Malignant gastrointestinal neuroectodermal tumor with BRAF mutation and a history of malignant melanoma: A case report

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Abstract. Malignant gastrointestinal neuroectodermal tumors (GNETs), also called clear-cell sarcoma-like tumors of the gastrointestinal tract, are rare and highly aggressive tumors originating in the gastrointestinal tract. These tumors are generally immunohistochemically positive for S-100 protein (S-100) and SRY-related HMG-box 10 (SOX10), and often contain EWSR1-ATF1 or EWSR1-CREB1. The histological features of GNETs overlap with those of clear-cell sarcoma of the tendons and aponeuroses. However, GNETs immunohistochemically lack melanocyte-specific markers and often demonstrate positivity for CD56, synaptophysin and neuron-specific enolase. The present case reports a woman with a history of desmoplastic malignant melanoma exhibiting a BRAF mutation, which later transformed into a GNET of the small intestine with both a BRAF mutation and two subtypes of EWSR1-ATF1 fusion genes. Tumor cells were revealed to be weakly immunoreactive or negative for S-100 and SOX10, lacked markers of melanocytic differentiation and were focally positive for CD56. Combination therapy with dabrafenib mesylate and trametinib dimethyl sulfoxide proved to be temporarily effective against this tumor. The present case is relatively unique as, to the best of our knowledge, there is no case of GNET with a history of melanoma. Furthermore, there is no report of GNET exhibiting both a BRAF mutation and an EWSR1-ATF1 fusion gene. Further accumulation of similar cases is necessary to elucidate the pathological significance of this GNET having a BRAF mutation.

Introduction

Malignant gastrointestinal neuroectodermal tumor (GNET) is a term designated by Stockman et al for rare, clear-cell sarcoma-like tumors of the gastrointestinal tract (CCSLGT) (1). They reappraised CCSLGT cases and clarified the following features. This tumor is histologically characterized by a sheet-like or nested population of epithelioid or oval-to-spindle-shaped cells with small nucleoli and scattered mitoses. These tumor cells are positive for S-100 protein (S-100), SRY-related HMG-box 10 (SOX10), and vimentin and sometimes positive for CD56, synaptophysin, neuroblastoma 84, neuron-specific enolase (NSE), and neurofilament proteins. They usually contain EWSR1 gene rearrangements, such as EWSR1-ATF1 or EWSR1-CREB1. In general, GNET lacks melanocyte-specific markers, making it clearly different from clear-cell sarcoma (CCS) of the tendons and aponeuroses.

Because GNETs are extremely rare and reports on them are only available in the form of case reports or small size reviews, their morbidity is unclear. They tend to occur mainly in young to middle-aged adults. The most common site of tumor origin is the small intestine (57.9%), followed by the stomach, colon, and other sites of the gastrointestinal tract (2). Surgery is often the choice for resectable lesions, but there are currently no standard chemotherapeutic or targeted therapeutic options for this disease in the metastatic setting. The prognosis is generally poor, and the median survival was reported to be 9.5 months (3). Here, we present a unique case of a GNET that has a history of desmoplastic malignant melanoma exhibiting...
a BRAF mutation, which later transformed into a GNET of the small intestine with both a BRAF mutation and two subtypes of the EWSR1-ATF1 fusion gene.

**Case report**

In April 2018, a 66-year-old woman with multiple metastatic tumors was referred to our hospital for further diagnosis and treatment. She had suffered from lower abdominal pain in February 2018 and consulted a nearby hospital. Imaging examinations revealed a tumor in the small intestine, a soft-tissue mass in her left forearm, a bilateral pleural mass, and a left breast mass. A small intestinal tumor of 8 cm in diameter was resected in early March 2018, and the histological diagnosis was undifferentiated carcinoma. She was diagnosed with carcinoma of unknown primary and transferred to our hospital.

