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Host sphingosine kinase 1 worsens pancreatic cancer peritoneal carcinomatosis

Hiroaki Aoki, MD, PhD^a, Masayo Aoki, MD, PhD^a, Eriko Katsuta, MD, PhD^{a,b}, Rajesh Ramanathan, MD^a, Michael O. Idowu, MD, MPH^c, Sarah Spiegel, PhD^d, and Kazuaki Takabe, MD, PhD, FACS^{a,b,*}

^aDivision of Surgical Oncology, Department of Surgery, Virginia Commonwealth University School of Medicine and Massey Cancer Center, Richmond, Virginia

^bBreast Surgery, Roswell Park Cancer Institute, Buffalo, New York

^cDepartment of Pathology, Virginia Commonwealth University School of Medicine and Massey Cancer Center, Richmond, Virginia

^dDepartment of Biochemistry & Molecular Biology, Virginia Commonwealth University School of Medicine and Massey Cancer Center, Richmond, Virginia

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.

* Corresponding author. Tel.: +1 716 845 2367; fax: +1 716 845 5705. kazuaki.takabe@roswellpark.org (K. Takabe).

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Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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.

Keywords

Pancreatic cancer; Carcinomatosis; Sphingosine-1-phosphate; Sphingosine kinase; S1P; SphK1; Mouse; Animal; Peritoneal; Dissemination

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 sphingosine-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 (unpublished data). Murine pancreatic cancer panc02-luc cells were generated as previously described.¹⁷ Panc02-luc cells were chosen for this study because they can be implanted into immune-intact mice, which is critical for S1P because of its known roles in inflammation and immune response.¹⁸ 1×10^6 of panc02-luc cells were intraperitoneally administered in 1 mL of PBS into 11 wk-old SphK1 KO male mice. Animals were monitored every day with weight measurement every 3 d. Animals were sacrificed on day 14 after implantation, and all the peritoneal carcinomatosis nodules were collected, weighed, counted, and fixed in formalin.

Cell culture

The murine pancreatic adenocarcinoma panc02-luc cell line was engineered to express luciferase. Cells were cultured in Dulbecco's Modified Eagle Medium with 10% FBS and 500 ug/mL of G418 and maintained at 37°C in a mixture of 5% CO₂ and 95% air. Panc02-luc cells express S1P receptor 2 (S1PR2) and proliferate in response to S1P stimulus *in vitro* (data not shown).

Bioluminescent quantification of tumor burden

Bioluminescence imaging with an IVIS imager was conducted as previously described.^{12,19,20} Briefly, D-Luciferin, potassium salt (0.2 mL of 15 mg/mL stock; GOLDBIO COM), was injected intraperitoneally. Living Image Software (Xenogen) was used to quantify the photons/s emitted by the cells. Bioluminescence was measured and

quantified at 5 min intervals over 30 min using a subject height of 1.5 cm, medium binning and an exposure time of 1 min. Bioluminescence was determined by the peak number of photons/s calculated over this time frame.

Pathologic analyses

Immediately after sacrifice, PC nodules were removed and fixed in 10% neutral buffered formalin for immunohistochemical analyses. Cell proliferation was determined by staining with rabbit monoclonal antibodies against Ki67 (Dako), a nuclear protein expressed in proliferating cells. Apoptosis was determined by terminal uridine deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using the ApopTag Peroxidase In Situ Apoptosis Detection Kit S7100 (Millipore). Lymphocyte infiltration was determined by counting the number of CD45 positive cells in $\times 400$ high power field.

Statistics

Data were analyzed for statistical significance with unpaired two-tail Student *t* test. *P* values < 0.05 were considered statistically significant in all analyses.

