

TSLP Expression and High Serum TSLP Level Indicate a Poor Prognosis in Gastric Cancer Patients

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ABSTRACT

Background Thymic stromal lymphopoietin (TSLP) plays an important role in promoting tumor survival, by manipulating the immune response and angiogenesis. However, the clinical significance of TSLP in gastric cancer is unclear.

Methods Immunohistochemistry was used to investigate TSLP expression in non-cancerous gastric mucosa and gastric cancer tissue from patients with gastric cancer. Serum TSLP levels were measured using an enzyme-linked immunosorbent assay.

Results Tumors with TSLP expression were significantly larger than those without TSLP expression. TSLP expression was observed more frequently in advanced (T2/T3/T4) than in early (T1) gastric cancer and in stage 3/4 than in stage 1/2. Lymph node metastasis, liver metastasis, positive peritoneal lavage cytology, lymphatic invasion, and vascular invasion occurred significantly more often in TSLP-expressing than in non-expressing tumors. The prognosis of patients with TSLP-positive tumors was significantly worse than that of patients with TSLP-negative tumors. Patients with high serum TSLP concentrations also had a significantly worse prognosis than those with low concentrations. Multivariate analysis identified serum TSLP level as an independent prognostic indicator.

Conclusion TSLP is closely related to the progression of gastric cancer and may predict survival in these patients.

Key words Gastric cancer; Prognosis; TSLP; Tumor

Thymic stromal lymphopoietin (TSLP) is an interleukin-7 (IL-7)-like cytokine originally identified by its ability to promote the proliferation and development of immature B cells.¹ Several types of cells respond to TSLP, such as basophils,² mast cells,³ B cells,⁴ CD4+,⁵ CD8+,⁶ natural killer T cells,⁷ and dendritic cells (DCs).⁸ Moreover, a major subset of TSLP-responsive cells identified in both humans⁸ and mice⁹ are myeloid-derived DCs that express high levels of TSLP receptor (TSLPR)

complex. This heterodimer of TSLPR and IL-7R alpha is functional and has a high affinity for TSLP.⁸

CD4+ effector T cells play a central role in initiating and maintaining antitumor immunological responses. They are categorized into two subsets based on their cytokine profile. Thus, in mice and humans type 1 (Th1) or type 2 (Th2) have been identified.^{10, 11} Th1 cells produce interferon-gamma and mainly confer protection against microbial infections, whereas Th2 cells produce IL-4, -5, -9, and -13 and help protect against infections by gastrointestinal nematodes, but they are also responsible for allergic disorders.^{12, 13} TSLP plays an extremely important role in the induction of Th2. TSLP-activated DCs are primed for inflammatory Th2 cell differentiation through their expression of the OX40 ligand.¹⁴ Thus, dysregulated TSLP expression can result in the development of type 2 inflammatory responses leading to allergic disease.

The Th2 response predominates over the Th1 response not only in allergic disease but also in many types of cancers.¹⁵ Tumors associated with a Th2-type response generally carry a worse prognosis than those with a predominantly Th1-type response.^{16, 17} However, the mechanism by which Th2-biased immune responses are initiated in tumors is largely unknown. De Monte et al. recently demonstrated that in pancreatic cancer, TSLP produced by cancer-associated fibroblasts activates DCs to undergo Th2 differentiation.¹⁷ Pedroza-Gonzalez et al. showed that TSLP produced by breast cancer cells was capable of inducing OX40 ligand expression in DCs, which in turn promoted Th2 differentiation by naïve CD4+ T cells.¹⁸ These observations

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Abbreviations: CA19-9, carbohydrate antigen 19-9; CEA, carcino-embryonic antigen; DCs, dendritic cells; ELISA, Enzyme-Linked ImmunoSorbent Assay; IL-7, interleukin-7; Th1, humans type 1 helper T cells; Th2, human type 2 helper T cells; Tregs, regulatory T cells; TSLP, Thymic Stromal Lymphopoietin; TSLPR, Thymic Stromal Lymphopoietin receptor

suggest an important role for TSLP in promoting Th2-biased immune responses in the tumor microenvironment. Indeed, TSLP expression has been confirmed in several types of cancer, including breast cancer,^{19, 20} cervical cancer,²¹ lung cancer,²² and leukemia,^{23, 24} and is associated with the progression and metastasis of these tumors.^{19–21}

Gastric cancer is one of the most common cancers in Asia and ranks second among all cancer deaths worldwide.²⁵ *Helicobacter pylori* infection promotes TSLP production by gastric epithelial cells and induces DC-mediated inflammatory Th2-type responses,²⁶ which have been shown to predominate in gastric cancer.²⁷ Since *H. pylori* infection is closely related to the oncogenesis of gastric cancer, we hypothesized that TSLP expression similarly correlates with tumor progression and prognosis in these patients. Therefore, in this study the clinical significance of TSLP expression in patients with gastric cancer was determined by comparing their non-cancerous gastric mucosa and gastric cancer tissue and by measuring serum TSLP concentrations.

