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Original article

Relationship between interleukin-1-alpha concentration in tumors and cell growth in gastric cancer, determined using flow cytometry

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Abstract

Background. **Interleukin-1-alpha (IL-1α) produced by tumor cells stimulates the proliferation or the growth of several cancer cell lines. The aim of this study was to clarify the relationship between growth activity evaluated by DNA analysis and the concentration of tumor-derived IL-1α in gastric cancers in clinical cases.**

Methods. **We measured the concentration of IL-1α in homogenized tumor samples obtained from 49 patients with gastric cancer, using an enzyme-linked immunosorbent assay, and we analyzed the cellular DNA content of paraffin-embedded tumor sections using flow cytometry.**

Results. **Both the IL-1α concentration and the percentage of S-phase fraction were significantly correlated with liver metastasis, histologic type, pattern of tumor infiltration, quantity of stroma, and venous invasion. A good correlation was found between IL-1α concentration in tumors and the percentage of S-phase fraction ($R = 0.604$, $P < 0.0001$).**

Conclusion. **IL-1α may be related to the stimulation of cell growth of gastric cancer in clinical cases.**

Key words Interleukin-1 · Gastric cancer · Cell growth · S-phase fraction · DNA flowcytometry

Introduction

Interleukin-1-alpha (IL-1α), a cytokine mainly produced by macrophages, has multiple biologic activities and plays an important role not only in the immune system [1] but also in the proliferation and metastasis of cancers. We previously reported, using stepwise logistic regression, that among various clinicopathologic characteristics, IL-1α concentration in tumors may be one of the most useful determinants of liver metastasis of gastric cancers [2]. It has recently been reported that IL-1α is produced in many human cancer cell lines, including those of bladder cancer [3], thyroid carcinoma [4,5], gastric carcinoma [6], ovarian cancer [7], squamous cell carcinoma [8–10], and pancreatic carcinoma [11]. We have revealed that patients with tumors stained with IL-1 antibody by immunohistochemistry showed a higher incidence of liver metastasis of gastric cancers [12]. IL-1α produced by tumor cells stimulates the proliferation or the growth of thyroid cancer [4,5], gastric cancer [6], ovarian cancer [7], and colon cancer cell lines [13], and is considered to act as a promoter of invasion or metastasis via the expression of E-selectin on vascular endothelial cells [14] or via the production of matrix metalloproteinase from the tumor cells [15]. However, it has also been reported that IL-1α inhibited the growth of cell lines in thyroid cancer [16], breast cancer [17,18], and colon cancer [19].

In the present study, to clarify the clinical role of IL-1α, we investigated the relationship between growth activity, evaluated by DNA analysis, and the concentration of tumor-derived IL-1α in gastric cancers.

Patients and methods

Patients

A total of 410 patients underwent gastrectomy for gastric cancer at the First Department of Surgery, National Defense Medical College Hospital, between 1994 and 1997. Fresh frozen samples of the tumors were obtained from 49 of these patients.

Assay of IL-1α concentrations

After being thawed, the tissue samples were homogenized in 0.1 M phosphate-buffered saline, using scissors and an Ultra Turrax (Polytron RT10-35 homogenizer; Kinematica AG, Lucerne, Switzerland) and

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centrifuged at 1800*g* for 10 min. The IL-1α concentration in the supernatants was determined by enzyme-linked immunosorbent assay (Biotrak IL-1α ELISA System; RPN.2140; Amersham, Buckingham shire, United Kingdom, and corrected for protein concentration, using the Lowry method, with minor modification [20].

