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G-CSF PRODUCING PAROTID TUMOR; A CASE REPORT

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We reported a rare case of malignant parotid tumor with a severe granulocytosis, which was induced by granulocyte colony stimulating factor (G-CSF) produced from tumor cells. A 59-year-old male patient had shown a marked leukocytosis with up to 27,100/mm^3 and a larger number of mature granulocytes in peripheral blood leukocytes. The level of G-CSF in serum was obviously high (263.0 pg/ml). We undertook a total parotidectomy and left radical neck dissection, but the tumor resection was not completely performed because of an extensive invasion to the left carotid artery and parapharyngeal space as well. The pathological diagnosis was poorly-differentiated adenocarcinoma. The patient died about 4 months after being admitted to our hospital, even though a systemic chemotherapy was followed after the surgical treatment. We diagnosed this case as a G-CSF producing malignant parotid tumor, because high G-CSF activity was detected in the tumor secretion, cancerous pleural effusion and culture supernatant of tumor cells, respectively. This diagnosis was further confirmed by the finding that G-CSF gene transcripts were detected in tumor cells of this patient.

Key words: leukocytosis, granulocyte colony stimulating factor (G-CSF), malignant parotid tumor

INTRODUCTION

G-CSF plays an important role in a survival, growth, and differentiation of mature granulocytes from hematopoietic progenitor cells both *in vitro* and *in vivo* (1). Patients with leukocytosis sometimes accompany malignant solid tumors in the absence of infection. In these cases, G-CSF had been implicated in the tumor-induced leukocytosis(2). G-CSF is especially produced by monocytes/macrophages, fibroblasts, endothelial cells and it stimulates the growth of neutrophils. G-CSF production in cancer patients is frequently associated with an aggressive tumor cell growth and a detrimental clinical outcome. However, there have been a few reports of head and neck cancer with leukocytosis, which actually showed the G-CSF production from tumor cells, particularly in malignant parotid tumors. This report introduces a rare case of G-CSF producing parotid cancer, by employing with an examination of G-CSF specific messenger RNA expression in tumor cells and measurement of G-CSF activity in culture supernatants of tumor cells.

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CASE REPORT

A 59-year-old male patient presented with a one-month history of swelling and spontaneous pain on the left side of his neck. He received an antibiotics therapy in another hospital, because of the leukocytosis and the elevation of C-reactive protein (CRP). However, his condition did not improve and therefore he was introduced to our hospital on November 29, 1994.

Physical examination on his admission revealed a tumor formation from the left infraauricular region to the upper neck with red overlying skin (Fig. 1). The tumor was elastic and hard, and had poor mobility. Several swollen lymph nodes were palpable around the tumor on the left side of the neck. The patient's ears, nose and throat were fine. No facial nerve palsy was found.

Peripheral blood analysis on admission showed a marked leukocytosis (27,100/mm^3) and 85% of leukocytes were neutrophils. CRP was positive at 16.8 mg/dl and erythrocyte sedimentation rate (ESR)

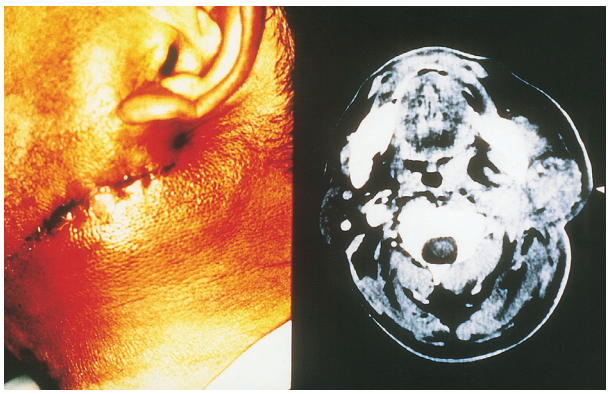

Fig. 1. A tumor formation was seen from the left infraauricular region to the upper neck with red overlying skin(left side). Open biopsy was already done in another hospital before admission. The tumor was elastic and hard, and had poor mobility. Several swollen lymph nodes were palpable around the tumor on the left side of the neck. CT scan indicates this tumor under the skin around left parotid region(right side).

was accelerated (108 mm/hour).