MATERIALS AND METHODS

Patients

One hundred and thirty-two and 110 patients who treated at Tottori University Hospital and pathologically diagnosed with gastric cancer were enrolled for immunohistochemistry and Enzyme-Linked Immunosorbent Assay (ELISA), respectively, in the current study. None of the patients had received radiotherapy, chemotherapy, or other medical interventions before surgery. The clinicopathological findings were determined according to the Japanese Classification of Gastric Carcinoma.²⁸ This study was approved by the Ethical Committee of the Tottori University Hospital (approval number: 2573) and written informed consent was obtained from all patients.

Immunostaining analysis

Serial sections (4 μ m thick) were dewaxed in xylene, dehydrated in ethanol, placed in citrate buffer pH 6, and heated in a microwave oven (700 W) for 15 min to retrieve the antigens. The tissues were then incubated in 3% hydrogen peroxide in methanol to block endogenous peroxide. Immunohistochemical staining for TSLP was performed using anti-human TSLP antibody (ab47943; Abcam, Cambridge, MA) at a dilution of 1:100 and a secondary antibody (ab97080; Abcam) at a dilution of 1:200, according to the manufacturer's instructions. The tissue sections were evaluated by a pathologist blinded to the clinical data and considered positive when > 10% of the cells expressed TSLP.

Measurement of serum TSLP level

Serum TSLP levels were measured in an ELISA using a human TSLP immunoassay (DY1398, R&D SYSTEMS, Minneapolis, MN). All samples were measured in duplicate and the mean concentration was calculated.

Statistical analysis

Associations among factors were evaluated using the χ^2 test. Mann-Whitney *U*-tests were used to determine statistical differences between groups. Five-year survival rates were calculated according to the Kaplan-Meier method and differences between survival curves were examined with the log-rank test. Survival data represent cancer-specific survival. Thus, patients who died from causes other than gastric cancer were considered lost to follow-up as of the time of death. Multivariate analysis was performed using the Cox proportional hazards model. The accepted level of significance was defined as a *P*-value < 0.05. Statistical analyses were performed using SPSS Statistics 22.0 for Windows (IBM, Armonk, NY).

RESULTS

TSLP expression in the tissue of non-cancerous gastric mucosa and gastric cancer

The immunohistochemically demonstrated expression of TSLP in non-cancerous gastric tissue and cancer tissue is shown in Fig. 1. TSLP expression was observed in the cytoplasm of cells from non-cancerous gastric mucosa obtained from 23 patients (20.9%) and in the cancer tissues of 57 patients (51.8%). There was no significant correlation between the clinicopathological characteristics of the patients and TSLP expression in non-cancerous gastric mucosa (Table 1). However, TSLP expression in the malignant tissue was associated with significantly larger tumors and occurred more frequently in advanced (T2/T3/T4) than in early (T1) gastric cancer and in stage 3/4 than in stage 1/2 disease. Lymph node metastasis, liver metastasis, positive peritoneal lavage cytology, lymphatic invasion, and vascular invasion also occurred significantly more often in patients with TSLP-expressing cancer tissue (Table 1).

The 5-year survival rates of patients with TSLP-positive tumors and TSLP-negative tumors were 21.8% and 76.9%, respectively (*P* = 0.0011; Fig. 2). By contrast, there was no significant difference in the prognosis of patients with TSLP-positive and TSLP-negative non-cancerous mucosa (*P* = 0.4; data not shown).