DNA analysis

Materials were obtained from paraffin-embedded blocks. Single-cell suspensions from these specimens were prepared according to the method of Hedley et al. [21], with minor modification. Three 50-μm slices were prepared from the cancer tissues. After deparaffinization, the cancer tissues were minced with scissors in 0.1 M phosphate-buffered saline and centrifuged at 1800*g* for 5 min, and the necrotic tissues were then excluded. The remaining tissues were incubated in 0.25% trypsin solution at 37°C for 120 min. After filtration through a 50-μm nylon mesh, the specimens were centrifuged at 400*g* for 5 min. DNA staining was performed using CycleTEST (Becton Dickinson, Mountainview CA, USA). The DNA content of individual cell nuclei, at 10000 per sample, was measured with a FACScan/CellFIT DNA System (Becton Dickinson). On the histograms, DNA diploidy was defined as a single G0/G1 peak, and aneuploidy as more than two G0/G1 peaks. Histograms with coefficients of variation of more than 8% were excluded from the study.

Statistical analysis

IL-1α concentration is expressed as picograms per milligram protein, and S-phase fractions are expressed as percentages. Any difference in the means of continuous variables between two groups was evaluated using Student's *t*-test or Welch's *t*-test.

Clinicopathologic findings were described according to the *Japanese classification of gastric carcinoma* proposed by the Japanese Research Society for Gastric Cancer [22].

Results

Relationship between clinicopathologic findings and IL-1α concentration or S-phase fraction of tumors

Both IL-1α concentration and S-phase fraction were significantly higher in differentiated type tumors, in tumors associated with liver metastasis, and in tumors with an expansive or intermediate growth pattern (INFα, INFβ) and small or intermediate amount of interstitial connective tissue (medullary, intermediate). In tumors associated with severe to moderate venous invasion (v3, v2), only IL-1α concentration showed a significantly higher level. No significant correlation was observed between IL-1α concentration or S-phase fraction and other clinicopathologic factors such as depth of invasion, lymph node metastasis, peritoneal dissemination, and lymphatic invasion (Table 1).

Relationship between IL-1α concentration and S-phase fraction and DNA ploidy pattern

When we compared the IL-1α concentration of the tumors with their S-phase fraction (%S), we found a positive correlation between these parameters ($R = 0.604$; $P < 0.0001$). The average S-phase fraction was 11.0% and the average IL-1α concentration was 9.0 pg/mg protein. Tumors in four of the five patients

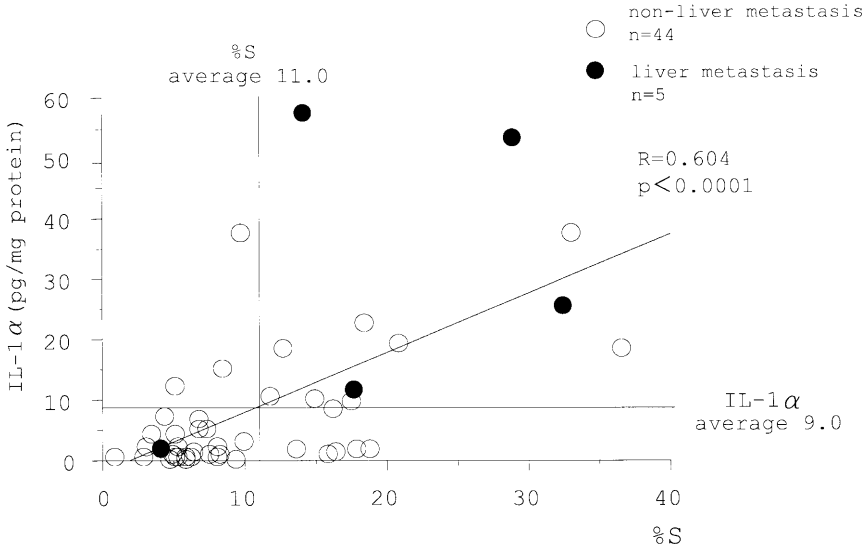

Fig. 1. Relationship between interleukin 1α concentration in tumor and S-phase fraction

Table 1. Relationship between clinicopathologic findings and interleukin (IL)-1α concentration and S-phase fractions (%S) of tumors