Peripheral blood T lymphocytes($CD3^+$) were only 55.0% of the total lymphocytes and helper/inducer T cells ($CD4^+$) decreased to 26.0% of the total T lymphocytes. Tumor markers such as squamous cell carcinoma antigen(SCC Ag), carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 were within normal limits (Table 1), but G-CSF activity in serum measured by an enzyme-linked immunosorbent assay kit (Amersham, UK) was obviously high, being at 263.0 pg/ml. A bone marrow biopsy was done, because the severe granulocytosis was observed, but resulted in marked hypercellular bone marrow and no leukemic cells were identified.

Serial enhanced CT scan and magnetic resonance imaging (MRI) revealed a heterogeneously enhancing mass in the left parotid gland and multiple swollen lymph nodes in the left upper side of the neck, that had a mixed appearance of solid and cystic portions (Fig. 2).

Table 1. Laboratory dataon a dmission

WBC	27.100 ↑	/mm³	T.P	7.6	g/dl	C3	103 ↑	mg/dl
Band	5	%	ALB	3.3	g/dl	C4	45 ↑	mg/dl
Seg	80 ↑	%	T.BIL	0.3	mg/dl	IgG	2302 ↑	mg/dl
Eos	2	%	AST	19	IU/l	IgA	680 ↑	mg/dl
Baso	0	%	ALT	15	IU/l	IgM	300	mg/dl
Mono	2	%	LDH	276	IU/l			
Lymph	11 ↓	%	ALP	116	IU/l	CD3	55.0 ↓	%
RBC	432×10⁴	/mm³	ChE	193	IU/l	CD20	24.0 ↑	%
Hgb	14.1	g/dl	CK	40	IU/l	CD4	26.0 ↓	%
Hct	41.6	%	Amy	302	IU/l	CD8	29.0	%
PLT	42.2×10⁴	/mm³	BUN	8	mg/dl	Th/Ts	0.8 ↓	
			Crea	0.7	mg/dl			
CRP	16.8 ↑	mg/dl	Na	141	mEq/l	SCC	1.0	ng/ml
ESR	1hr 108 ↑	mm	K	4.3	mEq/l	CEA	2.4	ng/ml
	2hr 122 ↑	mm	Cl	103	mEq/l	CA19-9	11	U/ml
			Ca	8.9	mg/dl			
			Gluc	89	mg/dl			

^{67}Ga scintigraphy showed the abnormal ^{67}Ga uptake in the left parotid gland and the upper neck. A biopsy of the tumor was performed after his admission, and malignant neoplasm was suspected but a definite pathological diagnosis could not be achieved. On December 16, 1994, left total parotidectomy and radical neck dissection was carried out. At the operation, the tumor extensively invaded to the overlying skin, facial nerve trunk, internal carotid artery, and parapharyngeal space as well on the left side. The facial nerve and the tumor-infiltrated skin were excised, but the tumor resection was not completely performed. The histological diagnosis was poorly-differentiated adenocarcinoma (Fig. 3). On day 3 after the operation, the white blood cells (WBC) in

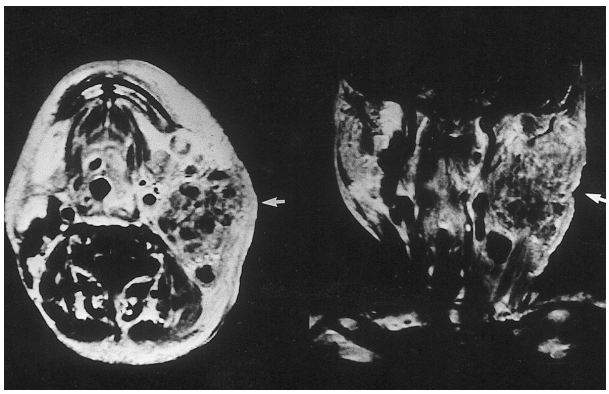

Fig. 2. Magnetic resonance imaging (MRI) revealed a heterogeneously enhancing mass in the left parotid gland and multiple swollen lymph nodes in the left upper side of the neck, that had a mixed appearance of solid and cystic portion (horizontal section, left and coronary section, right).