Results

Tumor burden of pancreatic cancer PC is reduced in SphK1 knockout mice

Previous studies showed that SphK1 is a major player in the progression of breast, colon, and bladder cancers.^{8,12,21} However, much less is known of the role of SphK1 in the tumor microenvironment. To examine its involvement in pancreatic cancer PC progression *in vivo*, we used a syngeneic panc02-luc carcinomatosis model. SphK1 KO and littermate wildtype (WT) mice (11 wk) were injected intraperitoneally with 1×10^6 panc02-luc murine pancreatic cancer cells suspended in 1 mL of PBS. Tumor burden was determined by bioluminescence imaging and was significantly suppressed in SphK1 KO mice compared to its littermate WT (Fig. 1). Both the number and total weight of PC nodules on days 14 were significantly lower in SphK1 KO mice. (Fig. 2A–C).

Host SphK1 contributes to infiltration of lymphocytes and promotion of cancer cell proliferation, rather than inhibition of apoptosis

It is well known that S1P generated by SphK1 is anti-apoptotic and promotes cancer cell proliferation.^{22,23} Moreover, SphK1 KO mice have reduced levels of S1P.⁸ Therefore, it was of interest to determine the proliferation and apoptosis of PC in SphK1 KO mice.

H & E staining revealed less inflammatory cell infiltration around the PC tumors in SphK1 KO mice compared to littermate control (Fig. 3A). Lymphocyte infiltration, quantified by CD45 stain, was significantly less in tumors from SphK1 KO (Fig. 3B). In addition, pancreatic cancer cell proliferation, as determined by Ki67 staining, was significantly reduced in SphK1 KO mice (Fig. 4A). On the other hand, there was no difference in apoptosis detected by TUNEL staining of PC nodules of WT compared to SphK1 KO mice (Fig. 4B).

Discussion

To date, there is no conventional treatment that has been proven to prolong life after diagnosis of pancreatic cancer PC. Systemic chemotherapy has been used for only palliative purposes. Despite increased use of chemotherapy from 10% in mid-1990s to 27% in 2010 in Netherlands, median survival has remained unchanged at around 8 weeks.³ Although cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy is beneficial for PC of less aggressive cancers such as appendiceal and colorectal cancers, it is not as effective for pancreatic cancer PC.²⁴ Intraperitoneal gemcitabine was demonstrated to provide effective control of local disease both in an animal model²⁵ and in a prospective clinical trial. However, this was only after a complete R0 resection.²⁶ Given the lack of effective current treatment modalities for pancreatic cancer PC, we decided to study the role of S1P in pancreatic cancer PC to deepen our understanding of the biology with a hope of developing future novel treatments against this devastating disease.

S1P is a signaling lipid mediator which is generated by sphingosine kinases. One such sphingosine kinase, SphK1, is located close to the cell membrane in the cytoplasm²³ and is translocated to the plasma membrane on activation.²⁷ We have previously shown that secretion of S1P out of breast cancer cells is regulated by SphK1 and SphK2,¹¹ and secreted S1P enhances lymphangiogenesis and lymph node metastasis.¹² In contrast to SphK1, SphK2 is localized to the nucleus and mitochondria. Hence, the current dogma is that SphK1 is mainly responsible for proinflammatory responses, and SphK2 is involved in epigenetic control of cell functions including lipid metabolism.²⁸

Recently, there has been increased elucidation of the role of nontumor-derived S1P in the tumor microenvironment of metastatic disease. Ponnusamy et al. have shown that S1P generated by host SphK1, rather than cancer-derived S1P, regulates lung colonization of bladder cancer MB49 cells injected intravenously.²¹ They reported that genetic loss of SphK1 in the tumor microenvironment activates a master metastasis suppressor, Brms1 (breast carcinoma metastasis suppressor 1), via modulation of S1PR2 in cancer cells. This increases its metastatic potential. In addition, lack of S1P in the tumor microenvironment prevented the development of tumors from orthotopically injected melanoma cells, and tumor growth and dissemination were suppressed by coinjection of fibroblasts from SphK1^{-/-} mice.²⁹ These results suggest that SphK1 in the host tumor microenvironment contributes to the progression of cancer.