	No. of patients	IL-1α	*P* Value	%S	*P* Value
Histologic type[a]					
Differentiated	22	12.9 ± 16.5	***0.035***	14.1 ± 8.9	***0.017***
Undifferentiated	27	4.7 ± 5.7		8.5 ± 6.9	
Depth of invasion[a]					
t1 and t2	17	7.5 ± 7.8	0.727	10.9 ± 8.6	0.938
t3 and t4	32	9.7 ± 15.1		11.1 ± 8.3	
Lymph node metastasis[a]					
Negative	8	12.7 ± 19.1	0.486	12.9 ± 8.5	0.479
Positive	41	7.6 ± 11.6		10.6 ± 8.3	
Liver metastasis[a]					
Negative	44	6.0 ± 7.8	***0.045***	10.1 ± 7.4	***0.017***
Positive	5	30.3 ± 18.7		19.2 ± 11.5	
Peritoneal dissemination[a]					
Negative	45	8.2 ± 13.2	0.651	10.6 ± 8.0	0.317
Positive	4	11.3 ± 10.5		15.0 ± 12.2	
INF[a]					
α and β	36	11.0 ± 15.0	***0.006***	12.9 ± 9.2	***0.002***
γ	13	3.2 ± 3.8		7.0 ± 3.4	
Stroma[a]					
Med. and inter.	36	10.3 ± 14.5	***0.010***	12.3 ± 9.1	***0.009***
Sci.	13	3.8 ± 3.9		7.3 ± 3.7	
Lymphatic invasion[a]					
ly0 and ly1	6	5.8 ± 8.6	0.480	11.0 ± 6.3	1.000
ly2 and ly3	43	8.8 ± 13.5		11.0 ± 8.6	
Venous invasion[a]					
v0 and v1	24	4.1 ± 4.8	***0.021***	9.8 ± 7.4	0.317
v2 and v3	25	12.6 ± 16.7		12.2 ± 9.1	

[a] According to the *Japanese classification of gastric carcinoma* of the Japanese Research Society for Gastric Cancer [22]

with liver metastasis had higher than average values for both parameters and a histology of differentiated type. The one remaining patient had values below the averages for both parameters; this tumor was of the undifferentiated type (Fig. 1). There was no relationship between IL-1α concentration and DNA ploidy pattern.

Discussion

We previously reported that, for gastric cancer, IL-1α concentration in tumor tissue was significantly correlated with liver metastasis [2]. In gastric cancer patients, the IL-1α concentration in the tumor tissue has been reported to be higher than that in the normal gastric mucosa [23]. Recently, Kimura et al. [24], using the techniques of immunohistochemical staining and in-situ hybridization, revealed that IL-1α was strongly expressed not on interstitial cells but on gastric cancer cells themselves [24]. However, the relationship between IL-1α expression and tumor malignancy, which is estimated by tumor growth capacity, is still unclear, particularly from the viewpoint of clinical evaluation.

Here, we have demonstrated that the percentage of S-phase fraction, one of the most reliable markers of tumor growth capacity, is well correlated with IL-1α concentration. Tumors with a high percentage of S-phase fraction and a high concentration of IL-1α were associated with differentiated histology, scanty stroma, and expanding tumor growth. Ikeguchi et al. [25] reported that the mean size of the S-phase fraction

in carcinomas in differentiated tumors was significantly higher than that in tumors of the undifferentiated type. With regard to undifferentiated type tumors, an abundance of non-proliferative cells having plenty of mucin was found, which would explain why the S-phase fraction in differentiated type tumors is significantly higher than that in undifferentiated type tumors.

Our study has revealed that IL-1α is one of the growth stimulators for gastric cancer. The tumor-promoting mechanism of IL-1α has been ascribed to its stimulatory effect on tumor cell proliferation through a Ca^{2+}/calmodulin-dependent pathway, which has been demonstrated in a thyroid cancer cell line [4], or through an epidermal growth factor (EGF) receptor-dependent pathway, which has been demonstrated in malignant cervical epithelial cells [26]. The EGF/transforming growth factor-α (TGFα) receptor system may be involved in the growth modulation exerted by IL-1α in gastric cancer [6]. IL-1α has also been reported to act as an autocrine growth stimulator for an ovarian cancer cell line [7]. Recently, cyclooxygenase-2 (COX-2), which is increased by stimulation with IL-1, was shown to play an important role in the progression of gastric cancer [27]. Stimulation of tumor growth may not be the only mechanism through which IL-1α promotes cancer metastasis. IL-1α has been reported to induce the expression of urokinase-type plasminogen activator [28], matrix metalloproteinase-9 [15], vascular endothelial growth factor [29], and thymidine phosphorylase [30], all of which have the ability to promote cancer metastasis.