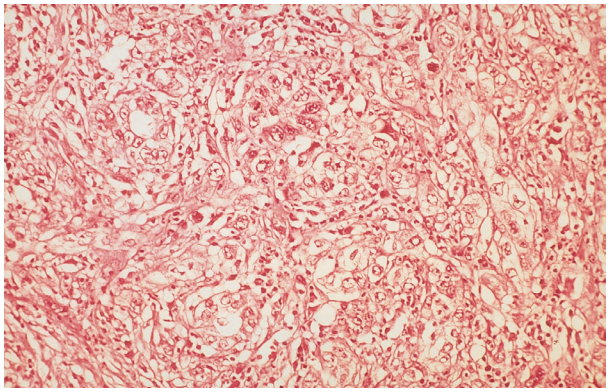

Fig. 3. Histological finding (H&E staining). The diagnosis was poorly-differentiated adenocarcinoma.

his peripheral blood decreased to 17,900/mm^3. However, the tumor regrew gradually afterwards. Therefore, the systemic chemotherapy with cisplatin, THP-adriamycin and 5-Fu was done in 2 courses. The size of the tumor did reduce for about one month. But it was unfortunate that the patient finally died of a lung metastasis and pleuritis carcinomatosa on April 3, 1996. An autopsy was not performed but carcinomatous pleural effusion was aspirated and brought into a primary cell culture.

Fig. 4 showed the clinical course of this patient, including WBC count in the peripheral blood and the serum G-CSF activity. The WBC once decreased after the operation and chemotherapy. However it eventually increased up to 225,900/mm^3. On the other hand, serum G-CSF activity was constantly high, regardless of the treatment, the tumor size and WBC count in his peripheral blood.

Because of the marked leukocytosis in the absence of infection and the constant high G-CSF activity in the patient's, sera, we examined the G-CSF activity in the other clinical materials of this patient. As shown in Table 2, G-CSF activities in the tumor secretion, and the carcinomatous pleural effusion were very high (234000 pg/ml and 4800 pg/ml, respectively). Furthermore, G-CSF activity in the culture supernatant of tumor cells derived from pleural effusion was also very high (4030 pg/ml). Fig. 5 shows the phase contrast microscopic findings of the primary culture of carcinomatous pleural effusion obtained from this patient. Polygonal-shaped cells have a cobble-stone appearance. Table 3 shows G-CSF levels in culture supernatants of cancer cell lines maintained in our laboratory, including this case. In vitro culture of cancer cell lines was performed as follows. 1x10^6 cells were distilled in 5 ml of RPMI1640 medium supplemented with 10% fetal calf serum and incubated for 48 hours at 37 ℃ in 5% CO_2 incubator. And thereafter culture supernatants were obtained. G-CSF activity varies from low to high levels among these cell lines and the highest activity is shown in this case. But there was no difference in G-CSF activity of culture super-

Fig. 4. WBC coumt in clinical course

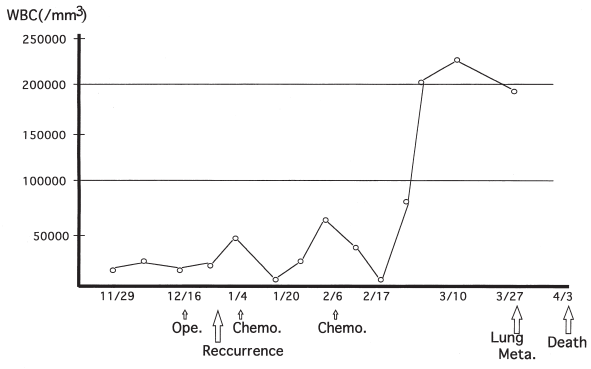

Table 2. G-CSF Activities in sample

	G-CSF Activity (pg/ml)
Serum	263
Tumor Secretion	234000
Pleural Effusion	4800
Culture Supernatant	4030

