CASE REPORT

Granulocyte Colony-stimulating Factor- and Interleukin-6-producing Large-cell Carcinoma of the Lung with Sarcomatoid Changes Suggestive of Epithelial-mesenchymal Transition: An Autopsy Case Report

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Abstract:
A rare case of lung cancer with the simultaneous production of granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6) is reported. A 79-year-old man was admitted to our hospital due to cachectic symptoms and an increased inflammatory response. Laboratory tests and imaging studies suggested metastatic lung cancer with high serum levels of G-CSF and IL-6. He died of progressive disease, and an autopsy showed that the lung tumor had positive protein expression of both cytokines and a solid growth of large-cell carcinoma with sarcomatoid changes, possibly resulting from the epithelial-mesenchymal transition mediated by IL-6 and leading to widespread metastases.

Key words: granulocyte colony-stimulating factor (G-CSF)-producing, interleukin-6 (IL-6)-producing, large-cell carcinoma (LCC) of the lung, sarcomatoid component, epithelial-mesenchymal transition (EMT)

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Introduction
Lung cancer is a neoplasia that potentially produces various cytokines, most of them being granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6) (1-4). Since 1977, when Asano et al. first described a lung cancer producing G-CSF that was responsible for marked neutrophilia (5), many cases of G-CSF-producing tumors have been published. A high serum level of IL-6 has been shown to cause inflammatory responses in a subset of patients with lung cancer, including those with tumors producing IL-6 (6, 7). However, there have been few case reports of lung cancers simultaneously producing both cytokines.

We herein report a rare case of a G-CSF and IL-6 double-producing large-cell carcinoma (LCC) of the lung. Intrigu-ingly, an autopsy showed that the tumor, in which the expression of both cytokines was confirmed immunohistochemically, consisted of a solid growth of LCC with sarcomatoid changes suggestive of epithelial-mesenchymal transition (EMT).

Case Report
A 79-year-old Japanese man visited our hospital with complaints of general fatigue, anorexia, and weight loss of 8 kg (11.4% of body mass) over a month in June 201X. On laboratory testing, blood counts showed marked leukocytosis (white blood cells: 33,210/μL, with 88.0% neutrophils), anemia (hemoglobin: 8.5 g/dL), and thrombocytosis (platelets: 66.0×10⁴/μL), and biochemistry and serology showed hypoalbuminemia (albumin: 3.1 g/dL), polyclonal hyper-γ-
globulinemia (31.1% γ-globulin fraction), and an inflammatory response [C-reactive protein (CRP): 6.95 mg/dL, fibrinogen: 562 mg/dL]. He was admitted for a further examination.

He had smoked 20 cigarettes a day for 60 years and had a history of pulmonary tuberculosis at 74 years of age. A physical examination showed mild anemia and a slight systolic heart murmur without rales. No superficial lymphadenopathy was present. His body mass index and body temperature were 23.2 kg/m$^2$ and 36.0 °C, respectively. On pulse oximetry, O$_2$ saturation was 97% on room air. Bacterial cultures of blood, urine, and sputa were all negative, and polymerase chain reaction of the sputa did not detect *Mycobacterium tuberculosis*, indicating non-infectious inflammation.

Regarding imaging studies, chest X-ray showed abnormal shadows in the hilar region and middle area of the left lung (Fig. 1a), and plain and contrast-enhanced computed tomography (CT) of the chest showed swollen lymph nodes of the left hilum, mediastinum (Fig. 1c), and bilateral supraclavicular fossae, in addition to a subpleural mass (2 cm in diameter) in the upper lobe of the left lung (Fig. 1b and c). $^{18}$F-fluorodeoxyglucose (FDG) positron emission tomography-CT showed the significant uptake of FDG within the lesions indicated by CT (Fig. 2). Abdominal CT showed mild hepatosplenomegaly with no lymphadenopathy and no ascites. Gastrointestinal endoscopy showed no abnormal findings. Repeated sputum cytology showed Class I findings, whereas the serum squamous cell carcinoma antigen level was increased to 6.3 ng/mL (<1.5 ng/mL). Subsequently, serum G-CSF and IL-6 concentrations were measured to search for the cause of the leukocytosis and sterile inflammation, and G-CSF and IL-6 levels were increased to 69.3 pg/mL (<39.0 pg/mL) and 204 pg/mL (<8 pg/mL), respectively; the latter showed a marked elevation of more than 20 times the cut-off value. An aspiration biopsy of the bone marrow showed hypercellularity, with no neoplastic cells and no Philadelphia chromosome, so chronic myeloproliferative disorders were excluded. Despite the absence of specific autoimmune diseases, antinuclear antibody (×160) was detected in the patient’s serum.

