Case Report

A case of neutrophilia related to a cytokine-producing relapsed squamous cell carcinoma of the uterine cervix arising from the rectovaginal septum

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Paraneoplastic neutrophilia caused by a squamous cell carcinoma of the uterine cervix has been seen rarely. We report a case of relapsed squamous cell carcinoma of the uterine cervix with severe neutrophilia, rapid tumor growth and aggressive clinical course, possibly due to autocrine stimulation of cell growth by G-CSF and IL-6 without other possible causes of neutrophilia.

Key Words: Squamous cell carcinoma, Uterine cervix, Paraneoplastic syndrome, G-CSF, IL-6

INTRODUCTION

Paraneoplastic syndrome is a heterogeneous collection of disease manifestations that are caused by non-metastatic systemic effects that accompany malignant tumors.¹ Paraneoplastic hematological syndromes have hematological abnormal features which are leukocytosis, erythrocytosis, thrombocytosis, and pancytopenia due to cytokines or immunologic mechanisms.² Paraneoplastic leukemoid reaction is caused by nonhematopoietic tumor-producing hematopoietic growth factors, including granulocyte colony-stimulating factor (G-CSF), without bone marrow involvement.³ It has been reported that various nonhematopoietic tumors produces G-CSF or hematopoietic cytokines. However, squamous cell carcinoma of the uterine cervix producing these cytokines are very rare.^{4,5} Herein, we report a relapsed squamous cell carcinoma of the uterine cervix with severe neutrophilia, rapid tumor growth and aggressive clinical course, possibly due to autocrine stimulation of cell growth by G-CSF and IL-6.

CASE REPORT

A 68-year-old-woman, gravida 7, para 2, diabetic patient was admitted to the Department of obstetrics and gynecology at Chosun University Hospital for routine follow-up on October 10, 2007. She had been previously diagnosed with squamous cell carcinoma of the uterine cervix, FIGO stage IA1 on September 11, 2004. But, an MRI study of the abdomen and pelvis showed a cervical mass that was suspicious for parametrial involvement. Additionally, SCC Ag serum level on admission was 3.9 ng/ml. Therefore, she was treated with three cycles of neoadjuvant chemotherapy and radical hysterectomy. One year later, she was diagnosed with recurrent squamous cell carcinoma in situ of the posterior vagina wall on September 14, 2005 and was treated by photodynamic therapy. The Pap smear returned to normal on follow-up.

Despite demonstrating no sign of recurrent disease during the two years after photodynamic therapy, squamous cell carcinoma recurred on the rectovaginal septum presenting as a palpable cystic pelvic mass confirmed by aspiration cytology on this admission date, October 10, 2007 (#0 day after second recurrence) (Fig. 1). She complained of slight bowel discomfort and constipation without abdominal pains, and she was provided with laxatives. A chest X-ray revealed no abnormality. Her complete blood cell count (CBC) was in the normal reference range except for mild anemia. A trans-rectal ultrasonography showed a 8.2×6.3 cm cystic mass in the rectovaginal septum (Fig. 1). Due to uncontrolled diabetic hyperglycemia, the confirmative surgical biopsy and chemotherapy were delayed. During control time of serum glucose, simple drainage and dressing was performed.

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Fig. 1. Serial monitoring data of WBC count, SCC, C-reactive protein (CRP), several cervicographic images (A, B, and E), transrectal ultrasound finding (C) and cytologic finding (D).

After one month from the diagnosis of the second recurrence, the patient presented with leukocytosis (more than $12,000/\mu\text{l}$) and increased levels of C-reactive protein (more than 5 mg/dl) and SCC Ag (more than 2 ng/ml), concomitant with intermittent mild fever (less than 38.5°C) (Fig. 1). A blood sample showed the following findings on November 23, 2007 (#44 days after second recurrence): WBC $17.4 \times 10^3/\mu\text{l}$ (neutrophil 88.0%, lymphocyte 6.5%, monocyte 3.1%, eosinophil 1.2%); RBC $2.70 \times 10^6/\mu\text{l}$; Hb 8.5 g/dl ; Hct 26.2%; platelets $330 \times 10^3/\mu\text{l}$; total protein 7.55 g/dl ; albumin 4.30 g/dl ; AST 18.4 IU/L ; ALT 23.6 IU/L ; alkaline phosphatase 62 IU/L ; BUN 13.0 mg/dl ; serum creatinine 1.01 mg/dl ; C-reactive protein (CRP) 10.40 mg/dl . There was no evidence of syphilis, HIV and hepatitis viral infection on the serologic studies. In order to exclude bacterial infectious diseases, various specimens such as aspirates of the rectovaginal mass, vaginal discharge, urine, sputum, venous blood and bone marrow aspirate were stained and cultured during this admission period. However, there were no positive signs of bacterial infection. Leukocytosis was resistant to empirical therapy of various antibiotics with vancomycin. Physical examination and blood analyses showed no evidence of collagen diseases such as arthritis, mucosal ulcer and high values for rheumatoid factor. The peripheral blood smear was remarkable for

