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Upregulation of phosphorylated sphingosine kinase 1 expression in colitis-associated cancer

Kizuki Yuza, MD,[a] *Masayuki Nagahashi, MD, PhD,*[a,*] *Yoshifumi Shimada, MD, PhD,*[a] *Mae Nakano, MD, PhD,*[a] *Yosuke Tajima, MD, PhD,*[a] *Hitoshi Kameyama, MD, PhD,*[a] *Masato Nakajima, MD, PhD,*[a] *Kazuaki Takabe, MD, PhD, FACS,*[a,b,c,d,e] *and Toshifumi Wakai, MD, PhD, FACS*[a]

[a] *Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata City, Japan*

[b] *Breast Surgery, Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, New York*

[c] *Department of Surgery, University at Buffalo Jacobs School of Medicine and Biomedical Sciences, The State University of New York, Buffalo, New York*

[d] *Department of Breast Surgery and Oncology, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan*

[e] *Department of Surgery, Yokohama City University, Kanazawa-ku, Yokohama, Japan*

ARTICLE INFO

Article history:
Received 2 March 2018
Received in revised form
8 May 2018
Accepted 31 May 2018
Available online xxx

Keywords:
Colitis-associated cancer
Colorectal cancer
Sphingosine-1-phosphate
Phosphorylated sphingosine kinase 1
STAT3
IL-6

ABSTRACT

Background: Colitis-associated cancer (CAC) is the most serious complication of inflammatory bowel disease. Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator that is generated by sphingosine kinase 1 (SphK1) and is known to play an important role in inflammation and cancer progression. Moreover, SphK1 and S1P act as upstream mediators of proinflammatory cytokine interleukin 6 (IL-6) and signal transducer and activator of transcription-3 (STAT3). We hypothesized that the expression levels of phosphorylated SphK1 (pSphK1), phosphorylated STAT3 (pSTAT3), and IL-6 are universally higher in CAC patients than in sporadic colorectal cancer (CRC) patients because all of these factors are associated with inflammation. In this study, we determined the expression levels of pSphK1 in patients with sporadic CRC and CAC and clarified the importance of S1P in CAC patients.
Materials and methods: We randomly selected 10 sporadic CRC patients and 10 CAC patients who underwent curative resection, and we examined their surgical specimens by immunohistochemistry. We determined the expression levels of pSphK1, pSTAT3, and IL-6 in these samples.
Results: We found pSphK1 expression to be more prevalent in CAC patients ($P = 0.019$) and to have a higher immunohistochemistry score ($P = 0.005$) than in sporadic CRC patients. However, the expression of pSTAT3 and IL-6 did not differ between the patient groups.
Conclusions: To our knowledge, this is the first report comparing pSphK1 expression levels in CAC with those in sporadic CRC. The high levels of pSphK1 expression in CAC suggest an important role of S1P in the disease process of CAC.

* *Corresponding author*. Associate Professor, Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-ku, Niigata City, 951-8510, Japan. Tel.: +81 25 227 2228; fax: +81 25 227 0779.
E-mail address: mnagahashi@med.niigata-u.ac.jp (M. Nagahashi).

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https://doi.org/10.1016/j.jss.2018.05.085

Introduction

Ulcerative colitis (UC) is a phenotype of inflammatory bowel disease (IBD) characterized by chronic recurrent colonic inflammation, and patients with UC are at a substantially increased risk of developing colorectal cancer (CRC).[1] Colitis-associated cancer (CAC) is a cancer developed in patients with IBD, including UC and Crohn's disease. The mechanism of CAC development is different to that of sporadic CRC: while CAC is caused by an inflammation-dysplasia-carcinoma sequence, sporadic CRC development is caused by an adenoma-carcinoma sequence.[2] Chronic inflammation in the intestine can lead to epithelial damage. Cytokines that are produced locally to aid in the replacement of lost epithelial cells can cause additional inflammation, which stimulates the proliferation of crypt cells. This chronically stimulated state of the epithelium may eventually lead to the development of CAC.[3] Although experimental models have provided important clues to the roles of inflammatory mediators and molecular events leading to development of CAC, the mechanism is yet not fully understood in human patients.

