Granulocyte-colony-stimulating factor-producing metaplastic carcinoma of the breast with significant elevation of serum interleukin-17 and vascular endothelial growth factor levels

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Abstract

Granulocyte-colony-stimulating factor (G-CSF) production in carcinomas is associated with a very aggressive phenotype. Interleukin (IL)-17 secreted from tumor-infiltrating lymphocytes induces the production of G-CSF and vascular endothelial growth factor (VEGF) in cancer tissue. We present a case of a G-CSF-producing metaplastic breast carcinoma (MpBC) accompanied by systemic elevation of IL-17 and VEGF levels. A 56-year-old woman presented with a rapidly growing tumor measuring > 10 cm in her left breast. Core needle biopsy confirmed the diagnosis as MpBC with triple-negative features. Diffuse fluorodeoxyglucose uptake in the long bones and marked leukocytosis suggested that the G-CSF was produced by the primary tumor, which showed upregulated G-CSF mRNA and protein levels. Multiplex cytokine assessment identified increased serum IL-17, VEGF, and G-CSF levels. After radical mastectomy and skin grafting, the leukocyte count and serum G-CSF, IL-17, and VEGF levels were normalized. She underwent postmastectomy radiotherapy (50 Gy/25 Fr) and adjuvant chemotherapy (90 mg/m² of epirubicin and 600 mg/m² of cyclophosphamide followed by 80 mg/m² of paclitaxel) and is alive without recurrence. This is the first in vivo observation that describes the systemic elevation of IL-17 and VEGF levels with concomitant G-CSF production. Further research is warranted to study the IL-17/G-CSF/VEGF axis as a potential therapeutic target for this aggressive type of breast cancer.

Keywords Granulocyte-colony-stimulating factor · Metaplastic breast carcinoma · Interleukin-17 · Vascular endothelial growth factor

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Introduction

Several solid tumors secrete substances with colony-stimulating activity such as granulocyte-colony-stimulating factor (G-CSF) [1]. In particular, G-CSF-producing carcinomas show rapid progression and poor prognosis. However, its etiology, diagnosis, and adequate treatment remain unclear [2]. Several studies have presented cases of G-CSF-producing carcinomas originating from various organs such as the lung [3], bladder [4], stomach [5], and breast [6]. G-CSF produced by cancer tissue is released into the systemic circulation and induces marked neutrophilia. G-CSF-producing tumors can be diagnosed on the basis of the following findings:

1. high white blood cell (WBC) counts without signs of infection;

2. high serum G-CSF level;
3. normalization of WBC counts and serum G-CSF level after tumor excision;
4. immunohistochemical detection of G-CSF expression in tumor cells [2].

Although G-CSF production occurs autonomously in cancer cells [7–9], recent research indicates that G-CSF and vascular endothelial growth factor (VEGF) production may be induced by Interleukin (IL)-17 secreted from tumor-infiltrating lymphocytes [10].

We present a case of a rapidly growing G-CSF-producing metaplastic breast carcinoma (MpBC) accompanied by increased serum VEGF and IL-17 levels.

Case report

A 56-year-old woman was referred to our hospital with a rapidly growing breast mass. She noticed a small nodule in her left breast a half year prior to presentation that rapidly grew over the past 2 months. On physical examination, the tumor measured > 10 cm in diameter and bled easily owing to ulceration (Fig. 1a). No axillary lymph node swelling was detected. Contrast-enhanced magnetic resonance imaging revealed a $101 \times 62 \times 85$ mm mass without apparent infiltration to the cortex of the adjacent ribs (Fig. 1b). Several cystic components observed inside the mass on T2-weighted imaging indicated MpBC (Fig. 1c). Fluorodeoxyglucose (FDG) positron emission tomography–computed tomography

Fig. 1 Clinical and imaging examination findings. **a** A 56-year-old woman had a large tumor with skin ulceration in her left breast. **b** Axial fat-suppressed T2-weighted magnetic resonance image showed left breast mass with multiple internal cystic components. **c** Axial contrast-enhanced magnetic resonance image showed left breast mass with rim enhanced. **d** Fluorodeoxyglucose (FDG) positron emission tomography–computed tomography image showed multiple FDG uptakes in the left breast tumor, brain, urinary system, bones and so on. Unlike bone metastases, uptakes in the bones were diffusely distributed within the spine, pelvic bone, and long bones

(PET–CT) revealed no distant metastases, although mild to moderate and homogenous FDG uptake was detected in the spine, pelvic bone, and long bones, suggesting bone marrow hyperactivity (Fig. 1d). Laboratory tests showed a high WBC count (15,560 cells/ μ L with 80.5% neutrophils) without signs of an infection. Core needle biopsy confirmed the diagnosis as MpBC cT4bN0M0 cStage IIIB negative for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Since the tumor was considered resectable despite its rapid local progression, left mastectomy with greater pectoral muscle resection and axially lymph node dissection (level I, II, and III) were performed. The skin and muscle defects were repaired by artificial skin grafting. Histopathological findings showed MpBC with cystic changes, predominantly composed of spindle cells and partially of squamous cells (Fig. 2a–c). The tumor was completely resected with a negative margin; lymph node metastasis was not identified (0 out of 16). Subsequently, the WBC count was normalized (4480 cells/ μ L with 55.4% neutrophils).

