

## Case Report

# Adenocarcinoma with Neuroendocrine Differentiation of the Colon Accompanying Osteoclast-Like Giant Cells

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**Introduction.** Neuroendocrine differentiation in colorectal cancer is reportedly associated with poorer grade of tumor differentiation, nodal and distant metastasis, and other unfavorable features, contributing to a worse clinical outcome. Colorectal cancer with osteoclast-like giant cells (OGCs) is extremely rare. **Case Presentation.** An 86-year-old woman was diagnosed as double cancer of the transverse and sigmoid colon. Both tumors were simultaneously removed. The transverse colon cancer directly invaded the area of the right gastroepiploic vessels and spread to the nodes and histologically consisted of both the tubuloglandular and solid components. CD8/granzyme B-positive tumor-infiltrating lymphocytes and CD163/CD68-positive macrophages, frequently forming OGCs, were observed particularly at the invasion front. The carcinoma cells were labeled focally for synaptophysin and diffusely for the DR locus of the human leukocyte antigen and programmed death-ligand 1 (PD-L1). Deficient expression of DNA mismatch repair (dMMR) proteins was immunohistochemically confirmed. The patient died 16 months after surgery. **Conclusion.** This is the first report of colonic adenocarcinoma with neuroendocrine differentiation accompanying OGCs. Histopathologic factors of the poor prognosis in the present case included (a) the presence of more than 2% cells with neuroendocrine differentiation, (b) infiltration of CD163/CD68-positive OGCs at the invasion front, (c) deficiency of dMMR proteins, and (d) PD-L1 expression.

## 1. Introduction

Colorectal carcinoma is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related death worldwide, accounting for about 700,000 deaths/year [1, 2]. About 30% of patients with colorectal carcinoma present with local lymph node spread but no distant metastasis at the time of diagnosis (Union Internationale Contre le Cancer, stage III). The five-year survival in stage III colorectal cancer ranges from 89% for stage IIIA to 36% for stage IIIC [3]. Neuroendocrine marker-positive cells are occasionally distributed in adenocarcinoma of the colon and rectum [4–6]. It has been clarified that neuroendocrine differentiation in colorectal cancer is associated with poorer grade of tumor differentiation, nodal and distant metastasis, and other unfavorable features, contributing to a worse clinical outcome [7].

Approximately 4% of metastatic colorectal cancers are associated with high microsatellite instability (MSI-H), indicating a deficient function of DNA mismatch repair (dMMR) proteins [8]. Expression of the immune checkpoint protein, programmed death-ligand 1 (PD-L1), on the tumor cells is well correlated with dysfunction of dMMR proteins [9, 10].

Osteoclast-like giant cells (OGCs) of histiocytic lineage are morphologically and immunophenotypically distinguished from multinucleated stromal giant cells or neoplastic multinucleated cells [11]. OGCs may represent an immune cellular population involved in the tumor growth. Some colorectal carcinomas may accompany tumor-infiltrating lymphocytes (TILs) in the stroma, representing tumor-specific immune responses [12]. TILs have been proposed as a crucial prognostic indicator. The density of TILs has a strong power for predicting patients' survival, as comparable

with the well-established tumor, node, and metastasis classification system [13].

The present report describes clinicopathological features of adenocarcinoma with neuroendocrine differentiation of the transverse colon, accompanying OGCs and TILs. We believe that the cooccurrence of neuroendocrine features and OGCs in colorectal cancer has not been reported so far.

## 2. Case Presentation

An 86-year-old Japanese woman complaining of lower abdominal pain was referred to Shimada Municipal Hospital, Shimada, Shizuoka, Japan. She had no particular familial or environmental history. Ultrasonography, abdominal computed tomography, and colonoscopy suggested double cancer of the transverse and sigmoid colon. Both tumors were simultaneously removed by right hemicolectomy and sigmoidectomy with lymphadenectomy. The sigmoid colon cancer was ulcer-forming, 4 × 3 cm sized, well-differentiated papillotubular adenocarcinoma of common type, reaching the subserosal layer. Lymphatic, venous, and perineural invasions were observed but without lymph node metastasis. The transverse colon cancer grossly showed a circumferential 7 × 6 cm mass with gray- to black-colored ulceration (Figure 1(a)). The cancer directly invaded the area of the right gastroepiploic artery and vein and spread to the regional lymph nodes. Histologically, the mildly pleomorphic tumor cells formed both tubuloglandular components and irregular-shaped medullary solid nests (Figure 1(b)). Lymphovascular invasion was positive.