prominent neutrophilia without a left shift. For evaluation of neutrophilia, bone marrow examination, cytogenetic study, molecular *JAK2 V617F* mutation study and *BCR/ABL* translocation reverse transcriptase chain reaction (RT-PCR) were performed on December 27, 2007 (#78 days after second recurrence). The findings of bone marrow aspiration and section revealed normal levels of myeloblast (0.5% of 500 nucleated cells), general hypocellular bone marrow with multi-focal hypercellular bone marrow (5-50%) for her age, extremely active granulopoiesis with high M:E ratio (3.6:1), no dysplastic features and no metastatic features (Fig. 2). Cytogenetic finding of bone marrow aspirate showed 46, XX. No *JAK2 V617F* mutation and *BCR/ABL* translocation of bone marrow aspirate was detected by Seeplex *JAK2 ACE* Genotyping kit (Seegene, Seoul, Korea) and Seeplex Leukemia *BCR/ABL* kit (Seegene), respectively. Measurement of serum levels of G-CSF and Interleukin (IL)-6 were entrusted to a commercial laboratory on December 27, 2007 (#78 days after second recurrence). The commercial laboratory measure levels of G-CSF and IL-6 using enzyme-linked immunosorbent assay (Human Quantikine kits, R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. We received the results from commercial laboratory on January 10, 2008 (#92 days after second recurrence).

Fig. 2. Peripheral blood smear (A) and bone marrow aspiration smear (B) showed neutrophilia and reactive marrow, respectively (Wright-Giemsa stain, $\times 200$).

Fig. 3. Microscopic finding of 2nd relapsed squamous cell carcinoma arising from rectovaginal septum (H-E stain, $\times 200$ (A) and $\times 400$ (B)).

Serum levels of G-CSF and IL-6 in this patient were 2,450 pg/ml (normal 18.1 pg/ml) and 141 pg/ml (normal 8.0 pg/ml), respectively. The excisional biopsy was performed on December 28, 2007 (#79 days after second recurrence) (Fig. 3).

For detection of the expression of G-CSF, G-CSF receptor, IL-6 and IL-6 receptor mRNA, RT-PCR were performed of the fresh tumor biopsy samples on December 28, 2007. Briefly, total RNA extracted from the fresh surgical tissue specimens using EasyRed (Intron, Seoul, Korea) according to the manufacturer's protocol. Synthesis of cDNA was performed by QuantiTect Reverse Transcription Kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol. PCR was performed by Anydirect RedMax premix (BioQuest, Seoul, Korea) using 2 μ l aliquots of the reverse-transcribed cDNA. Each PCR cycle consisted of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 60 sec.

The target genes, the oligonucleotide primers used, and the sizes of the amplicons are summarized in Table 1. RT-PCR analyses revealed specific bands of G-CSF and IL-6 as well as their receptor mRNAs in tumor samples (Fig. 4).

Due to unknown origin of neutrophilia, she was not treated with chemotherapy. She was given hydroxyurea (500 mg/day) on two different days on December 30, 2007 (#81 days after second recurrence) and January 9, 2008 (#91 days after second recurrence) because the WBC count was very high ($63.0 \times 10^3/\mu$ l) on December 29, 2007 (#80 days after second recurrence), and did not return to normal range on January 8, 2008 (#90 days after second recurrence), respectively. After therapy with hydroxyurea, the serial WBC count showed rapid decline. However, severe pancytopenia (WBC $0.2 \times 10^3/\mu$ l, Hb 9.2 g/dl, platelets $14 \times 10^3/\mu$ l) emerged on January 9, 2008 (#91 days after second re-

Table 1. The summary of the targets, the oligonucleotide primers used, annealing temperatures (Ta) and the sizes of the amplicons