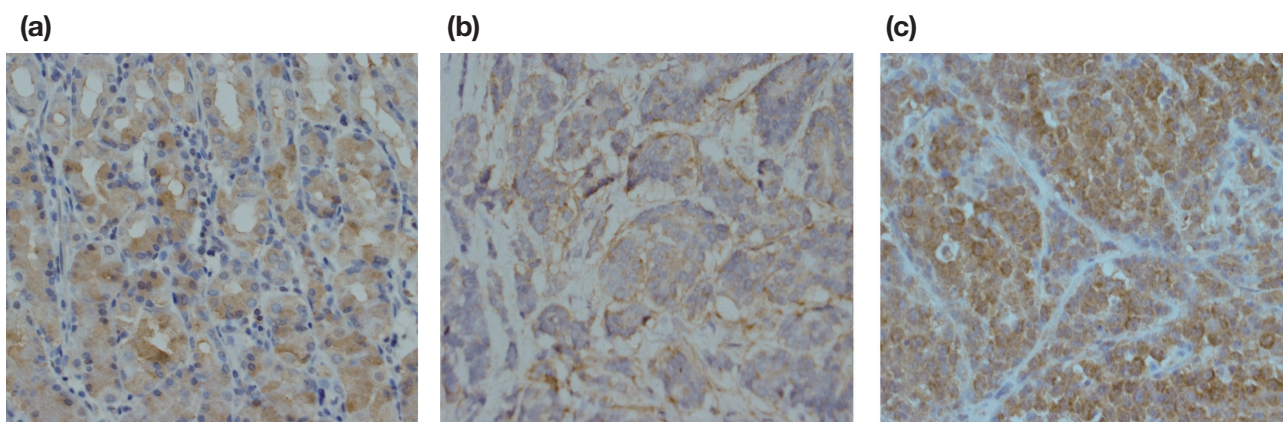


Fig. 1. TSLP expression in non-cancerous gastric mucosa and gastric cancer tissue. (a) Non-cancerous gastric mucosa, $\times 400$; (b) tubular adenocarcinoma, $\times 400$; (c) poorly differentiated adenocarcinoma, $\times 400$.

Table 1. TSLP expression in gastric cancer and the clinicopathological characteristics of the patients

	TSLP expression					
	Non-cancerous gastric mucosa			Cancer tissue		
	Negative	Positive	<i>P</i> -value	Negative	Positive	<i>P</i> -value
Tumor size (mm)	48.8 \pm 31.7	44.0 \pm 29.5	0.430	42.8 \pm 28.3	59.5 \pm 34.3	0.023
Age (years)	68.5 \pm 10.2	64.8 \pm 13.3	0.108	65.8 \pm 12.4	69.4 \pm 9.6	0.195
Male	42	34	0.026	63	14	0.283
Female	11	23		24	9	
Histology						
Differentiated	30	27	0.333	41	16	0.055
Undifferentiated	23	30		46	7	
Depth of invasion						
T1	27	29	0.994	52	4	< 0.001
T2/T3/T4	26	28		35	19	
Lymph node metastasis						
Absent	34	32	0.391	59	7	0.001
Present	19	25		28	16	
Peritoneal metastasis						
P0	50	50	0.227	82	19	0.070
P1	3	7		5	4	
Liver metastasis						
H0	49	55	0.351	87	17	< 0.001
H1	4	2		0	6	
Cytology						
CY0	31	36	0.600	55	11	0.016
CY1	5	4		5	5	
Lymphatic invasion						
Absent	20	23	0.779	41	2	0.001
Present	33	34		46	21	
Vascular invasion						
Absent	24	29	0.557	49	4	0.001
Present	29	28		38	19	
Disease stage						
1/2	37	41	0.807	68	10	0.001
3/4	16	16		19	13	

TSLP, Thymic Stromal Lymphopoietin.

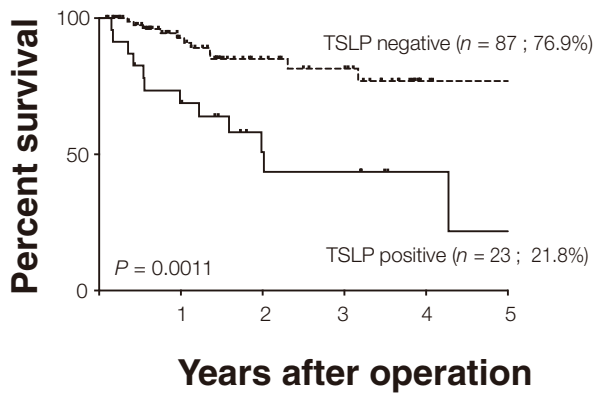


Fig. 2. Survival curves of gastric cancer patients according to TSLP expression in gastric cancer tissue. TSLP, Thymic Stromal Lymphopoietin.