The patient also suffered from idiopathic thrombocytopenic purpura and had a history of cutaneous malignant melanoma (MM). Her MM history was as follows: She underwent extended resection of MM at her left thigh in April 2010 at another hospital; she also received adjuvant combination chemotherapy containing dacarbazine, nimustine hydrochloride, vincristine and interferon-β. However, the patient experienced a local recurrence of MM in May 2011 and underwent additional extended resection, though no adjuvant chemotherapy was administered after the second operation.

The patient's pleural mass was rapidly growing; hence, she was admitted to our hospital in April 2018. On admission, she was slightly obese (the body mass index was 28.06). Physical examination demonstrated a soft, painful mass of 5 cm in diameter in the flexor muscle side of her left forearm. In addition, a hard mass of 2 cm in diameter was palpable in the left upper portion of her left breast. Computed tomography revealed a large necrotic tumor of 15 cm in diameter in the left upper portion of her chest, with bilateral multiple lung metastatic masses and left pleural effusion. Furthermore, a 47-mm tumor without contrast effect in the left forearm and a 17-mm nodule in the left breast were detected, but no lymphadenopathy was found.

Laboratory studies yielded the following results: White blood cells=7.96x10⁹/l, hemoglobin=12.8 g/dl, platelet count=79x10⁹/l (normal range [NR]: 158-348x10⁹/l), lactate dehydrogenase=438 IU/l (NR: 124-222 IU/l), C-reactive protein=6.03 mg/dl (NR ≤0.14 mg/dl), CA-125=38 U/ml (NR ≤35 U/ml),NSE=19.4 ng/ml (NR ≤16.3 ng/ml), and platelet-associated IgG=59 ng/10⁷ cells (NR ≤46 ng/10⁷ cells).

Reexamination of the resected small intestinal tumor by our hospital pathologists resulted in the new diagnosis of a GNET with both a BRAF mutation and two subtypes of the EWSR1-ATF1 fusion gene.

At this point, we questioned whether the previous diagnosis of MM was valid because it showed phenotypic and immunohistochemical overlap with GNET and CCS. Therefore, we asked the hospital which had treated her MM to provide samples. Because of the rapid progression of her pleural mass, chemotherapy with doxorubicin was started even before the samples were examined. Two courses of doxorubicin were administered, stabilizing the case. However, the patient further suffered from pneumocystis pneumonia, and it was important to discontinue chemotherapy. She was further treated using a drug combination of sulfamethoxazole and trimethoprim and recovered from pneumonia.

In June 2018, the histological diagnosis of the previous samples at the time of recurrence of MM was finally established as desmoplastic MM with a BRAF mutation. Therefore, the BRAF gene status of the small intestinal and left forearm tumors were checked, and both tissues were found to exhibit a BRAF mutation. Her GNET was diagnosed as double-positive for BRAF mutation and EWSR1-ATF1 fusion transcript. Dabrafenib mesylate and trametinib dimethyl sulfoxide were started, a combination therapy that proved effective and achieved a partial response three months later (Fig. 1). The size of the pleural mass increased in January 2019, and these drugs were replaced by immunotherapy with nivolumab and ipilimumab. This immunotherapy was performed only once because of the rapid progression of her pleural mass, the occurrence of multiple brain metastases, and the deterioration of her health.
She was transferred to a hospice facility in February 2019 and died in January 2020 because of the deterioration of her brain metastasis.

Pathological findings

In total, four tissue samples were reviewed (Table 1): A primary cutaneous lesion of the left thigh resected in April 2010 (Sample 1), a recurrent tumor embedded in the former operation scar excised in July 2011 (Sample 2), a small intestinal mass resected in March 2018 (Sample 3) and a core-needle biopsy of a soft-tissue mass that arose in the left forearm in April 2018 (Sample 4).