Based on these results, a primary lung cancer of clinical stage T1N3M0 (stage IIIIB), possibly producing both G-CSF and IL-6, was strongly suspected. It could be assumed that the leukocytosis and inflammatory response were paraneoplastic syndromes derived from the hypercytokinemia. The present case resembled multicentric Castleman’s disease (MCD), a rare lymphoproliferative disorder characterized by systemic lymphadenopathy and excessive IL-6 production with inflammatory responses (8), but it was ruled out because the patient did not present with systemic lymphadenopathy. The patient refused further investigation for a definitive diagnosis and possible treatment and decided to receive best supportive care. While the patient had suffered from an intermittent fever of 38 to 39 °C since immediately after admission, general fatigue, and anorexia, the oral administration of non-steroidal anti-inflammatory drugs and corticosteroid provided a degree of relief for his cachectic condition for several months. However, he died of progressive disease six months after admission. To make a definitive diagnosis, an autopsy was performed after obtaining written, informed consent from his family.

Macroscopically, a peripheral mass (measuring 30 mm) with pleural invasion in the upper lobe of the left lung, multiple nodal swellings in the left hilum and mediastinum, and a small nodule in the left adrenal gland were observed. A histopathological examination showed that two tumor components coexisted in the mass lesion at the upper lobe of the left lung: a solid component of poorly differentiated carcinoma consisting of atypical large cells and a sarcomatoid...
component (Fig. 3). The sarcomatoid component was composed of atypical spindle-shaped cells and was predominantly found in metastases that included swollen lymph nodes, the left adrenal gland, and bone marrow. In the transitional zone between the two tumor components, an area of discohesive tumor growth was also observed. A further immunohistochemical analysis was then performed. The tumor showed positive staining with anti-G-CSF and anti-IL-6 monoclonal antibodies in tumor cells of both components (Fig. 4a, b, d and e), indicating that the lung tumor had produced both cytokines simultaneously. The overexpression of p53 protein was detected in nuclei of cells of both tumor types (Fig. 4c and f). Adenocarcinoma, squamous cell carcinoma, and mesothelioma were excluded because both tumor components were negative for thyroid transcription factor (TTF)-1, p63, and calretinin, respectively. Thus, the final diagnosis was metastatic LCC of the lung with a sarcomatoid component, which simultaneously produced G-CSF and IL-6.

The histomorphological transition in the primary lesion was reminiscent of the EMT phenomenon, which is defined as a conversion of epithelial cells into mesenchymal cells, where cell polarity and cell-cell adhesion of tumor epithelium are lost in cancer, and the tumor cells acquire invasive and metastatic properties (9). Therefore, the immunohistochemical expression of keratin and E-cadherin as representative markers of the epithelial phenotype and the expression of vimentin as a representative marker of the mesenchymal phenotype were evaluated in both tumor components. In particular, the loss of E-cadherin expression has been reported to be a central event in the EMT process. These investigations showed that keratin expression was strongly present in the LCC but decreased in the sarcomatoid component (Fig. 5b and f), and E-cadherin expression was mostly retained in the LCC but was entirely lost in the sarcomatoid component (Fig. 5c and g). The vimentin expression was weakly detected in LCC tumor cells and markedly positive in sarcomatoid tumor cells (Fig. 5d and h). These findings were consistent with the features of phenotypic alteration of tumor cells observed during EMT, suggesting that the histological change of LCC to a sarcomatoid component may have resulted from EMT.

**Discussion**

The diagnosis in the present case was primary lung can-
Figure 3. Histopathological findings of the lung tumor in the left upper lobe are shown. The growth of two tumor components—(a and b) a solid component of large-cell carcinoma (LCC) and (c and f) a sarcomatoid component—is seen in the lung tumor. The sarcomatoid component is composed of spindle-shaped cells with a hyperchromatic nucleus embedded in haphazard fascicles. In the transitional zone between the two tumor components, (c and d) LCC loses its solid structure and is separated into small groups of tumor cells (lower left), which transition to an area of tumor growth made of discohesive cells (upper right). 100× magnification in a and c, 200× magnification in b, d, and e, and 400× magnification in f.

Figure 4. An immunohistochemical analysis of the tumor tissues shows positive staining for both G-CSF and IL-6 in not only (a and b) LCC cells but also (d and e) sarcomatoid tumor cells. (c and f) The overexpression of p53 protein is shown in the nuclei of tumor cells of both components. 200× magnification in a, b, d and e and 100× magnification in c and f.
cer simultaneously producing G-CSF and IL-6 based on the pathological and immunohistochemical findings obtained by a postmortem analysis, in addition to high serum levels of both cytokines with unexplained leukocytosis and inflammation. Although there have been many case reports of neoplasms producing each cytokine, those with a G-CSF and IL-6 double-producing tumor are very rare. To our knowledge, only seven cases, including four with lung cancer (excluding cell lines) (10-16), in which the expression of both cytokines was detected in tumor cells, have been reported so far in the English-language medical literature (summarized in Table). This is the first case of a G-CSF and IL-6 double-producing tumor showing a histological change suggestive of EMT, with supportive immunohistochemical findings.

