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*Case report*

# Urinary bladder carcinoma producing granulocyte colony stimulating factor (G-CSF): a case report with immunohistochemistry

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**Abstract.** A rare case of urinary bladder carcinoma with granulocyte colony stimulating factor (G-CSF) production was reported. In an 83-year-old female, marked neutrophilia in the peripheral blood decreased from 132,500/mm$^3$ to 3,300/mm$^3$ after tumour resection. The tumour was a transitional cell carcinoma. The serum G-CSF level reduced from 238 pg/ml pre-operatively to normal (60 pg/ml) after the operation. Immunohistochemical investigation of the resected tumour with monoclonal antibody specific for G-CSF revealed positive staining in the carcinoma cells, confirming G-CSF secretion.

**Key words:** Urinary bladder carcinoma – Granulocytosis – Granulocyte colony stimulating factor

## Introduction

Leukocytosis in patients with non-haematological malignancies is caused in part by colony stimulating factors (CSF) produced in the malignancies. Production of granulocyte-CSF (G-CSF), one of the CSFs which stimulates granulopoiesis and differentiation of granulocytes, has been observed in malignancies including bladder carcinoma (Souza et al. 1986), oral cavity carcinoma (Nomura et al. 1986), malignant melanoma (Lilly et al. 1987), malignant mesothelioma (Demetri et al. 1989; Svet-Moldavskaya et al. 1989), ovarian tumours (Ishiwata et al. 1988), hepatoma (Tohyama et al. 1989), mammary carcinoma (Lee et al. 1989) and lower jaw cancer (Sato et al. 1989). G-CSF production was demonstrated by bioassay or Northern blot analysis using tumour cell lines established from the malignancies or transplanted into nude mice. In the past, absence of antibodies specific for G-CSF prevented us from investigating surgical material from patients with granulocytosis. We recently developed a monoclonal antibody specific for G-CSF (Shimamura et al. 1990) and in this article, we report a case of bladder carcinoma with marked granulocytosis and immunohistochemical demonstration of G-CSF in the tumour cells, using this antibody.

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## Clinical history

A 83-year-old female was referred to Nippon-Kokan Hospital on July 26 1988, because of continuous haematuria for 2 months. Past and family histories were unremarkable. The leukocyte count then was 62,500/mm$^3$ and increased to 132,500/mm$^3$ (Fig. 1). The granulocytosis consisted mainly of neutrophils. Bladder cancer was demonstrated by cytoscopic examination and she was admitted for the therapy on September 19, when there were no further abnormal findings on physical examination. Blood chemistry was unremarkable except for high alkaline phosphatase activity. Bone marrow smear showed granulocytic hyperplasia with maturation (Table 1). Computed tomographic scan revealed a low density mass, 36 × 28 mm in diameter, on the right anterior wall of the

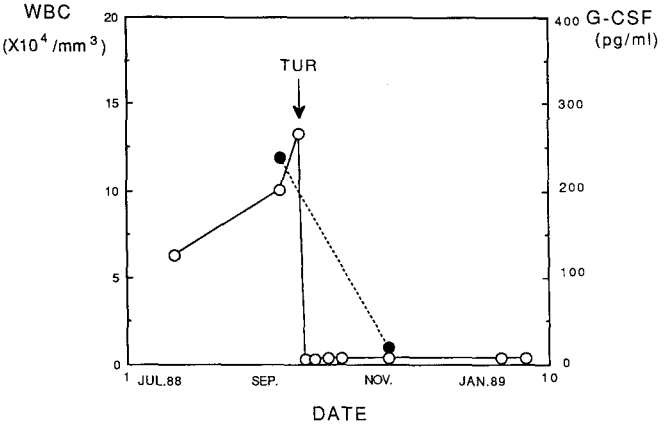


**Fig. 1.** Changes of white blood cell count in peripheral blood (○) and serum G-CSF level (●) before and after the transurethral resection of bladder carcinoma. Measurement of human G-CSF level in the patient sera was performed by sandwich enzyme immunoassay using rabbit anti-rhG-CSF