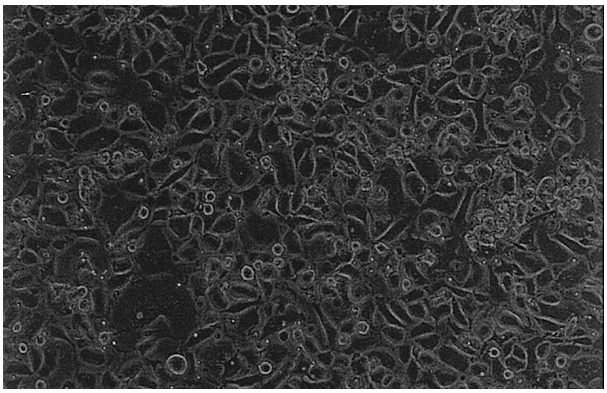

Fig. 5. Phase contrast microscopic findings of the primary culture of carcinomatous pleural effusion obtained from this patient. Polygonal-shaped cells have a cobble-stone appearance, and these tumor cells are considered to produce the G-CSF.

Table 3. G-CSF Activities in culture sup of various cell lines

Cell Line	G-CSF Activity(pg/ml)
MCS-2 (Maxilla, SCC)	<9.8
MCS-4 (Maxilla, SCC)	30.5
HYS-4 (Hypopharynx, SCC)	<9.8
LS-14 (Larynx, SCC)	<9.8
KIMURA (Hypopharynx, SCC)	30.5
YASUHARA (Thyroid Adeno)	513.0
MCF-7 (Mamma, Adeno)	<9.8
A549 (Lung, Adeno)	<9.8
H520 (Lung, SCC)	<9.8
NONAKA (Parotid, Adeno)	4030.0

※SCC : Squamous cell carcinoma Adeno:Adenocarcinoma

natant, in terms of tumor site, histological type, or differentiation degree. Fig. 6 shows the results of reversed transcription-polymerase chain reaction (RT-PCR) of G-CSF transcripts in these cell lines including this case. G-CSF-specific messenger RNA gene expression are actually detected in the tumor cells (NONAKA) obtained from this patient.

Based on these findings taken all together, we confirmed this case as a G-CSF producing parotid tumor.

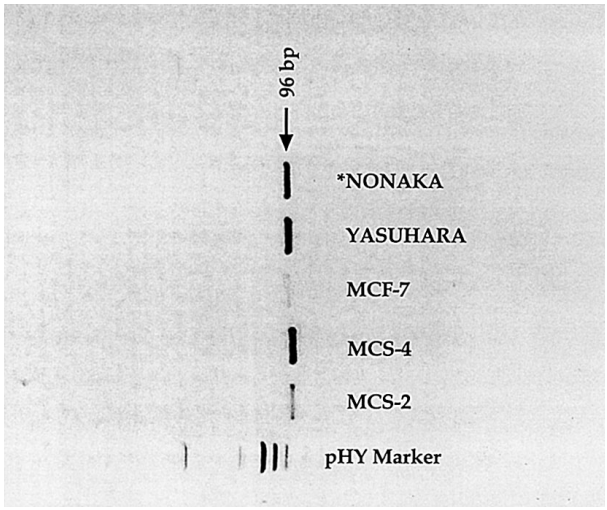

Fig. 6. RT-PCR of G-CSF transcripts (Northern blot analysis). G-CSF-specific mRNA gene expression are actually detected in the tumor cells (NONAKA) obtained from this patient.