There is growing evidence that sphingosine-1-phosphate (S1P), a bioactive lipid mediator, is involved in inflammation and cancer.[4] S1P is generated intracellularly by sphingosine kinases and regulates many important cellular processes involved in cancer, including cell growth, survival, invasion, lymphocyte trafficking, angiogenesis, and cytokine and chemokine production.[5] S1P can function intracellularly as a second messenger or extracellularly in the tumor microenvironment as a ligand for S1P receptors in an autocrine and/or paracrine manner.[6] Numerous studies suggest that this process, referred to as "inside-out" signaling by S1P, plays an important role in cancer progression.[5] Recently it was demonstrated that sphingosine kinase 1 (SphK1) and intracellular S1P also play a direct role as upstream mediators of the canonical nuclear factor-kappa B (NF-κB) activation pathway, which is important in inflammatory, antiapoptotic, and immune processes.[7] Transcriptional NF-κB and signal transducer and activator of transcription-3 (STAT3) have been highlighted for their key roles in the development of CAC. NF-κB activation in intestinal epithelial cells promotes survival pathways that are required for the growth of premalignant cells and the subsequent formation of tumors.[8] Activation of NF-κB in myeloid-derived inflammatory cells enhances inflammation in the tumor microenvironment, mainly by increasing the expression of proinflammatory cytokines, such as tumor necrosis factor-α and interleukin 6 (IL-6), which in turn stimulate the proliferation of tumor and stromal cells to further promote CAC.[8,9] STAT3 is a key transcription factor involved in inflammation and carcinogenesis.[10] Its phosphorylation by cytokines, including its major activator IL-6, enables its nuclear translocation and function. Crucially, S1P signaling participates in a reciprocal positive feedback loop with STAT3.[11] Cross-talk between S1P and the STAT3 pathway can sustain persistent STAT3 signaling in cancer, thereby promoting tumor progression and metastasis (Fig. 1).[12] The involvement of S1P signaling in these events in human patients is still not understood.

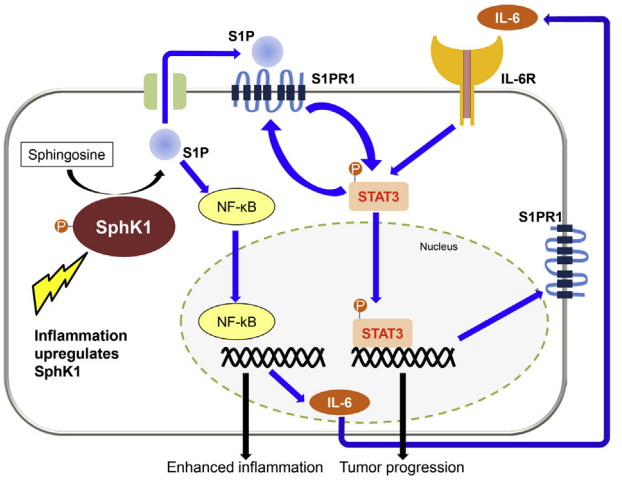

Fig. 1 – Role of the SphK1/S1P/S1PR1 axis in the activation of NF-κB and STAT3, linking inflammation and cancer. S1P is increased in colitis and colitis-associated cancer, which is produced by upregulated SphK1. S1P is exported to the outside of cell through its transporters and activates its receptor, S1PR1, leading to the activation of the STAT3. Reciprocally, STAT3 enhances the transcription of its target genes, including S1PR1. Intracellular and/or extracellular S1P also induces the activation of NF-κB. NF-κB induces the transcription of proinflammatory cytokines such as IL-6, which induces the activation of STAT3 by binding to its receptor, IL-6R. In summary, increased levels of SphK1 and S1P during colitis lead to a feed-forward amplification loop driving persistent NF-κB and STAT3 activation linked to tumorigenesis during CAC. (Color version of figure is available online.)

We have recently shown that S1P produced by the upregulation of SphK1 is the link between chronic intestinal inflammation and CAC in animal models.[13] However, the role of S1P in human CAC has never been investigated due to the difficulty of measuring S1P levels in human samples, as S1P is a lipid. We have previously shown that high expression of phosphorylated SphK1 (pSphK1) is associated with higher levels of S1P in human tissue, so detecting pSphK1 by immunohistochemistry could be an alternative method of examining the role of S1P in human patients.[14] We hypothesized that the expression levels of phosphorylated STAT3 (pSTAT3), IL-6, and pSphK1 would be universally higher in CAC patients than in sporadic CRC patients. The aim of this study was to determine the expression levels of pSTAT3, IL-6, and pSphK1 in sporadic CRC and CAC patients and to clarify the importance of S1P in CAC patients.

Materials and methods

Human CRC tissue samples

We randomly selected 10 sporadic CRC patients and 10 ulcerative CAC patients, who underwent curative resection at Niigata University Medical and Dental Hospital, Japan, between April 2012 and May 2017. Specimens were fixed in