In conclusion, a clearly positive relationship was found between the concentration of tumor IL-1α and S-phase fraction. Given this relationship, IL-1α may be one of the growth stimulators of gastric cancer cells.

References

1. Platanias LC, Vogelzang NJ. Interleukin-1: biology, pathophysiology, and clinical prospects. Am J Med 1990;89:621–9.
2. Furuya Y, Ichikura T, Mochizuki H. IL-1α concentration in tumor as a risk factor for liver metastasis in gastric cancer. Surgery Today 1999;29:288–9.
3. Hayashi O, Akashi M, Fujime M, Hanazawa K, Kitagawa R. Detection of interleukin-1 activity in human bladder cancer cell lines. J Urol 1994;151:750–3.
4. Inokuchi N, Zeki K, Morimoto I, Nakano Y, Fujihira T, Yamashita U, et al. Stimulatory effect of interleukin-1 alpha on proliferation through a Ca^{2+}/calmodulin-dependent pathway of a human thyroid carcinoma cell line, NIM 1. Jpn J Cancer Res 1995;86:670–6.
5. Zeki K, Nakano Y, Inokuchi N, Watanabe K, Morimoto I, Yamashita U, et al. Autocrine stimulation of interleukin-1 in the growth of human thyroid carcinoma cell line NIM1. J Clin Endocrinol Metab 1993;76:127–33.
6. Ito R, Kitadai Y, Kyo E, Yokozaki H, Yasui W, Yamashita U, et al. Interleukin 1 alpha acts as an autocrine stimulator for human gastric carcinoma cells. Cancer Res 1993;53:4102–6.
7. Kawakami Y, Nagai N, Ota S, Ohama K, Yamashita U. Interleukin-1 as an autocrine stimulator in the growth of human ovarian cancer cells. Hiroshima J Med Sci 1997;44:51–9.
8. Knerer S, Hulla W, Martinek H, Formanek M, Temmel A, Kornfehl J. IL-1 and TNF-alpha but no IL-2 expression is found in squamous cell carcinomas of the head and neck by RT-PCR. Acta Oto-Laryngol (Stockh) 1996;116:132–6.
9. Nowak RA, Morrison NE, Goad DL, Gaffney EV, Tashjian AH Jr. Squamous cell carcinomas often produce more than a single bone resorption-stimulating factor: role of interleukin-1 alpha. Endocrinology 1990;127:3061–9.
10. Partridge M, Chantry D, Turner M, Feldmann M. Production of interleukin-1 and interleukin-6 by human keratinocytes and squamous cell carcinoma cell lines. J Invest Dermatol 1991;96: 771–6.
11. Kaji M, Ishikura H, Kishimoto T, Omi M, Ishizu A, Kimura C, et al. E-Selectin expression induced by pancreas-carcinoma-derived interleukin-1 alpha results in enhanced adhesion of pancreas-carcinoma cells to endothelial cells. Int J Cancer 1995;60:712–7.
12. Tomimatsu S, Ichikura T, Furuya Y, Mukouda K, Kimura M, Okusa Y, et al. A study of the relationship between expression of interleukin 1α and liver metastasis. Jpn J Gastroenterol Surg 1998;31:273.
13. Lahm H, Petral-Malec D, Yilmaz-Ceyhan A, Fisher JR, Lorenzoni M, Givel JC, Odartchenko N. Growth stimulation of a human colorectal carcinoma cell line by interleukin-1 and -6 and antagonistic effects of transforming growth factor beta-1. Eur J Cancer 1992;28:1894–9.
14. Srinimas U, Pahlsson P, Lundbland A. E-Selectin: sialyl Lewis, a dependent adhesion of colon cancer cells, is inhibited differently by antibodies against E-selectin ligands. Scand J Immunol 1996;44:197–203.
15. Shimizu S, Nishikawa Y, Kuroda K, Takagi S, Kozaki K, Hyuga S, et al. Involvement of transforming growth factor beta 1 in autocrine enhancement of gelatinase B secretion by murine metastatic colon carcinoma cells. Cancer Res 1996;56:3366–70.
16. Kimura H, Yamashita S, Namba H, Tominaga T, Tsuruta M, Yokoyama N, et al. Interleukin-1 inhibits human thyroid carcinoma cell growth. J Clin Endocrinol Metab 1992;75:596–602.
17. Gaffy EV, Koch G, Tsai SC, Loucks T, Lingenfelter SE. Correlation between human cell growth response to interleukin-1 and receptor binding. Cancer Res 1988;48:5455–9.
18. Sgagias MK, Kasid A, Danforth DN Jr. Interleukin-1 alpha and tumor necrosis factor-alpha (TNF alpha) inhibit growth and induce TNF messenger RNA in MCF-7 human breast cancer cells. Mol Endocrinol 1991;5:1740–7.
19. Raitano AB, Korc M. Growth inhibition of a human colorectal carcinoma cell line by interleukin 1 is associated with enhanced expression of gamma-interferon receptors. Cancer Res 1993;53: 636–40.
20. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1992;83:346–56.
21. Hedley DW, Friedlander ML, Tailor LW, Rugg CA, Musgrove EA. Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 1983;31:1333–5.
22. Japanese Research Society for Gastric Cancer. Japanese classification of gastric carcinoma. 2nd English ed. Gastric Cancer 1998;1:10–24.
23. Kimura T, Kobayashi T, Nakaya Y, Iida T, Uchimura M, Murohisa B, et al. Effect of 5′-deoxy-5-fluorouridine (5′-DFUR) on the activity of pyrimidine nucleoside phosphorylase (PyNPase) in normal and tumor tissue of human stomach. Jpn J Cancer Chemother 22:1051–6.
24. Kimura M, Ichikura T, Uefuji K, Tomimatsu S, Okusa Y, Majima T, et al. Examination for the expression of interleukin-1 α mRNA by tissue biopsy specimen and cell lines in gastric cancer. Jpn J Gastroenterol Surg 1999;32:423.