In general, tumors producing G-CSF or IL-6 are aggressive and have a poor outcome. One possible reason for this is that both cytokines exert growth effects on tumor cells via autocrine and/or paracrine loops. G-CSF normally acts as a hematopoietic cytokine that stimulates the production of neutrophils, but it is also known as a growth factor that is produced by different types of solid tumors, including lung cancer. Inoue et al. were the first to report that G-CSF secreted by lung cancer cells can affect the proliferation of cancer cells expressing G-CSF receptor (17). As for IL-6, two independent studies using non-small-cell lung cancer (NSCLC) cell lines expressing IL-6/IL-6 receptor (IL-6R) have recently indicated the promoter role of IL-6 in the growth of cancer stem-like cells (18, 19). Hence, in lung cancers with simultaneous production of G-CSF and IL-6, the cytokines might cooperate in enhancing the proliferation of tumor cells in a different way, contributing to a more aggressive behavior of these tumors.

Of note, IL-6 is implicated in EMT, a key process during cancer progression, although the role of G-CSF in EMT re-
In lung cancer, several recent studies have demonstrated the positive regulation of EMT by IL-6 (19-21). The hallmarks of the molecular mechanisms underlying EMT include a functional loss of E-cadherin, a critical adhesion molecule of epithelial cells, and it has been shown that IL-6 abrogated E-cadherin expression through STAT3 activation in EMT induced by transforming growth factor-β in lung cancer cells, thereby promoting EMT (20).

In the present case, a histological change of LCC to a sarcomatoid component in the primary lesion and predominant growth of the sarcomatoid tumor cells in metastases were observed. Furthermore, immunohistochemistry demonstrated the loss of E-cadherin expression in the sarcomatoid component, along with the positive expression of vimentin, indicating that the sarcomatoid cells obviously had a mesenchymal phenotype. Taken together, these findings suggest that the sarcomatoid change of LCC in the present case may have resulted from EMT, likely mediated by IL-6 produced by the tumor cells themselves, and sarcomatoid tumor cells acquiring a metastatic ability may have spread to lymph nodes and distant organs.

The overexpression of p53 protein was shown in both LCC and sarcomatoid tumor cells in this case. A driver mutation in the TP53 gene, one of the most frequently mutated genes in lung cancer, has been reported to be almost always clonal and early (22). It is therefore reasonable to postulate that the two tumor components have a clonal relationship with the same TP53 mutation. In addition, the mutant p53 protein may be complicit in the constitutive IL-6 production of the tumor cells (23).

Clinically, the present patient had an inflammatory paraneoplastic syndrome with a markedly elevated serum IL-6 level, resembling MCD except for systemic lymphadenopathy. IL-6, a pro-inflammatory cytokine, has essentially pleiotropic effects, e.g. the production of the acute-phase proteins, hepcidin and thrombopoietin, a reduction in albumin synthesis in hepatocytes, the induction of activated B cells to antibody-producing cells, and the inhibition of regulatory T cell differentiation (24). These are in turn reflected by laboratory value abnormalities, i.e. an elevated CRP level, anemia, thrombocytosis, hypoalbuminemia, polyclonal hyper-gamma-globulinemia, and autoantibody-positivity, respectively, as was also seen in the present case. Together with these clinical data, MCD is known to result in a cachectic condition, with pyrexia, fatigue, and weight loss, due to IL-6 overproduction by proliferative lymphoid tissues (25). Thus, IL-6 is also recognized as a cachexia-inducing substance. The deregulated exposure of excessive IL-6 in the present patient was likely involved in his refractory cachexia and unfavorable prognosis.

In the treatment of cancer patients with IL-6-producing tumors, inhibiting IL-6 signaling is considered to not only reduce the tumor growth and EMT activity but to also ameliorate cancer cachexia (26). In addition, since IL-6 has been implicated in chemoresistance (27), IL-6 blockade therapy, e.g. anti-IL-6R antibody, combined with chemotherapy, can be expected to attenuate drug resistance (28), although such an approach has not yet been approved clinically.

In conclusion, we encountered a rare case of LCC of the lung with simultaneous production of G-CSF and IL-6, showing sarcomatoid changes suggestive of IL-6-mediated EMT, as well as paraneoplastic leukocytosis and inflammatory syndrome similar to MCD.

The authors state that they have no Conflict of Interest (COI).

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