COMMENT

Leukocytosis is a well-recognized hematological abnormality that develops in cancer patients even in the absence of infection. A number of malignant tumors are reported to produce colony-stimulating factor (CSF), a kind of cytokine, which brings about marked leukocytosis in tumor-bearing patients (3). G-CSF producing tumors have been reported in lung cancers(4-7), as well as in cancers of the stomach(8), bladder(9,10), oral cavity(11) and thyroid gland(12-14). However, few parotid cancers that produce G-CSF have been reported. We demonstrated herein an unusual case of a 59-year-old patient with a G-CSF producing poorly differentiated adenocarcinoma originated from parotid gland. We diagnosed this case as a G-CSF producing tumor for several reasons mentioned as follows: 1. The patient continued to have marked leukocytosis. 2. Serum G-CSF levels of this patient were significantly elevated. 3. G-CSF level in the culture supernatant of tumor cells sampled from his pleural effusion was also significantly elevated. Furthermore, the G-CSF transcript was detected in cultured tumor cells of this patient by RT-PCR method. These results strongly suggest that the autonomous G-CSF production by the tumor cells induced leukocytosis in this patient.

The leukemoid reaction has been widely observed clinically to appear at an advanced stage of cancer in association with the aggressive cell growth. It is, therefore, in all likelihood that the G-CSF production and G-CSF receptor expression exhibited by cancer cells play a crucial role in mediating the malignant progression of the non-hematopoietic cancer cells. Some authors have reported that the proliferation of the cultured non-hematopoietic cancer cells are stimulated by exogenous G-CSF administration and suggested that G-CSF production by cancer cells autocrine growth(10,15). On the other hand, it was reported that exogenous G-CSF had no effect on the proliferation of the cultured cancer cells (11,13).

Katoh *et al.* (16) commented that the production of G-CSF is a common event in human tumor xenograft, but that factors other than G-CSF are also likely involved. There are some reports that non-hematopoietic cancer cells also produce interleukin 1 (IL-1) (17), or interleukin 6 (IL-6) (11), or both of them(4,7) in addition to G-CSF. We successfully detected G-CSF activities in a number of cultured cancer cell lines maintained in our laboratory but did not examine other cytokines such as IL-1 and IL-6. Leukocytosis induced by neoplasm seems to be a heterogeneous and complex disorder.

It is generally accepted that G-CSF production in cancer patients associated with an aggressive tumor cell growth have a detrimental clinical outcome. The patient in this report died only about 4 months after his admission to our hospital, regardless of the multimodality treatments. Further precise study will be needed to fully understand the biological significance of tumor-producing G-CSF and consequently to have an appropriate clinical management of forthcoming patients for pursuing a better prognosis.

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REFERENCES

1) Metcalf D (1984) The hematopoietic colony stimulating factors. Elsevier Science Publishers Amsterdam-New York.

2) Okabe T, Sato N, Kondo Y, Asano S, Ohsawa N, Kosaka K and Ueyama Y (1978) Establishment and characterization of a human cancer cell line that produced human colony stimulating factor. *Cancer Res* 38: 1085-1088.

3) Asano S, Urabe A, Okabe T, Sato N and Kondo Y (1977) Demonstration of granulopoietic factor(s) in the plasma of nude mice transplanted with a human lung cancer and in the tumor tissue. *Blood* 49: 845-852.

4) Suzuki A, Takahashi T, Okuno Y, Nakamura K, Tashiro H, Fukumoto M, Konaka Y and Imura H (1991) Analysis of abnormal expression of G-CSF gene in a novel tumor cell line (KHC 287) elaborating G-CSF, IL-1 and IL-6 with co-amplification of c-myc and c-ki-ras. *Int J Cancer* 48: 428-433.

5) Nagatomo A, Ooike H, Harada A, Sasaki T, Hanada T, Matuoka Y, Irimajiri S and Kishimoto H (1993) Direct identification of lung cancer cells producing granulocyte colony stimulating factor with monoclonal anti-G-CSF antibodies. *Internal Medicine* 32: 468-471.