25. Ikeguchi M, Katano K, Oka A, Kurayoshi K, Tsujitani S, Maeta M, et al. Relationship between the cell-proliferative activity of gastric cancers and that of the normal epithelium. Anticancer Res 1995;15:821–5.
26. Woodworth CD, McMullin E, Iglesias M, Plowman GD. Interleukin 1 alpha and tumor necrosis factor alpha stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells. Proc Natl Acad Sci USA 1995;92:2840–4.
27. Sawada H, Kawano S, Tsuji S, Tsujii M, Murata H, Hori M. Effects of NSAIDs on proliferation of gastric cancer cells in vitro: possible implication of cyclooxygenase-2 in gastric cancer development. J Clin Gastroenterol 1998;27:47–52.
28. van Hinsbergh VW, van den Berg EA, Fiers W, Dooijewaard G. Tumor necrosis factor induces the production of urokinase-type plasminogen activator by human endothelial cells. Blood 1990;75: 1991–8.
29. Yamamoto S, Yasui W, Kitadai Y, Yokozaki H, Haruma K, Kajiyama G, et al. Expression of vascular endothelial growth factor in human gastric carcinomas. Pathol Int 1998;48:499–506.
30. Takebayashi Y, Yamada K, Ohmoto Y, Sameshima T, Miyadera K, Yamada Y, et al. The correlation of thymidine phosphorylase activity with the expression of interleukin 1 alpha, interferon alpha and interferon gamma in human colorectal carcinoma. Cancer Lett 1995;95:57–62.