Since the trend in WBC count and diffuse uptake of FDG in the bone marrow suggested a G-CSF-producing primary tumor, we conducted a few additional tests. The serum G-CSF level was high at 208 pg/mL (reference < 28 pg/mL) and decreased to 22.8 pg/mL after the surgery. The serum G-CSF concentration before the surgery was also higher than that in consecutive patients diagnosed with stage I to III invasive ductal carcinoma from December 2008 to April 2011 (Fig. 3a, the assay details were provided in the figure legends). G-CSF overexpression in the resected tumor tissue was confirmed by both immunohistochemistry and quantitative reverse transcription–polymerase chain reaction (qRT-PCR) (Fig. 3b, c, the assay details were provided in the figure legends). In immunohistochemical staining, G-CSF expression was mainly localized within spindle-like cancer cells (Fig. 3b). The qRT-PCR showed that mRNA expression of G-CSF was significantly higher in the cancer tissue compared to the adjacent normal breast tissue (Fig. 3c). These findings confirmed the presence of a G-CSF-producing primary tumor.

To further investigate the pathology of the rapid progression, we measured a panel of serum cytokines (Table 1). We used the serum collected from this patient and the above-mentioned consecutive patients harboring invasive ductal carcinoma before starting their treatment. A significant increase in serum IL-17 and VEGF concentrations was identified (440.2 and 437.7 pg/mL, respectively; Fig. 3d, e), which were normalized after surgery (189.9 and 115.2 pg/mL, respectively). This strongly suggested that the production of IL-17 and VEGF associated with the primary tumor. Immunohistochemistry confirmed IL-17 expression in the tumor-infiltrating inflammatory cells (Fig. 3f). VEGF-A expression was not observed in the tested tumor section

Fig. 2 Histopathological findings of the resected tumor. **a** The low magnification image ($\times 1$) shows a clear margin and cyst formation within the mass. **b** Photomicrograph shows that the tumor was mainly composed of spindle-like cells ($\times 400$; scale bar 300 μ m). **c** Photomicrograph shows squamous cells in a limited part of the tumor ($\times 200$; scale bar 300 μ m)

(Fig. 3g). These findings indicated the tumor-infiltrating inflammatory cells as an origin of IL-17 although the origin of VEGF was undetermined.

Fig. 3 Cytokine assessment of the resected tissue and patients' blood. **a** Serum G-CSF levels were measured using the Bio-Plex (Bio-Rad, Hercules, CA) multiplex assay system according to the manufacturer's instructions. The black dot represents the data for the present patient; the white dots indicate 72 patients diagnosed with stage I to III invasive ductal carcinoma. **b** G-CSF expression in cancer cells: hematoxylin eosin staining of a tumor section (left); immunohistochemical staining using an anti-G-CSF monoclonal antibody (rabbit polyclonal [catalog number #9691]) diluted at 1:500 (Abcam, Cambridge, MA) (right): a positive signal was observed in the cytoplasm of several cancer cells ($\times 100$; scale bar 1000 μm). **c** Quantitative reverse transcription–polymerase chain reaction findings for G-CSF mRNA: the total RNA was extracted from the (1) primary tumor and (2) adjacent normal tissue using formalin-fixed surgical specimens (left). mRNA expression of G-CSF (right). G-CSF expression was significantly higher in the cancer tissue. G-CSF was measured using the TaqMan gene expression assay (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. 18 s ribosomal RNA was used as the reference gene. *ND* not detected. Error bar; standard deviation. **d** Serum IL-17 levels measured using the Bio-Plex multiplex assay system. **e** Serum VEGF levels measured using the Bio-Plex multiplex assay system. **f** Immunohistochemical staining using an anti-IL-17 antibody (rabbit polyclonal [catalog number #H-132]) diluted at 1:500 (Santa Cruz, Dallas, TX): a positive signal was observed in the cytoplasm of several inflammatory cells ($\times 400$; scale bar 300 μm). **g** immunohistochemical staining using an anti-VEGF-A antibody (rabbit polyclonal [catalog number #ab9570]) diluted at 1:200 (Abcam, Cambridge, MA): a positive signal was not observed in the section ($\times 400$; scale bar 300 μm)

Subsequently, irradiation was administered to the chest wall and regional lymph nodes (50 Gy/25 Fr) as adjuvant radiotherapy, followed by 4 cycles of triweekly EC (90 mg/m² of epirubicin and 600 mg/m² of cyclophosphamide) plus 12 cycles of weekly paclitaxel (80 mg/m²). The patient is currently alive 3.5 years after the operation.