The associations between inflammation and cancer were reported as early as in the 19th century by Rudolf Virchow.³⁰ It has continued to be a topic of great interest, as the modulation of inflammation has been shown to enhance or suppress tumor growth and metastasis.³¹ It is well known that S1P plays important role in inflammation and cancer.^{32,33} For instance, S1P and SphK1 have a reciprocal relationship with proinflammatory cytokines, such that TNF- α . Activation by such cytokines activates and translocates SphK1 to the plasma membrane,³⁴ leading to production and secretion of S1P that promotes certain TNF- α inflammatory functions.^{35,36} Moreover, S1P and SphK1 levels are elevated in colitis-associated colon cancer induced by colonotropic mutagen azoxymethane and luminal toxin

dextran sodium sulfate.^{8,37,38} Both dextran sodium sulfate–induced colitis³⁸ and development of colon cancer in this model³⁷ were reduced in SphK1 knockout mice.

Our current preliminary study demonstrates that the pancreatic cancer PC tumor burden was reduced in SphK1 knockout mice containing cancer cells with intact SphK1. We further found that the host SphK1 contributes to proliferation of cancer and recruitment of lymphocytes into the PC tumors. We have previously shown that a specific inhibitor of SphK1 reduced not only tumor burden but also metastasis of breast cancer cells to lymph nodes and lung.¹² Taken together, our studies support the notion that potent SphK1 inhibitors might prove useful as adjuvant therapy for pancreatic cancer PC.

In summary, we have identified that SphK1 in host mice worsens progression of peritoneal spread of a murine syngeneic pancreatic cancer model. This preliminary study shows promise and further investigation with rescue experiments (gain-of-function in SphK1 knockout mice), orthotopic models with subsequent metastatic spread, and clinical samples are warranted.

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References

1. Siegel R, Miller K, Jemal A. Cancer statistics 2016. *CA Cancer J Clin.* 2016; 66:7–30. [PubMed: 26742998]
2. Møller H, Linklater KM, Robinson D. A visual summary of the EURO CARE-4 results: a UK perspective. *Br J Cancer.* 2009; 101(Suppl 2):S110–S114. [PubMed: 19956154]
3. Bernards N, Haj Mohammad N, Creemers GJ, de Hingh IH, van Laarhoven HW, Lemmens VE. Ten weeks to live: a population-based study on treatment and survival of patients with metastatic pancreatic cancer in the south of the Netherlands. *Acta Oncol.* 2015; 54:403–410. [PubMed: 25263080]
4. Warshaw A, Castillo CF. Pancreatic carcinoma. *N Engl J Med.* 1992; 326:455–465. [PubMed: 1732772]
5. Del Castillo CF, Warshaw AL. Peritoneal metastasis in pancreatic carcinoma. *Hepatogastroenterology.* 1993; 40:430–432. [PubMed: 8270231]
6. Sadeghi B, Arvieux C, Glehen O, et al. Peritoneal carcinomatosis from non-gynecologic malignancies: results of the EVOCAPE 1 multicentric prospective study. *Cancer.* 2000; 88:358–363. [PubMed: 10640968]
7. Aoyagi T, Terracina KP, Raza A, Takabe K. Current treatment options for colon cancer peritoneal carcinomatosis. *World J Gastroenterol.* 2014; 20:12493–12500. [PubMed: 25253949]
8. Liang J, Nagahashi M, Kim EY, et al. Sphingosine-1-Phosphate links persistent STAT3 activation, Chronic Intestinal inflammation, and development of colitis-associated Cancer. *Cancer Cell.* 2013; 23:107–120. [PubMed: 23273921]
9. Takabe K, Spiegel S. Export of sphingosine-1-phosphate and Cancer progression. *J Lipid Res.* 2014; 55:1839–1846. [PubMed: 24474820]
10. Takabe K, Paugh SW, Milstien S, Spiegel S. “Inside-out” signaling of sphingosine-1-Phosphate. *Pharmacol Rev.* 2008; 60:181–195.