Target genes	Forward primer	Reverse primer	Product size (bp)	References
<i>CSF3</i>	caagtgccttagagcaagtggag	aggagaatgaaactcagaaatg	620	In this study
<i>CSF3R</i>	atccaaggttatgtggtttct	agaagatgggttagtgggtaag	595	In this study
<i>IL6</i>	tagccgccccacacagacag	ggctggcatttggttggg	408	Kyo, et al ⁴
<i>IL6R</i>	cattgccattgttctgaggttc	agtagtctgtattgctgatgc	251	Kyo, et al ⁴
<i>GAPDH</i>	tggaaatcccatcaccatct	gtcttctgggtggcagtgat	351	Cools, et al

Fig. 4. Results of RT-PCR assay revealed expression of mRNA of G-CSF (L1, 620 bp), G-CSF receptor (L2, 595 bp), IL-6 (L3, 408 bp), IL-6 receptor (L4, 251 bp) and GAPDH (L5, 351 bp). M, Φ X 174-*Hae* III digest size marker (TaKaRa Bio, Shiga, Japan).

currence). She was injected with recombinant human granulocyte colony-stimulating factor (Neutrogin, Choongwae Pharm. Co., Seoul, Korea) for three times on January 11 to 13, 2008 (#93 to #95 days after second recurrence) followed by return to $10.3 \times 10^3 / \mu\text{l}$ of WBC count on January 13, 2008 (#95 days after second recurrence). The size of the tumor mass and the level of CRP were aggressively increased irrespective of the neutrophil count after hydroxyurea ingestions. Finally, the tumor mass protruded through the vaginal orifice and serum level of CRP reached 21.5 mg/dl (Fig. 1). She took a sudden turn for the worse and died of multiple organ failure on January 17, 2008 (#99 days after second recurrence).

DISCUSSION

There are many etiologic factors for neutrophilia. The possible causes of the extreme leukocytosis of the patient are infectious diseases, drug, enteric necrosis, myeloproliferative disorders and paraneoplastic syndrome.⁶ Despite having a mild fever, there was no evidence of infection because staining and culture of various specimens were negative, and leukocytosis was resistant to the empirical antibiotic therapy in the patient. Drug-induced neutrophilia can be excluded because she was treated with only laxatives. Enteric necrosis can be excluded because of the absence of typical symptoms such as abdominal pain and tenderness. There was no evidence of a mye-

loproliferative disorder because of the absence of splenomegaly, acute developed neutrophilia, wild *JAK2* genotype and no *BCR/ABL* translocation. These exclusive findings suggested that neutrophilia observed in this patient was caused by a paraneoplastic syndrome. Moreover, the squamous cell carcinoma of the patient secreted G-CSF and IL-6. G-CSF stimulates the proliferation of myeloid lineage, especially neutrophilic series and consequently resulted in induced neutrophilia in this patient.⁷ IL-6 is the chief stimulator of the production of most acute phase proteins including CRP. IL-6 stimulates synthesis of CRP from the liver.⁸ The function of CRP is thought to be related to its role in the innate immune system. In this patient, the size of the tumor mass synchronized with serum levels of CRP and WBC count before hydroxyurea therapy. Hydroxyurea is an anticancer drug that blocks the synthesis of DNA by inhibiting ribonucleotide reductase, thus arresting cells in the S-phase. Therefore, hydroxyurea blocks proliferation of hematopoietic progenitor cells and induces suppression of bone marrow that is a highly proliferative tissue. Hydroxyurea is not indicated for leukemoid reaction because paraneoplastic neutrophilia does not cause hyperviscosity from leukostasis.⁹ Hydroxyurea may be a radiosensitizer of radiotherapy for squamous cell carcinoma of the uterine cervix. But, hydroxyurea alone is not used as a therapeutic drug for squamous cell carcinoma of the uterine cervix.¹⁰ This patient was treated with hydroxyurea to reduce the neutrophil count because the cause of neutrophilia was obscure and her general condition progressively worsened. However, hydroxyurea induced severe neutropenia and did not improve her general condition. The above evidence suggested that neutrophilia of the patient was due to a paraneoplastic neutrophilia. The rapid tumor progression and poor clinical outcome is frequently observed for cytokine-producing tumors, as in this patient.⁴ Ahn et al.⁵ reported a case of uterine cervical cancer presenting with granulocytosis but did not determine serum levels of G-CSF and IL-6 and did not detect mRNA of G-CSF and IL-6. We report a patient with an aggressive clinical presentation of relapsed squamous cell carcinoma of the uterine cervix arising from the rectovaginal septum as a cystic pelvic mass, possibly due to autocrine stimulation of cell growth by G-CSF and IL-6.

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