Table 2. Serum concentration of TSLP in gastric cancer patients and their clinicopathologic characteristics

	n	TSLP (pg/mL) ± SD	P-value
Male	92	226.6 ± 583.8	0.741
Female	40	189.4 ± 614.5	
Tumor size (mm)			
> 40	48	233.4 ± 705.3	0.576
< 40	66	170.5 ± 493.3	
Histology			
Differentiated	67	194.5 ± 580.0	0.683
Undifferentiated	65	236.8 ± 606.2	
Depth of invasion			
T1	67	165.3 ± 494.9	0.315
T2/T3/T4	60	272.6 ± 695.3	
Lymph node metastasis			
Absent	86	226.8 ± 652.5	0.762
Present	46	193.9 ± 460.9	
Lymphatic invasion			
Absent	53	133.1 ± 471.8	0.308
Present	66	242.5 ± 652.8	
Venous invasion			
Absent	67	133.7 ± 447.2	0.200
Present	52	271.2 ± 712.2	
Peritoneal metastasis			
P0	120	192.3 ± 577.3	0.152
P1	9	486.6 ± 770.3	
Liver metastasis			
H0	126	169.7 ± 544.9	< 0.001
H1	6	1173.6 ± 761.8	
Peritoneal lavage cytology			
CY0	74	172.1 ± 633.7	0.043
CY1	9	650.6 ± 870.8	
Disease stage			
1/2	93	182.5 ± 587.5	0.326
3/4	39	293.7 ± 600.3	

TSLP, Thymic Stromal Lymphopoietin.

Table 3. Association of prognostic factors with disease-specific survival in patients with gastric cancer, as determined by the Cox proportional hazards model

Prognostic factors	Hazard ratio	95% CI	P-value
Age*	1.035	0.972–1.102	0.280
Sex	0.444	0.105–1.874	0.269
Histology†	1.372	0.395–4.761	0.619
Depth of invasion(T1–T4) ‡	2.569	0.861–7.666	0.091
Lymph node metastasis (n0–n3) §	1.023	0.494–2.118	0.951
Peritoneal metastasis (P0–P1)	2.214	0.131–37.286	0.581
Lymphathic invasion (ly0–ly3)	1.889	0.763–4.677	0.169
Vascular invasion (v0–v3) ¶	1.022	0.524–1.993	0.948
Tumor size*	1.010	0.990–1.030	0.342
TSLP concentration	1.001	1.000–1.002	0.003

*Continuous variable.

†Differentiated, papillary or tubular adenocarcinoma; undifferentiated, poorly differentiated or mucinous adenocarcinoma, or signet-ring cell carcinoma.

‡T3, penetrating the serosa; T4, invading adjacent organs.

§n0–n3, grade of lymph node metastasis.

||Lymphatic invasion: ly0–ly3, grade of lymphatic vessel invasion

¶Vascular invasion: v0–v3, grade of vascular invasion.

CI, confidence interval; TSLP, Thymic Stromal Lymphopoietin.

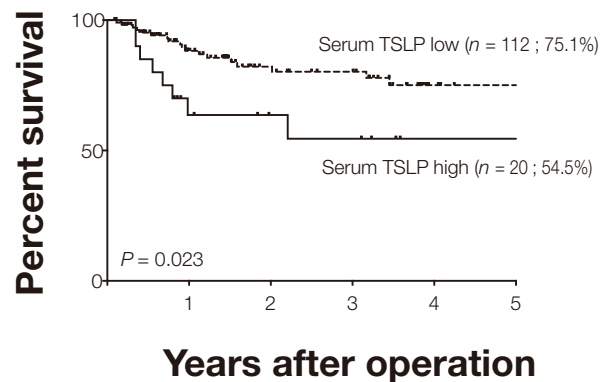


Fig. 3. Survival curves of gastric cancer patients according to their serum TSLP concentrations. TSLP, Thymic Stromal Lymphopoietin.

Serum concentration of TSLP in gastric cancer patients

The serum concentration of TSLP was significantly higher in patients with than in those without liver metastasis ($P < 0.001$; Table 2) and in patients with than without intraperitoneal free cancer cells ($P = 0.043$; Table 2). The serum TSLP concentrations in patients with TSLP-positive and TSLP-negative tumors were 178.2 ± 68.1 and 155.0 ± 122.2 pg/mL, respectively; the difference

was not statistically significant ($P = 0.39$). Serum TSLP concentrations were 130.8 ± 54.1 and 217.5 ± 107.4 pg/mL in patients with TSLP-positive and TSLP-negative non-cancerous tissue, respectively; again, the difference was not statistically significant ($P = 0.14$).

