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Phospho-Sphingosine Kinase 1 Expression in Lymphatic Spread of Esophageal Squamous Cell Carcinoma

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ABSTRACT

Background: Lymphatic spread is the main mode of progression of esophageal squamous cell carcinoma (ESCC). Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid mediator, which produced by sphingosine kinase 1 (SphK1) activated by phosphorylation. The SphK1-S1P axis has a crucial role in lymphangiogenesis. However, the significance of phospho-SphK1 (pSphK1) in the progression of ESCC has not been fully investigated.

Materials and methods: We evaluated pSphK1 expression in 92 surgically resected tumor tissues of ESCC by the immunohistochemistry. Fifty-nine (64%) patients with moderate or strong expression and 33 (36%) with negative or weak expression were classified in the pSphK1-high and pSphK1-low groups, respectively.

Results: Higher pathological N category (pN) was more frequently observed in the pSphK1-high group ($P < 0.01$). The median number of lymph node metastasis (pSphK1-high: 2 *versus* pSphK1-low: 0; $P < 0.01$), the proportion of patients with lymphatic invasion (69% *versus* 18%; $P < 0.01$) and that with intramural metastasis (27% *versus* 3%; $P < 0.01$) were significantly higher in the pSphK1-high group. The presence of lymphatic invasion (odds ratio [OR] 5.63; $P < 0.01$) and pN1–3 (OR 3.26; $P = 0.04$) were independently associated with high pSphK1 expression. The 5-y overall survival rate of the pSphK1-high group was significantly lower than that of the pSphK1-low group (50.8% *versus* 67.3%; $P = 0.01$). High pSphK1 expression was not identified as a significant independent prognostic factor.

Conclusions: We provide the first evidence of the association between high expression of pSphK1 and both lymphatic spread and patient outcomes in ESCC.

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Introduction

Esophageal carcinoma is the sixth most common cause of cancer-related mortality worldwide.[1] Squamous cell carcinoma is the predominant histological type in Eastern Asia with a high mortality rate from this disease. Esophageal squamous cell carcinoma (ESCC) is notorious for poor outcomes related to the high potential of metastasis.[2] Lymphatic spread is considered to be a main mode of progression of ESCC.[3] In fact, lymph node metastasis is frequently observed thorough the wide anatomical regions, such as the neck, mediastinal, and abdominal regions even in superficial ESCC.[4] Lymphatic invasion and lymph node metastasis is a strong, unfavorable prognostic factor of ESCC.[5] Therefore, a greater understanding of the molecular mechanisms underlying lymphatic spread is necessary to improve the outcome of patients with ESCC.

Lymphangiogenesis is an important step in the lymphatic spread of malignant tumors.[6,7] The molecular mechanisms of lymphangiogenesis have been elucidated by previous *in vivo* and *in vitro* experiments. Vascular endothelial growth factor receptor 2 (VEGFR2) and the VEGFR3 signaling pathway that activated by vascular endothelial growth factor C (VEGFC) and VEGFD are considered to be major drivers of lymphangiogenesis in cancer.[8] Sphingosine-1-phosphate (S1P), a pleiotropic bioactive lipid mediator, is one of the most important molecules in cancer progression.[9,10] We previously demonstrated that S1P, which is produced by sphingosine kinase 1 (SphK1), promotes lymphangiogenesis of breast cancer by *in vivo* and *in vitro* assays.[11] S1P is converted from sphingosine when SphK1 is activated by the phosphorylation on Ser-225 inside cancer cells.[12] We showed that the immunohistochemical expression of phospho-SphK1 (pSphK1) is associated with high levels of S1P as quantified by mass spectrometry in clinical samples of human breast cancer.[13] Currently the SphK1-S1P axis is recognized as having a crucial role in lymphangiogenesis of cancer as well as cell proliferation, migration, invasion, and hemangiogenesis.[8,10] However, the significance of this pathway in ESCC progression has not been fully investigated to date.

In this study, we hypothesized that the activation of SphK1 is associated with the progression of human ESCC. To test this hypothesis, we used immunohistochemistry to detect pSphK1 and investigated the association between pSphK1 expression and clinicopathological features and patient outcomes in ESCC.

Materials and methods

Patients

A total of 105 patients underwent esophagectomy and two or three field lymphadenectomy without preoperative therapy for ESCC at Niigata University Medical and Dental Hospital from January 2000 to December 2008. Among these, 92 patients with invasive tumor were enrolled in this study. There were 82 men and 10 women, with a median age of 66 y (range: 52-81 y). The clinicopathological features for 92 analyzed patients are shown in Table 1. This study was conducted in accordance with the provisions of the Declaration of Helsinki. Collection and use of all specimens and clinical information in this study were approved by the Ethics Committee of Niigata University (#2289). Written informed consent was omitted and the information of this study was disclosed on the website of Niigata University to guarantee an opportunity for refusal of participation in this study (opt-out method).

Surgical treatment and follow-up

Seventy-two and 20 patients underwent esophagectomy via the transthoracic and transhiatal approach, respectively. In patients who underwent a transthoracic esophagectomy, two-field and three-field lymphadenectomies were performed in 37 and 35 patients, respectively. Among 92 patients, 81, 7, and 4 patients achieved R0, R1, and R2 resection according to the seventh edition of the International Union against Cancer tumor-node-metastasis classification system, respectively.[14] All patients underwent physical and blood biochemistry examinations every 3 mo after esophagectomy as a regular checkup. Routine imaging investigations with computed tomography of the neck, chest, and abdomen were performed at least once a y. The overall survival (OS) was calculated from the date of esophagectomy until death of any cause. Patients alive at the time of final survival data collection were considered to be censored cases. The median follow-up period after esophagectomy was 64.5 mo (range: 0-188 mo) for surviving patients.

Histopathological examination and immunohistochemistry

The histological findings were described according to the seventh edition of International Union against Cancer tumor-node-metastasis classification system.[14] All of the surgically resected ESCC specimens were submitted to the Department of Surgical Pathology in our hospital and examined by two experienced pathologists.

pSphK1 and SphK1 expression was immunohistochemically examined using formalin-fixed paraffin-embedded blocks of surgically resected specimens. Three serial 4-μm sections were cut and used for staining with hematoxylin and eosin, pSphK1, and negative control. Antigen retrieval was performed by microwaving the slides under pressure in a citrate buffer for 10 min (pH 9.0). Endogenous peroxidase was blocked using 0.3% hydrogen peroxide for 20 min. After blocking nonspecific background, the sections were incubated overnight with the primary rabbit polyclonal antibody against pSphK1 (1:100 dilution; ECM Biosciences LLC, Versailles, KY) or Sphk1 (1:100 dilution; Sigma–Aldrich, St. Louis, MO) at 4°C. Then, the sections were incubated with biotinylated rabbit antimouse streptavidin-peroxidase complex for 7 min. Diaminobenzidine was used as the chromogen, and the sections were counterstained with hematoxylin. Normal mouse immunoglobulin was substituted as the primary antibodies in the negative control. Two independent investigators (M.N. and H.I.), both blinded to the clinical data, reviewed the sections stained with anti-pSphK1 and anti-SphK1. The vascular and lymphatic endothelial cells of all vessels reacted with the anti-pSphK1 and