urgent issue.

Sphinigosine-1-phosphate (S1P) is a pleiotropic lipid mediator which regulates numerous cellular processes, such as cell survival, migration, recruitment of immune cells, angiogenesis, and lymphangiogenesis, which are all factors involved in cancer progression.[4-7] S1P is generated by two types of sphingosine kinases, SphK1 and SphK2.[8] S1P produced by SphK1 is released from the cell via S1P transporters and exerts various functions.[8] We have reported that SphK1, but not SphK2, is responsible for S1P that is secreted from breast cancer cells.[9] We also found that secreted S1P is associated with lymphangiogenesis and lymph node metastasis, which suggests that S1P worsens cancer progression.[5,10-12] While the importance of S1P and its producing enzyme in progression of various types of cancer is becoming apparent, little is known about S1P in pancreatic cancer patients and the roles of sphingosine kinases in pancreatic cancer.[13]

We hypothesized that S1P and sphingosine kinases play an important role in the progression of pancreatic cancer. The aim of this study was to determine the S1P levels in pancreatic cancer patients and investigate the roles of sphingosine kinases in pancreatic cancer using knockout (KO) cells for these enzymes.

Materials and methods

Human pancreatic cancer tissue samples

Tumor and noncancerous pancreas tissue were obtained from 10 pancreatic cancer patients, who underwent surgical resection at Niigata University Medical and Dental Hospital, Japan, between 2015 and 2017. Both tumor and noncancerous pancreatic tissue were collected from the same resected specimen, and noncancerous tissue was collected from normal pancreas as far away as possible from the cancer site. All tissue samples were snap-frozen and stored at −80°C. Sphingolipids, including S1P and its metabolites, were measured by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). This study protocol was approved by the institutional review boards of Niigata University Graduate School of Medical and Dental Sciences. Written informed consent was obtained from all participants.

Quantification of sphingolipids by LC-ESI-MS/MS

Lipids were extracted from tissue samples, and sphingolipids were quantified by LC-ESI-MS/MS (4000QTRAP, ABI) at the Virginia Commonwealth University Lipidomics Core as described previously.[5,9,14] Internal standards were purchased from Avanti Polar Lipids (Alabaster, AL) and added to samples in 20 μL ethanol:methanol:water (7:2:1) in a cocktail of 500 pmol each. The high-performance liquid chromatography grade solvents were obtained from VWR (West Chester, PA).

Cell culture

PAN02 murine pancreatic cancer cell lines were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Thermo Fischer Scientific Inc, Waltham, MA), 100 U/mL penicillin, and 25 μg/mL amphotericin B (Thermo Fischer Scientific Inc) at 37°C in humidified air with 5% CO_2. For hypoxic culture, we applied the BIONIX-1 hypoxic culture kit (Sugiyamagen, Tokyo) according to the manufacturer's instructions. Cells were maintained at $pO_2 < 1\%$ and 37°C for 24 h before the experiment (preincubation), and after preincubation, cells were used for the experiment under hypoxic conditions.

Generation of cells with gene KO with clustered regularly interspaced short palindromic repeats (CRISPR)—CRISPR-associated system genes 9 (Cas9) system

First, we prepared the Cas9 and single-guide RNA (sgRNA) expression vectors for targeted mutagenesis using the CRISPR/Cas system. The Cas9 expression vector, CBh-hspCas9 plasmid, was constructed by subcloning the ubiquitous CBh promoter[15] and humanized spCas9 (System Biosciences, Mountain View, CA) into pGKPuro.[16] The sgRNA expression vector, hU6-sgRNA plasmid, was constructed by subcloning human U6 promotor and sgRNA scaffold sequence (System Biosciences) into pBluescript II. To produce Sphk1- or Sphk2-KO cell lines, we ligated synthetic oligo nucleotides of the sequence 5′- GTT GGG TCA CTG GGA CGC CC -3′ or 5′- TGC ATC TAC ACC TAC CCA AC -3′, which are identical to part of exon4 of *Sphk1* or exon3 of *Sphk2*, respectively, to sgRNA sequence in the expression vector. Transfections were carried out by incubating 450 ng of hspCas9 and 50 ng of hU6-sgRNA plasmid DNA with Lipofectamine LTX and Plus Reagent (Thermo