Based on a mean serum TSLP concentration in our gastric cancer patients of 215.3 pg/mL, a cut-off value of 200 pg/mL was used in this study to determine survival as a function of TSLP expression. The 5-year survival rates of patients with high (> 200 pg/mL) and low (≤ 200 pg/mL) serum TSLP levels were 54.5% and 75.1%, respectively (Fig. 3). The patients with high serum TSLP concentrations also had a significantly worse prognosis than those with low concentrations ($P = 0.023$; Fig. 3). In a multivariate analysis, serum TSLP level was an independent prognostic indicator ($P = 0.003$; Table 3).

Finally, we examined the correlation between the serum TSLP level and serum carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 levels. The latter are the most frequently used tumor markers in gastric cancer; however, there were no significant correlations between CEA, CA19-9, and TSLP expression ($P = 0.96$, $P = 0.55$, respectively; data not shown).

DISCUSSION

TSLP expression in both the non-cancerous gastric mucosa and the cancer tissue of our patients with gastric cancer was demonstrated using immunohistochemistry. TSLP expression in gastric cancer tissue, but not in non-cancerous gastric mucosa, was found to correlate significantly with cancer progression. Furthermore, patients with TSLP-positive had slightly worse prognosis than those with TSLP-negative, but the reason for this relationship is as yet unknown. However, TSLP plays a very important role in the induction of the Th2-based immune response and previous studies have shown that the Th1/Th2 ratio is a prognostic indicator in gastric cancer patients.²⁷ In addition, the polarization of group 2 innate lymphoid cells, which produce large amounts of Th2 cytokines, contributes to the immunosuppressive microenvironment in gastric cancer.²⁹ In cervical cancer, tumor-derived TSLP also act on TSLPR+ endothelial cells to promote angiogenesis.^{21, 30} Neo-angiogenesis is a critical process in cancer progression. In gastric cancer patients, high microvessel counts correlate significantly with a poor prognosis.³¹ Therefore, the TSLP-mediated increase in microvessels supplying the malignant tissue of gastric cancer may explain the relation between TSLP and a poor prognosis. Alternatively, regulatory T cells (Tregs) may play a role, based on in vitro studies demonstrating the involvement of TSLP in the generation of tolerogenic DCs that drive the differentiation of

Tregs.³²⁻³⁴ Kim et al. demonstrated that a higher intratumoral Tregs/helper T cells ratio was significantly related to poor prognosis in gastric cancer patients.³⁵ Further investigations are needed to clarify the role of TSLP in the progression of gastric cancer.

An analysis of the relationship between the serum concentration of TSLP and the prognosis of gastric cancer patients showed that patients with high serum TSLP concentrations had a significantly worse prognosis than low concentrations. The role of serum TSLP level as an independent indicator of prognosis in gastric cancer patients was confirmed in a multivariate analysis. Thus, the measurement of serum TSLP in patients with non-invasive gastric tumors may provide important prognostic information. However, the serum TSLP level was not related to the serum levels of two important tumor markers in gastric cancer, CEA and CA19-9. Therefore, measurement of the serum TSLP level may be useful to predict the prognosis of gastric cancer patients regardless of their serum CEA and CA19-9 concentrations.

To the best of our knowledge, ours is the first study to show a correlation between serum TSLP level and prognosis in gastric cancer patients. The absence of a relationship between TSLP expression in non-cancerous gastric mucosa, gastric cancer tissue, and the concentration of serum TSLP contrasts with the high serum TSLP concentrations in patients with liver metastasis and peritoneal lavage cytology. These observations suggest that TSLP is produced by tumor cells, fibroblasts, and epithelial cells at the metastatic site. In other words, TSLP expression reflects only a local progression of cancer, and serum TSLP reflects a systemic progression of cancer, such as metastasis and positive peritoneal lavage cytology. Because of this, we considered serum TSLP was only a prognostic indicator. The origin of the high concentration of serum TSLP remains to be determined.

There is currently no effective treatment for recurrent gastric cancer. Novel strategies for inhibiting tumor growth and thereby improving survival include the blockage of TSLP or OX40 ligand and thus IL-13 production, an approach recently evaluated in a xenotransfer model.³⁶ However, the close relationship between TSLP and the progression of gastric cancer suggests that TSLP is a potentially important and effective target for the treatment of gastric cancer. Measurement of serum TSLP levels might improve survival predictions for patients with gastric cancer.

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