Discussion

This case presents two major findings. First, the overexpression of both G-CSF mRNA and protein was confirmed in the primary tumor. Thus, G-CSF was initially produced in the primary tumor and released into the circulation, thereby stimulating the bone marrow and inducing neutrophilia. A G-CSF-producing tumor was first identified upon the transplantation of human lung cancer tissue into nude mice, wherein marked neutrophilia was induced through the secretion of G-CSF from the transplanted tumor [2]. Several clinical cases of G-CSF-producing tumors have subsequently been reported [3–5]. The diagnostic criteria mentioned earlier were applied in most of these cases, including that involving breast cancer [6]. However, these criteria could not rule out the possibility that G-CSF was produced systemically and not in the primary tumor tissue. In the present case, the detection of both the G-CSF mRNA and protein in the primary tumor indicates that the tumor tissue was the source of the circulating G-CSF.

Second, we identified a significant increase in serum IL-17 and VEGF levels. These cytokines had reported to promote tumor growth by enhancing angiogenesis [10]. Several studies have shown that increased levels of these cytokines are associated with chemotherapy and radiotherapy resistance [11–16]. One of the common explanations for aggressive G-CSF-producing carcinomas is that the secreted G-CSF can enhance cancer cell growth, invasion, and metastasis by stimulating cancer cells through an autocrine mechanism [7–9]. Therefore, the systemic administration of G-CSF is likely to have a negative effect on prognosis. In clinical practice, the systemic administration of G-CSF is widely used to support myelosuppression and maintain treatment intensity during chemotherapy. In such cases, the serum G-CSF level can increase transiently after its administration and the level could be higher than or as high as those in patients with G-CSF-producing carcinomas [3–6, 17]. However, there is no evidence of its negative effect on prognosis probably because of the rapid clearance of the administered G-CSF and the benefit of increasing dose intensity during chemotherapy [17, 18]. Our findings suggested that the sustained increase in G-CSF induced by IL-17 could explain the aggressive behavior of G-CSF-producing carcinomas. Although G-CSF and VEGF can be produced by tumor cells, IL-17 is generally secreted by immune cells. Recent studies claim that IL-17 secreted from tumor-infiltrating T helper type 17 (Th17) cells induces the production of G-CSF and VEGF from cancer-associated fibroblasts [10]. In the present case, immunohistochemistry revealed that IL-17 was expressed in the inflammatory cells, not in the tumor cells. Although we did not specify the subset of these inflammatory cells expressing IL-17, it was likely that tumor-infiltrating Th17 cells induced G-CSF production of tumor cells. G-CSF expression was mainly observed in the spindle-like tumor cells, which were supposed to undergo mesenchymal differentiation. In addition, a previous study of the G-CSF producing carcinomas also reported G-CSF production in spindle-like cells in MpBC [6]. On the other hand, the expression of VEGF-A was negative in the tumor cells regardless of the systematic elevation of VEGF. These findings raise the following hypotheses regarding the cause of the systemic elevation of VEGF. First, increased neutrophils in the circulation caused by G-CSF production were likely to secrete VEGF, since neutrophils and macrophages are known to secrete VEGF upon their activation [19–22]. Second, VEGF production could occur not in response to G-CSF production but in response to tumor hypoxia. Although VEGF-A expression was not detected in the tested tissue secretion, it is possible that there was more hypoxic lesion expressing VEGF-A within the rapidly growing bulky tumor.

The majority of MpBCs are triple-negative breast cancer (TNBC) and usually confer a worse prognosis than that

Table 1 Lists of tested serum cytokines before surgery

IL-1 β	IL-1ra	IL-2	IL-4	IL-5	IL-6	IL-7
IL-8	IL-9	IL-10	IL-12	IL-13	IL-15	IL-17
Eotaxin	FGF	G-CSF	GM-CSF	IFN- γ	IP-10	MCP-1
CCL2	CCL3	CCL4	CCL5	PDGF-BB	TNF- α	VEGF

observed in non-metaplastic TNBC [23]. Unlike for non-metaplastic TNBC, an effective chemotherapy and radiotherapy regimen for MpBC has not been established [23, 24]. A small case series has reported that a bevacizumab-containing regimen showed favorable efficacy against MpBC [25, 26]. Figure 3a, d shows that higher IL-17 levels did not always related to G-CSF production. This implicated that the aberrant production of G-CSF is limited to the specific subset of breast cancer, such as MpBC or breast cancer expressing IL-17 receptors (IL-17R). Several studies have reported that IL-17R expression was associated with poorer prognosis in breast cancer patients [27–30]. If elevated G-CSF, IL-17, and VEGF levels can be confirmed as a common features of aggressive MpBCs, targeting these cytokines, especially anti-angiogenic therapy, could be a promising option for MpBCs.

In conclusion, we described a case of a G-CSF-producing MpBC accompanied by increased serum IL-17 and VEGF level. Our findings suggest that the IL-17/G-CSF/VEGF axis could be a potential therapeutic target in rapidly growing MpBCs; however, further investigation is necessary.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants The tests presented in this report were performed under the approval of the Ethics Committee of Kyoto University Hospital (Protocol number; G424).

Informed consent Written informed consent for the usage of patient-derived resources was obtained from the patients prior to the research and the publication.

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