11. Takabe K, Kim RH, Allegood JC, et al. Estradiol induces export of sphingosine 1-phosphate from breast cancer cells via ABCC1 and ABCG2. *J Biol Chem*. 2010; 285:10477–10486. [PubMed: 20110355]
12. Nagahashi M, Ramachandran S, Kim EY, et al. Sphingosine-1-phosphate produced by sphingosine kinase 1 promotes breast cancer progression by stimulating angiogenesis and lymphangiogenesis. *Cancer Res*. 2012; 72:726–735. [PubMed: 22298596]
13. Huang W-C, Nagahashi M, Terracina K, Takabe K. Emerging role of sphingosine-1-phosphate in inflammation, Cancer, and lymphangiogenesis. *Biomolecules*. 2013; 3:408–434.
14. Takabe K, Yamada A, Rashid OM, et al. Twofer anti-vascular therapy targeting sphingosine-1-phosphate for breast cancer. *Gland Surg*. 2012; 1:80–83. [PubMed: 24855599]
15. Aoyagi T, Nagahashi M, Yamada A, Takabe K. The role of sphingosine-1-phosphate in breast Cancer tumor-induced lymphangiogenesis. *Lymphat Res Biol*. 2012; 10:97–106. [PubMed: 22984905]
16. Bi Y, Li J, Ji B, et al. Sphingosine-1-Phosphate mediates a reciprocal signaling pathway between stellate cells and Cancer cells that promotes pancreatic Cancer growth. *Am J Pathol*. 2014; 184:2791–2802. [PubMed: 25111230]
17. Roland CL, Dineen SP, Toombs JE, et al. Tumor-derived intercellular adhesion molecule-1 mediates tumor-associated leukocyte infiltration in orthotopic pancreatic xenografts. *Exp Biol Med* (Maywood). 2010; 235:263–270. [PubMed: 20404043]
18. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature*. 2014; 510:58–67. [PubMed: 24899305]
19. Rashid OM, Nagahashi M, Ramachandran S, et al. Resection of the primary tumor improves survival in metastatic breast cancer by reducing overall tumor burden. *Surgery*. 2013; 153:771–778. [PubMed: 23489938]
20. Rashid OM, Nagahashi M, Ramachandran S, et al. Is tail vein injection a relevant breast cancer lung metastasis model? *J Thorac Dis*. 2013; 5:385–392. [PubMed: 23991292]
21. Ponnusamy S, Selvam SP, Mehrotra S, et al. Communication between host organism and cancer cells is transduced by systemic sphingosine kinase 1/sphingosine 1-phosphate signalling to regulate tumour metastasis. *EMBO Mol Med*. 2012; 4:761–775. [PubMed: 22707406]
22. Datta A, Loo SY, Huang B, et al. SPHK1 regulates proliferation and survival responses in triple-negative breast cancer. *Oncotarget*. 2014; 5:5920–5933. [PubMed: 25153718]
23. Shida D, Takabe K, Kapitonov D, Milstien S, Spiegel S. Targeting SphK1 as a new strategy against cancer. *Curr Drug Targets*. 2008; 9:662–673. [PubMed: 18691013]
24. Loggie BB, Thomas P. Gastrointestinal cancers with peritoneal Carcinomatosis: surgery and hyperthermic intraperitoneal chemotherapy. *Oncology (Williston Park)*. 2015; 29:515–521. [PubMed: 26178339]
25. Ridwelski K, Meyer F, Hribaschek A, Kasper U, Lippert H. Intraoperative and early postoperative chemotherapy into the abdominal cavity using gemcitabine may prevent postoperative occurrence of peritoneal carcinomatosis. *J Surg Oncol*. 2002; 79:10–16. [PubMed: 11754372]
26. Tentes AAK, Kyziridis D, Kakolyris S, et al. Preliminary results of hyperthermic intraperitoneal intraoperative chemotherapy as an adjuvant in resectable pancreatic cancer. *Gastroenterol Res Pract*. 2012; 2012:506571. [PubMed: 22693491]
27. Hait NC, Allegood J, Maceyka M, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science*. 2009; 325:1254–1257. [PubMed: 19729656]
28. Nagahashi M, Takabe K, Liu R, et al. Conjugated bile acid-activated S1P receptor 2 is a key regulator of sphingosine kinase 2 and hepatic gene expression. *Hepatology*. 2015; 61:1216–1226. [PubMed: 25363242]
29. Albinet V, Bats ML, Huwiler A, et al. Dual role of sphingosine kinase-1 in promoting the differentiation of dermal fibroblasts and the dissemination of melanoma cells. *Oncogene*. 2014; 33:3364–3373. [PubMed: 23893239]
30. Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? *Lancet*. 2001; 357:539–545. [PubMed: 11229684]
31. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and Cancer. *Cell*. 2010; 140:883–899. [PubMed: 20303878]