i) Primary cutaneous lesion of the left thigh (Sample 1). Sections stained with hematoxylin and eosin (H&E) showed a subpedunculated polypoid lesion with a maximum diameter of 6 mm associated with intraepidermal neoplasm harboring brown pigments at the tumor border (Fig. 2A). The protrusion was mainly composed of spindle cells with relatively low atypia proliferating in fascicles (Fig. 2B). No lymphovascular invasion was identified. Immunohistochemically, the tumor cells were positive for S-100 but negative for human melanin black-45 (HMB45) (Fig. 2C and D). The histological diagnosis was desmoplastic MM with an unclear surgical margin. No tissue blocks were available for additional studies.

ii) Recurrent tumor beneath operation scar in the left thigh (Sample 2). Sections stained with H&E showed the proliferation of atypical spindle cells in the subcutaneous tissue. The morphological features resembled that of the invasive component of the previous tumor (Sample 1). Infiltrating cells were immunoreactive to S-100, SOX10, Melan A (+, scattered), CK AE1/3 (+, focal), CK CAM5.2 (+, focal), CD56 (+, focal) and negative for HMB45. The Ki-67 labeling index was 5%. These features are compatible with the local recurrence of desmoplastic MM.

iii) Small intestinal tumor (Sample 3). Sections stained with H&E demonstrated a well-demarcated tumor localized in the intestinal subserosa, which was composed of small round cells with a high nuclear/cytoplasm (N/C) ratio and frequent mitoses accompanied by central necrosis, focal hemorrhage, and myxoid changes (Fig. 3A). Neoplastic cells showed an immunophenotype with limited expressions of S-100, SOX10, Melan A, HMB45, desmin, α-SMA, HHF35, caldesmon, cKIT, DOG1, CD34, TTF-1, GCDFP-15, EMA, MyoD1, myogenin, synaptophysin, chromogranin A, PAX8, ER and negative for S-100, SOX10, Melan A, HMB45, CK7, CK20, CK AE1/3 (+, focal), CK CAM5.2 (+, scattered), CD56 (+, focal) and HMB45. The Ki-67 labeling index was 50%. These features are compatible with the local recurrence of desmoplastic MM.
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focal), CD56(+, focal; Fig. 3F), CK7(-), CK20(-), Desmin(-), α-SMA(-), HHF35(-), Caldesmon(-), cKIT(-), DOG1(-), CD34(-), TTF-1(-), GCDFP-15(-), EMA(-), MyoD1(-), Myogenin(-), Synaptophysin(-), Chromogranin A(-), PAX8(-) and ER(-). The Ki-67 labeling index was 50%. These features were suggestive of GNET, although the diagnosis was inconclusive.

iv) Soft-tissue mass of the left forearm (Sample 4). Sections stained with H&E revealed morphological features resembling those of Sample 3, composed of compact dysplastic cells with a high N/C ratio, which were immunohistochemically negative for S-100, SOX10, HMB45 and Melan A but focally positive for CD56 (Fig. 4A-F). A metastatic tumor was considered based on molecular rearrangement similar to that seen in the small-intestinal tumor (Sample 3), as described below.

Genetic findings

All BRAF mutation analyses were consigned to LSI Medience Corporation (Tokyo) and were performed using real-time PCR techniques. BRAF mutations (V600E) were detected in Samples 2, 3 and 4. To detect the type of EWSR1-ATF1 fusion transcript, RT-PCR was performed at our laboratory, as previously reported (4). In Sample 2, no fusion transcript was detected. However, in Sample 3, an EWSR1 exon8/ATF1 exon4 fusion transcript was identified. In addition, RT-PCR of Sample 4 revealed an EWSR1 exon10/ATF1 exon5 fusion transcript.