6) Adachi N, Yamaguchi K, Morikawa T, Suzuki M, Matsuda I and Abe MK (1994) Constitutive production of multiple colony-stimulating factors in patients with lung cancer associated with neutrophilia. *Br J Cancer* 69: 125-129.

7) Tsuyuoka R, Takahashi T, Sasaki Y, Taniguchi Y, Fukumoto M, Suzuki A, Nakamura K, Kobayashi S, Kudo T and Nakao K (1994) Colony-stimulating factor-producing tumors: Production of granulocyte colony-stimulating factor and interleukin-6 is secondary to interleukin-1 production. *Eur J Cancer* 30A: 2130-2136.

8) Baba M, Hasegawa H, Nakayabu M, Shimizu N, Suzuki S, Kamada N and Tani K (1995) Establishment and characteristics of a gastric cancer cell line (HuGC-OOHIRA) producing high levels of G-CSF, GM-CSF, and IL-6: The presence of autocrine growth control by G-CSF. *Am J Hematol* 49: 207-215.

9) Sato K, Terada K, Sugiyama T, Sugiyama T, Masuda H, Kakinuma H and Kato T (1994) Granulocyte colony-stimulating factor produced by bladder carcinoma of a patient with leukemoid reaction did not affect proliferation of the tumor cells. *J Urol* 151: 1687-1690.

10) Tachibana M, Miyakawa A, Tazaki H, Nakamura K, Kubo A, Hata J, Nishi T and Amano Y (1995) Autocrine growth of transitional cell carcinoma of the bladder induced by granulocyte-colony stimulating factor. *Cancer Res* 55: 3438-3443.

11) Matsuo K, Ishibashi Y, Kobayashi I, Ozeki S, Ohishi M, Tange T, Hirata J, Kiyoshima T and Sakai H (1994) New human oral squamous carcinoma cell line and its tumorigenic subline producing granulocyte colony-stimulating factor. *Jpn J Cancer Res* 85: 1257-1262.

12) Sato K, Fujii Y, Ono Y, Nomura H and Shizume K (1987) Production of interleukin 1-like factor and colony-stimulating factor by a squamous cell carcinoma of the thyroid (T3M-5) derived from a patient with hypercalcemia and leukocytosis. *Cancer Res* 47: 6474-6480.

13) Oka Y, Kobayashi T, Fujita S, Matsuura N, Okamoto S, Asakawa H, Murata A and Mori T (1993) Establishment of a human anaplastic thyroid cancer cell line secreting granulocyte colony-stimulating factor in response to cytokines. In Vitro *Cell Dev Biol* 29A: 537-542.

14) Iwasa K, Noguchi M, Mori K, Ohta N, Miyazaki I, Nonomura A, Mizukami Y, Nakamura S and Michigishi T (1995) Anaplastic thyroid carcinoma producing the granulocyte colony stimulating factor (G-CSF): Report of a case. *Surg Today Jpn J Surg* 25: 158-160.

15) Avalos BR, Gasson JC, Hedvat C, Quan SG. Baldwin GC, Weisbart RH, Williams RE, Golde DW and DiPersio JF (1990) Human granulocyte colony-stimulating factor: Biologic activities and

receptor characterization on hematopoietic cells and small cell lung cancer cell lines. *Blood* 75: 851-857.

16) Katoh Y, Nakamura M, Ohnishi Y, Shimamura K, Ueyama Y and Tamaoki N (1993) Autonomous production of granulocyte-colony stimulating factor in tumor xenograft associated with leukocytosis. *Br J Cancer* 68: 715-719

17) Sato K, Miura H, Han DC, Kakiuchi T, Ueyama Y, Ohkawa H, Okabe T, Kondo Y, Ohsawa N and Tsushima T (1986) Production of bone-resorbing activity and colony-stimulating activity in vivo and in vitro by a human squamous cell carcinoma associated with hypercalcemia and leukocytosis. *J Clin Invest* 78: 145-154.