TILs with phenotypes of killer T-lymphocytes were diffusely observed, particularly at the invasion front (Figure 1(c)). TILs were immunoreactive for CD3, CD8, granzyme B (Figure 1(c), inset), perforin, T-cell intracytoplasmic antigen-1 (TIA-1), and the DR locus of the human leukocyte antigen (HLA-DR)/class II major histocompatibility complex but negative for CD4, CD20, and CD56. Macrophages expressing CD68 and CD163 coexisted with TILs. The ratio of CD163/CD68 was 79.7%. Particularly at the invasion front, the macrophages frequently formed OGCs (Figure 1(d)). The OGCs were found in ten per one ×400 microscopic field. OGCs were immunoreactive for CD68/CD163 (Figure 1(d), inset) and HLA-DR (see Figure 1(h)). The percentages of stromal inflammatory cells positive for each phenotypic marker, CD8, TIA-1, HLA-DR, CD68, and CD163, were 60%, 20%, 50%, 30%, and 20%, respectively. OGCs were also observed in the lymph node metastasis.

The carcinoma cells with solid nest formation were focally labeled for synaptophysin (13% of the tumor cells) (Figure 1(e)). Chromogranin A and CD56 were negative. No synaptophysin-positive tumor cells were identified in the lymph node metastasis.

In order to confirm the neuroendocrine nature, the cancer cells with synaptophysin immunoreactivity were targeted at electron microscopic evaluation by digging a small piece from the formalin-fixed, paraffin-embedded block. With this approach, the fine morphologic preservation was fairly good even after paraffin embedding [14]. The tumor cells formed desmosomal junctions. Cellular process formation was not

evident. Neuroendocrine-type cored round electron-dense granules were dispersed among fine cytoskeletal filaments in the cytoplasm. The size of the granules ranged from 140 to 380 nm with the mean 220 nm (Figure 1(f)).

Immunohistochemical analysis for dMMR proteins, such as mutL homolog 1 (MLH1), postmeiotic segregation 1 homolog 2 (PMS2), mutS homolog 2 (MSH2), and mutS homolog 6 (MSH6), showed the complete loss of nuclear immunoreactivity of MLH1 (Figure 1(g)) and PMS2.

The cancer cells were strongly immunoreactive for HLA-DR (Figure 1(h)). PD-L1 decorated with a monoclonal antibody clone 22C3 was expressed on 70–80% of the tumor cells (Figure 1(i)). *In situ* hybridization analysis for Epstein-Barr virus-encoded small nuclear RNA was negative. Ki-67 (clone: MIB-1) labeling index was more than 80%.

The histopathological features of the transverse colon tumor were consistent with poorly differentiated adenocarcinoma with neuroendocrine differentiation accompanying OGCs and TILs, pT4bN2M0 (p-stage IIIC). Ten months after surgery, the tumor relapsed in the abdominal lymph nodes and disseminated onto the peritoneal cavity. At the 14th postoperative month, the patient vomited vast amounts of blood due to cancer invasion to the gastric prepylorus, and the patient expired 16 months after surgery. No autopsy was performed.