**Table 1.** Bone marrow cells before transurethral resection

| | Percent[a] | | Percent |
|---|---|---|---|
| Myeloblast | 0.4 | Erythrocyte | 7.8 |
| Promyelocyte | 8.4 | Lymphocyte | 3.8 |
| Myelocyte | 19.8 | Monocyte | 2.0 |
| Metamyelocyte | 12.0 | Eosinophil | 2.0 |
| Stab form | 28.8 | Basophil | 0.0 |
| Segmented | 14.4 | Plasma cell | 0.8 |
| | | Reticulum cell | 0.4 |

[a] Data were derived from a 400 cell count

bladder, but suggested no invasion of the adjacent organs. Re-examination by cystoscopy clarified the presence of a nodular tumour on the right of the bladder wall near the neck. One week after transurethral resection of the tumour on September 20, the leukocyte count in peripheral blood fell to 3,300/mm$^3$. There was no evidende of superimposed systemic infection or granulocytic leukaemia. She died on March 17, 1990 due to brain infarction and bronchopneumonia. During her clinical course, no post-operative leukocytosis was observed and no recurrence or metastasis was present at autopsy.

## Methods

The surgical specimen was fixed in 10% formalin and embedded in paraffin; sections were stained with haematoxylin and eosin.

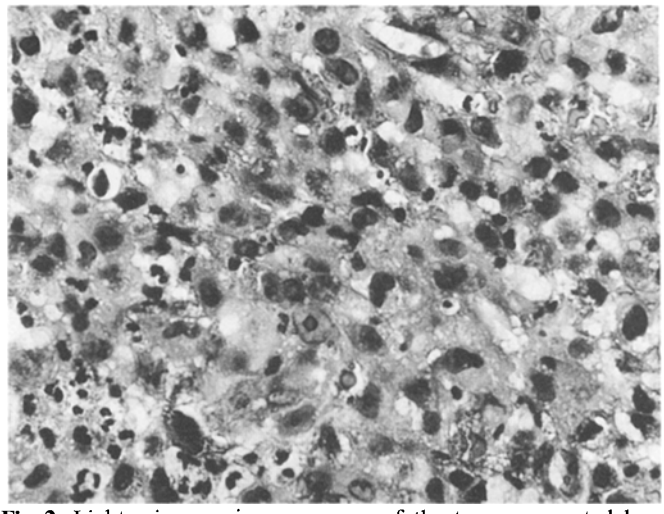

**Fig. 2.** Light microscopic appearance of the tumour resected by transurethral resection. Tumour cells arranged in clusters are accompanied by marked infiltration of granulocytes. ( × 600)

Establishment of the monoclonal antibody reactive with human G-CSF was described elsewhere (Shimamura et al. 1990). Briefly, the antibody was developed by cell hybridization between NS-1 myeloma cells and splenocytes of a (C57BL/6N × BALB/C) $F_1$ mouse immunized with recombinant human G-CSF(rhG-CSF).