32. Pyne NJ, Pyne S. Sphingosine 1-phosphate and cancer. *Nat Rev Cancer*. 2010; 10:489–503. [PubMed: 20555359]
33. Spiegel S, Milstien S. The outs and the ins of sphingosine-1-phosphate in immunity. *Nat Rev Immunol*. 2011; 11:403–415. [PubMed: 21546914]
34. Pitson SM, Moretti PA, Zebol JR, et al. Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J*. 2003; 22:5491–5500. [PubMed: 14532121]
35. De Palma C, Meacci E, Perrotta C, Bruni P, Clementi E. Endothelial nitric oxide synthase activation by tumor necrosis factor- α through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 phosphate receptors: a novel pathway relevant to the pathophysiology of endothelium. *Arterioscler Thromb Vasc Biol*. 2006; 26:99–105. [PubMed: 16269668]
36. Scherer EQ, Yang J, Canis M, et al. Tumor necrosis factor- α enhances microvascular tone and reduces blood flow in the cochlea via enhanced sphingosine-1-phosphate signaling. *Stroke*. 2010; 41:2618–2624. [PubMed: 20930159]
37. Kawamori T, Kaneshiro T, Okumura M, et al. Role for sphingosine kinase 1 in colon carcinogenesis. *FASEB J*. 2009; 23:405–414. [PubMed: 18824518]
38. Snider AJ, Kawamori T, Bradshaw SG, et al. A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. *FASEB J*. 2009; 23:143–152. [PubMed: 18815359]

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 $\pm$ SEM. * $P < 0.05$, ** $P < 0.001$. (Color version of figure is available online.)

Fig. 2.

Total weight and number of carcinomatous nodules are less in SphK1 knockout mice. SphK1 KO ($n = 5$) and WT mice ($n = 5$) were injected 1×10^6 panc02-luc cells i.p. (A) Macroscopic findings of carcinomatosis nodules 14th day after inoculation of cells. (B) Total weights of the nodules are compared. (C) Numbers of the nodules are compared. Data are expressed as mean $\pm$ SEM. * $P < 0.05$. (Color version of figure is available online.)

Fig. 3. Less infiltrating lymphocytes in tumors from SphK1 knockout mice. SphK1 KO ($n = 5$) and WT mice ($n = 5$) were injected 1×10^6 panc02-luc cells i.p., and carcinomatosis nodules

were harvested 14th day after inoculation of cells. (A) Hematoxylin and eosin stain of peritoneal carcinomatosis nodules demonstrate less infiltrating cells in tumor from SphK1 KO mice compared from WT controls. (B) Immunohistochemistry stain of CD 45 demonstrate significantly less lymphocyte infiltration in the tumor from SphK1 KO mice compared with that from WT mice. Data are expressed as mean $\pm$ SEM. * $P < 0.05$. (Color version of figure is available online.)

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Fig. 4.

Less cell proliferation, but no difference in apoptosis, is observed in tumors from SphK1 knockout mice. SphK1 KO ($n = 5$) and WT mice ($n = 5$) were injected 1×10^6 panc02-luc cells i.p. and carcinomatosis nodules were harvested 14th day after inoculation of cells. Paraffin-embedded tumor sections were immunostained with Ki67 (A) or stained by TUNEL (B). The Ki67 labeling index are defined as the percentage of positive cells per field, percent apoptotic cell was determined by TUNEL staining. Data are expressed as mean $\pm$ SEM. * $P < 0.05$. (Color version of figure is available online.)