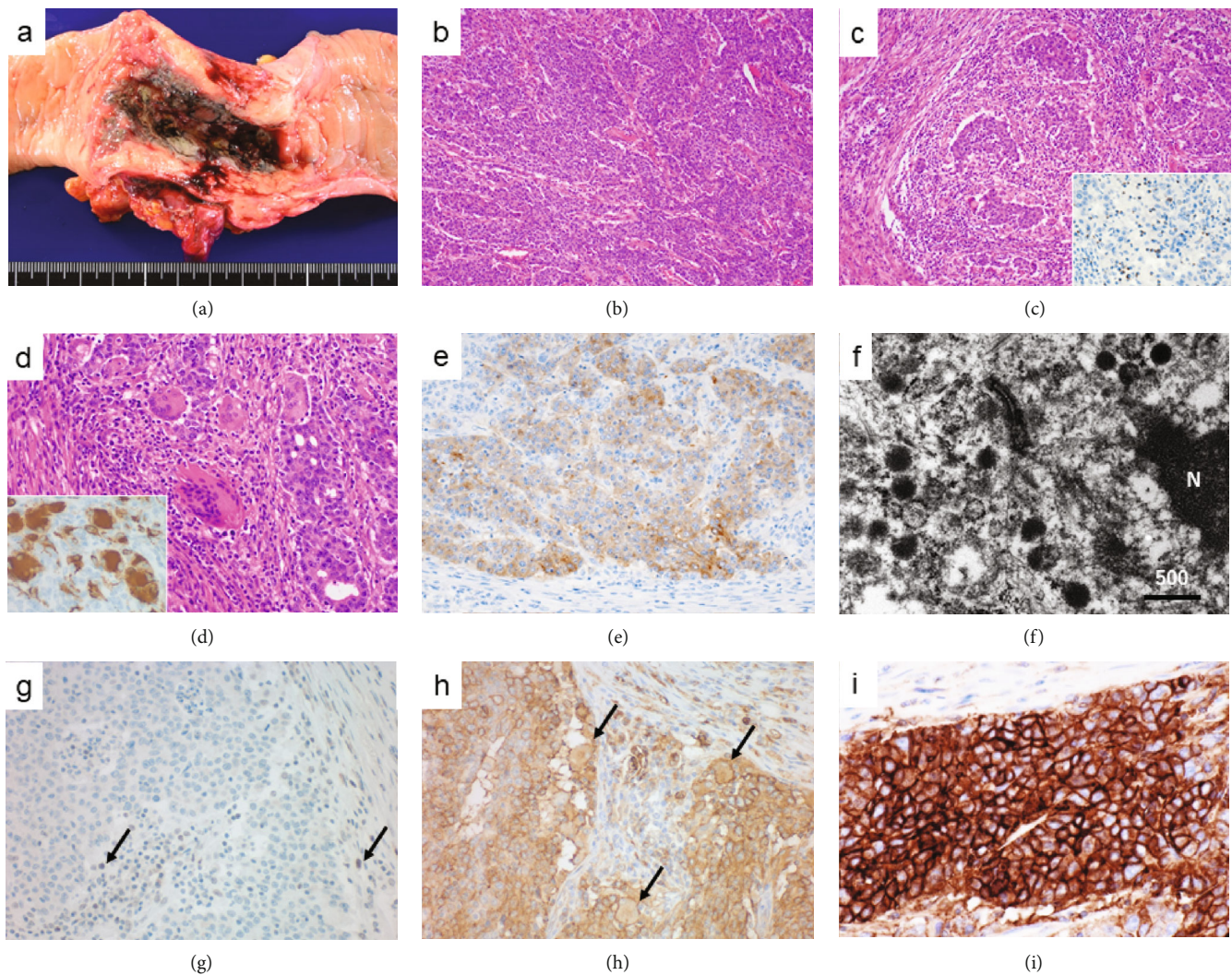
## 3. Discussion

The transverse colon cancer in the present case showed medullary poorly differentiated growth with deficient dMMR status. The deficient MMR status in colon cancer revealed by immunohistochemistry is commonly associated with right-sided localization, female sex, high histological grade, and dense inflammatory infiltration in the tumor stroma [8]. These features were reproduced in the present case. Furthermore, the tumor in the present case was quite unique for association of neuroendocrine differentiation and OGC reaction.

In sporadic colorectal cancer, neuroendocrine cells are detected in 8–77.5% of cases, largely depending on the method employed for assessing neuroendocrine cell population [7]. Neuroendocrine features are also noted in half of hereditary nonpolyposis colorectal cancers with defective dMMR proteins [15]. Using a multivariate Cox regression model, the presence of more than 2% of cells with neuroendocrine differentiation indicates a poor prognosis in stages III and IV colorectal cancer [16].