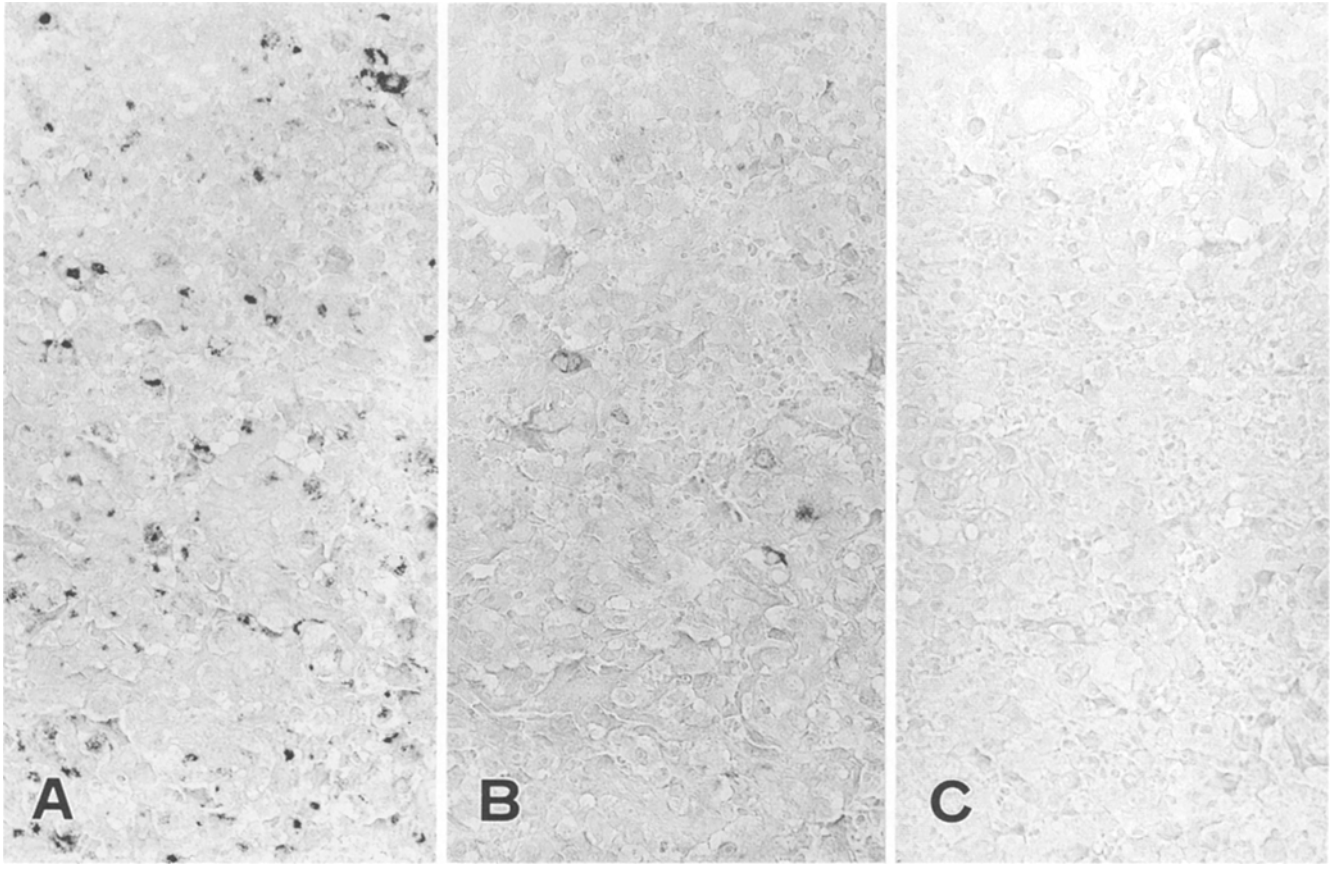


**Fig. 3A–C.** Immunohistochemical localization of G-CSF in the tumour cells. Immunostaining was performed by indirect immunoperoxidase method on paraffin embedded sections. A strongly positive reaction for G-CSF was observed in some of the carcinoma cells (**A**). A few neoplastic cells showed positive reaction for keratin (**B**). Control staining using a class-matched monoclonal antibody revealed no specific reaction (**C**). ( × 300)

They were reactive with human G-CSF but not with other CSFs or tissues which does not secrete G-CSF. Monoclonal antibodies against human keratin (KL 1) was purchased from Immunotech (Marseille, France).

The indirect horse radish peroxidase (HRP)-labelled antibody method was performed on the above paraffin sections after deparaffinization. The sections were incubated for 30 min with the monoclonal antibody after 30 min incubation with normal rabbit serum and washing in PBS. After further washing in PBS, they were reacted for 30 min with HRP-labelled rabbit anti mouse immunoglobulins and again washed in PBS. Finally, an emzymatic reaction was carried out in a solution containing 20 mg of 3,3'-diaminobenzidine-tetra hydrochloricacid and 0.005% hydrogen peroxide in 100 ml of TRIS buffer, pH 7.6. Counter-staining was performed with methyl-green. Control staining using a class-matched monoclonal antibody revealed no specific reaction. Measurement of human G-CSF level in the patient was performed by sandwich enzyme immunoassay according to the method described previously (Motojima et al. 1989). Anti-rhG-CSF rabbit IgG physically absorbed onto plastic tube was used as the capture antibody. The corresponding antibody-binding fragment, conjugated to HRP by means of a maleimide derivative, used as the detector antibody.

