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Host sphingosine kinase 1 worsens pancreatic cancer peritoneal carcinomatosis

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ABSTRACT

Background: There are no effective treatments for pancreatic cancer peritoneal carcinomatosis (PC) or cancer dissemination in abdominal cavity. Sphingosine-1-phosphate (S1P), a bioactive lipid mediator produced by sphingosine kinases (SphK1 and SphK2), plays critical roles in cancer progression. We reported that SphK1, but not SphK2, is responsible for S1P export from breast cancer cells and recently discovered that S1P is linked to inflammation and cancer in colitis-associated cancer progression. Given the fact that inflammation is known to be essential for the establishment and progression of PC, we hypothesized that SphK1 in the host animals is involved in progression of pancreatic cancer PC.

Methods: Murine pancreatic adenocarcinoma panc02-luc cells were intraperitoneally injected into wildtype or SphK1 knockout (KO) mice to generate a syngeneic PC model. Cell proliferation and apoptosis were determined by Ki67 and TUNEL staining, respectively.

Results: All the animals developed panc02-luc PC. SphK1 KO mice developed significantly less tumor burden, less total tumor weight, and fewer number of PC nodules at 14 d after implantation. Histologically, less inflammatory cell infiltration and less cancer cell proliferation were observed in the tumors. There was no difference in apoptosis.

Conclusions: Our results raise an intriguing possibility that S1P generated by SphK1 in the host promotes pancreatic cancer PC progression by stimulation of proliferation of cancer cells.

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Introduction

Pancreatic cancer is one of the most deadly cancers in the United States with more than 53,000 estimated new cases and close to 42,000 estimated deaths in 2016. 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% in Europe.[2] The only treatment that meaningfully prolongs life is complete surgical resection of the tumor, however, about 80% of the patients have advanced, unresectable disease at the time of diagnosis. A population-based study that included more than 3000 pancreatic cancer patients between 1993 and 2010 in Netherlands reported that approximately half of new patients with pancreatic cancer had metastatic disease at the time of diagnosis.[3]

Peritoneal carcinomatosis (PC), the dissemination of cancer cells throughout the abdominal cavity, is common in pancreatic cancer and is found in approximately 70%-80% of patients with unresectable cancers. The peritoneum is a site of recurrence in 40%-50% of the cases after potentially curative resection of pancreatic cancer.[4] From autopsy results of 974 pancreatic cancer patients at Massachusetts General Hospital, Del Castillo and Warshaw demonstrated that approximately 50% of patients had PC at time of death, and another 20%-30% patients were shown to have malignant cells in the peritoneal cavity.[5] Current treatment options for pancreatic cancer PC include systemic chemotherapy and cytoreductive surgery with hyperthermic intraperitoneal chemotherapy. Despite these advances in treatment, overall survival after pancreatic cancer PC remains dismal with crude median survival of 2-6 mo.[3,6] This lack of improvement in survival represents the poor efficacy of currently available treatment options. Given this persistent, grim prognosis, better understanding of the biology is of urgent need to guide the development of novel and effective treatments.

The tumor microenvironment that surrounds cancer cells is thought to play critical roles in PC. For instance, inflammation of the peritoneum is essential for the establishment and progression of PC. Inflammation likely contributes to cancer cells adhering to the peritoneum and forming nodules.[7] Recently, we have reported that sphinigosine-1-phosphate (S1P) links inflammation and cancer in colitis-associated cancer.[8] S1P is a bioactive lipid mediator generated by sphingosine kinases, SphK1, and SphK2. S1P regulates cell proliferation, invasion, and angiogenesis in cancer cells.[9,10] We have reported that SphK1, but not SphK2, is responsible for S1P that is secreted out of breast cancer cells.[11] We also found that secreted S1P is associated with lymphangiogenesis and lymph node metastasis, which suggests that S1P in the tumor microenvironment worsens cancer progression.[12-15] Indeed, pancreatic cancer–derived S1P has been shown to activate pancreatic stellate cells that, in turn, promote cancer cell growth and invasion.[16] Given that SphK1 and SphK2 are expressed in host stromal cells that surround the tumor, there has been growing interest in the role of S1P generated from the host cells in the tumor microenvironment. Defining the molecular signals that control the bidirectional dialog between cancer cells and the surrounding stroma is crucial for achieving a deeper understanding of the role of S1P in cancer biology.

The aim of this study was to investigate the impact of host SphK1 on the progression of pancreatic cancer PC and on survival using a murine model of PC. In the murine model, murine pancreatic cancer cells are implanted in the abdominal cavities of SphK1 knockout (KO) mice.

Material and methods

Animals

All animal studies were conducted in the Animal Research Core Facility at Virginia Commonwealth University School of Medicine in accordance with institutional guidelines. Animal procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC), accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. C57BL/6 background SphK1−/− mice and their wildtype littermates were generously provided by Dr. Richard Proia (The National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK] of National Institutes of Health [NIH]). Based on previous studies in colitis-associated colon cancer, S1P levels are lower in the tumors of SphK1−/− mice compared with wildtype mice.[8] Furthermore, S1P levels are lower in interstitial fluid of SphK1−/− mice compared from littermate wildtype control

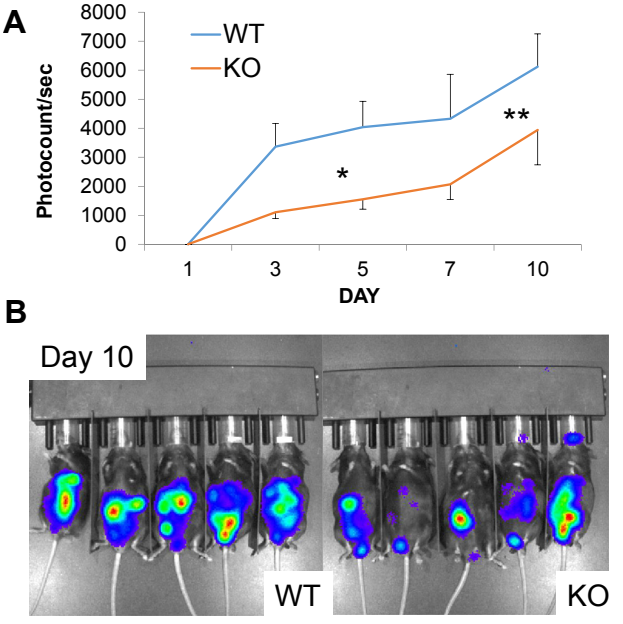

Fig. 1 – Tumor burden of pancreatic cancer PC is reduced in SphK1 knockout mice. SphK1 knockout (KO) ($n = 5$) and wildtype littermate control (WT) ($n = 5$) mice were injected 1×10^6 panc02-luc cells i.p. (A) Tumor burden was determined by *in vivo* bioluminescence. (B) Representative images on 10th day after i.p. are shown. Data are expressed as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.001$. (Color version of figure is available online.)