TILs at the invasive front of colorectal carcinoma play an important role in modulating invasion and progression of the cancer cells [12]. The extent of stromal TILs was negatively correlated with morphologic features of tumor progression, including venous, lymphatic, and perineural dissemination and nodal and distant metastases [17]. It is known that the expression of HLA-DR on the cancer cells is intimately related with cancer immunity via CD4-positive T-lymphocytes [18]. Colorectal cancer expressing HLA-DR is often associated with TILs and shows a better prognosis [19].

In contrast, an elevated ratio of CD163/CD68 in macrophages at the tumor invasion front was closely associated



**FIGURE 1:** Macroscopic, histologic, immunohistochemical, and electron microscopic images of colonic adenocarcinoma with neuroendocrine differentiation accompanying OGCs. (a) Grossly, the circumferential tumor of the transverse colon, measuring  $7 \times 6$  cm, forms a black- to gray-colored ulcer. (b) The tumor is microscopically composed of solid nests of mildly pleomorphic malignant cells (H&E). Small lymphocytes are dispersed in the tumor stroma. (c) At the invasion front of the tumor, TILs are richly distributed (H&E). The inset illustrates granzyme B expression in the TILs. (d) OGCs are noted particularly at the TIL-rich invasion front (H&E). CD68 immunoreactivity in the OGCs is illustrated in the inset. (e) Synaptophysin is focally immunoreactive in the neoplastic cells with solid nest formation. (f) Ultrastructurally, the tumor cells form desmosomal junctions, and neuroendocrine-type cored round electron-dense granules are identified, as shown in the inset (bar = 500 nm). (g) MLH-1, a representative dMMR protein, is not expressed in the nuclei of the cancer cells, while stromal/lymphoid cells are weakly positive (arrows). (h) The cancer cells, as well as OGCs (arrows), show strong membrane/cytoplasmic expression of HLA-DR. (i) PD-L1 is diffusely expressed on the tumor cells (monoclonal antibody 22C3).

with an aggressive phenotype and poor prognosis in colorectal cancer [20]. To the best of our knowledge, this is the second case of colorectal carcinoma with OGCs: the first case was described by Eshun-Wilson in 1973 [21]. Neuroendocrine tumors rarely accompany OGCs [22]. Correlation between the occurrence of OGCs and PD-L1 expression was studied in pancreatic cancer: in undifferentiated pancreatic carcinoma with OGCs, PD-L1 immunoreactivity was detected in the neoplastic cells in 63% cases, and PD-L1 expression indicated a poor prognosis [23]. MSI-H, caused by loss of function of MLH1 protein by CpG island hypermethylation in the promoter of *MLH1* gene [24], is seen in 10% to 13% of colorectal cancers. The MSI-H colorectal cancer is

poorly differentiated with a medullary growth pattern and is often associated with TILs [9]. Immune checkpoint inhibitors, such as nivolumab and pembrolizumab targeting programmed death 1 (PD-1) on the inflammatory cells, are effective against metastatic dMMR-deficient and MSI-H colorectal cancer [9, 10].

#### 4. Conclusion

As far as we know, this is the first report of colonic adenocarcinoma with neuroendocrine differentiation accompanying OGCs. The patient died 16 months after surgery. The histopathologic factors indicating the poor prognosis in the

present case included (a) the presence of more than 2% cells with neuroendocrine differentiation, (b) infiltration of CD163/CD68-positive OGCs at the invasion front, (c) deficiency of dMMR proteins in the cancer cell nucleus, and (d) PD-L1 expression on the cancer cells.

## Data Availability

All data generated or analyzed during this study are included in this published article.

## Disclosure

This case was presented at the 276th meeting of Shizuoka Pathology Seminar at Shizuoka, Japan, 2019.

## Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of the present paper.

## Authors' Contributions

Each author has sufficiently participated in the work to take public responsibility for appropriate portions of the content. MT performed histopathological diagnosis of the resected sample, analyzed the data, drafted the figure, and made a major contribution to writing the manuscript. TI performed clinical evaluations, surgical treatment, and clinical follow-up. YT analyzed histopathological features and brushed the manuscript up.

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