## Results

As shown in Fig. 1, the high leukocyte count in preoperative peripheral blood rapidly decreased to normal levels after tumour resection. Correspondingly, serum G-CSF level examined sandwich enzyme immunoassay was reduced from 238 pg/ml to 60 pg/ml after the operation.

Microscopic examination of the biopsy specimen from the bladder tumour demonstrated poorly differentiated transitional cell carcinoma with marked infiltration of granulocytes and focal haemorrhage. The tumour cells were arranged in clusters and demonstrated moderate nuclear atypia (Fig. 2). The indirect immunoperoxidase staining done on paraffin sections, disclosed strongly positive staining for G-CSF in carcinoma cells, some of which were observed in carcinoma nests. Small numbers of tumour cells (less than 10% of carcinoma cells) were positive for G-CSF. The staining pattern for G-CSF was granular and concentrated around nuclei in the tumour cells (Fig. 3 A). Positive staining for keratin in a few neoplastic cells confirmed expression of epithelial nature of the neoplasm (Fig. 3 B).

## Discussion

Our present case was diagnosed as G-CSF producing urinary bladder carcinoma since marked granulocytosis was reduced to normal with a corresponding reduction of high serum G-CSF level after tumour excision. G-CSF was immunohistochemically identified in tumour cells, even though detection of G-CSF mRNA not carried out. A granular staining pattern around nuclei was characteristic for G-CSF producing tumour cells and scarcity of positive cells was possibly due to rapid secretion of G-CSF from tumour cells (Akatsuka et al. 1991). Akatsuka et al. (1991) mentioned that only small number of cells (approximately 1%) were positive for the anti-G-CSF antibody even among a G-CSF secreting tumour cell line. Fahey (1951) pointed out the possibility of tumour cells producing substances stimulating bone marrow, and Ousawa et al. (1976) first reported a case of lung cancer producing CSF. Thereafter, probably reflecting the evidence that many non-neoplastic tissues have an ability to produce CSFs, malignant neoplasms arising in various sites were reported to produce CSFs. As for the production of CSF from urinary bladder carcinoma, Svet-Moldavskaya et al. (1980) reported two cases. Human G-CSF cDNA was cloned from cell line (‰ 5637) established from the one of the urinary bladder carcinoma (Souza et al. 1986).

Because granulocytosis in patients with non-haematological malignancies is caused by complex mechanisms including direct stimulation of haematopoietic stem cells or progenitor cells of CSFs produced by the tumour and/or indirect stimulation by another CSF-stimulating cytokine produced by the tumour cells. The detection of cytokines in the tumours is essential in understanding the pathogenesis of granulocytosis with malignancies. Ishizaka et al. (1986) reported that human urinary CSF has the ability to promote G-CSF production from monocytes or macrophages. In the present case, however, this possibility is unlikely because G-CSF was identified in tumour cells but not in macrophages or monocytes infiltrating the carcinoma. Cell lines established from a urinary bladder carcinoma were reported to produce a pluripotent haematopoietic CSF (interleukin-3; IL-3) and granulocyte-monocyte CSF in addition to G-CSF (Welte et al. 1985).

However, contribution of other CSFs to granulocytosis is also unlikely in this case because the examination of peripheral blood and bone marrow smears revealed granulocytosis with maturation and lack of proliferation in other haematopoietic series. CSF production in malignancies is usually detected by in vitro colony assays or molecular biological assay using tumour cell lines or tumours transplanted in nude mice. Thus in the past, malignancies suspected of producing CSF usually were not confirmed, due to a shortage of tumour cell lines or tumour tissue available for bioassay or molecular biological assay. In this case, we easily detected G-CSF in tumour cells by immunostaining on paraffin embedded sections, and in the patient's serum by using the sandwich immunoassay. In particular the application of this immunohistochemical method to cases of malignancy associated with granulocytosis may increase identification of G-CSF in the tumours as a causative factor of granulocytosis.

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