Discussion

Clear-cell sarcoma is a rare soft-tissue sarcoma that was first described by Enzinger (5). It typically involves the deep soft tissues of the extremities in close proximity with tendons and aponeurotic structures. Its distinctive features include a nested growth pattern and consistent melanocytic differentiation (6). From a pathological point of view, CCS and MM share many histological and immunohistochemical features. However, CCS and MM are currently considered to be two distinct disease entities because, in most cases, CCS involves specific fusion genes such as EWSR1-ATF1 or EWSR-CREB1. On the other hand, CCSLGT is an extremely rare condition that was originally described by Zambrano et al in 2003 as an osteoclast-rich tumor of the gastrointestinal tract with features resembling those of CCS of soft parts (7). Stockman et al (1) described the clinicopathological, immunohistochemical, ultrastructural and molecular analysis of 16 cases with CCSLGT. They reported that these tumors are positive for S-100, SOX10 but lack melanocyte-specific markers. Genetically, these tumors were characterized by EWSR1 gene rearrangements, including EWSR1-ATF1 or EWSR1-CREB1 fusion, similar to CCS of the tendons and aponeuroses. At the ultrastructural level, they lacked evidence of melanocytic differentiation and showed features of neural differentiation. In their study, Stockman et al suggested designating these tumors as "malignant gastrointestinal neuroectodermal tumors" (GNETs).

Our case exhibited the following unique characteristics: i) the patient had a history of BRAF-mutated desmoplastic MM; ii) her tumor cells were weakly immunoreactive or negative for S-100 and SOX10; iii) there was no evidence of melanocytic differentiation; iv) focal positivity for CD56 was present; and v) her tumor exhibited both a BRAF mutation and two subtypes of EWSR1-ATF1. Thway and Fisher (8) reviewed the clinicopathological and molecular features of
five diverse neoplasms that frequently exhibit EWSR1-CREB1 or EWSR1-ATF1 genetic fusion: Angiomatoid fibrous histiocytoma, conventional CCS (of tendons and aponeuroses), CCSLGT, hyalinizing CCS of the salivary gland and primary pulmonary myxoid sarcoma. Among these five neoplasms, S-100 negativity was a finding consistent with angiomatoid fibrous histiocytoma and hyalinizing CCS of the salivary gland, but their pathological and clinical features were clearly different from those of our case. Clinicopathologically, our case was more likely to be diagnosed with CCS or GNET, but both neoplasms were reported to be positive for S-100 without exception. Therefore, we could not reach a definite diagnosis for our case. However, considering the tumor site (small intestine), the absence of melanocytic differentiation, and the weak positivity for CD56, we determined that her tumor was very similar to a GNET.

There were some limitations in our diagnosis. First, the patient already had three large tumors when she visited the previous hospital for the first time: A pleural mass, a small intestinal mass, and a soft-tissue mass in her left forearm. No biopsy of her pleural mass was performed. The EWSR1-ATF1 fusion gene subtypes were different between the small intestinal mass and the forearm mass. As for CCS, several cases with two or three different types of EWS-ATF1 fusion have previously been reported (9,10). To the best of our knowledge, no case report of a GNET with more than two subtypes of EWSR1-ATF1 fusion exists so far. Therefore, it cannot be verified whether her tumor is really a single tumor or a collection of similar tumors. In addition, this tumor exhibited both a BRAF mutation and EWSR1-ATF1 fusion. Such cases have rarely been reported with CCS (11,12); however, to the best of our knowledge, no case report of a GNET with both a BRAF mutation and EWSR1-ATF1 fusion gene exists so far. This tumor is similar to GNET, but it may be a different type of neoplasm.

The unique highlight in our case is the patient's history of desmoplastic MM. It should be noted that the patient's past MM and GNET are morphologically and immunohistochemically different neoplasms, but both tumors exhibited BRAF mutations. Therefore, our case suggests one hypothesis for the pathogenetic process of her GNET: Her desmoplastic MM exhibiting a BRAF mutation acquired an additional

Figure 3. Photomicrographs of the small intestinal tumor. (A) A patternless proliferation of atypical cells characterized by scant pale cytoplasm with microvesicular degeneration (H&E stain; magnification, x200). The tumor was immunohistochemically partially positive for (B) S-100 (IHC stain; magnification, x100) and (C) SOX10 (IHC stain; magnification, x100), but negative for (D) HMB45 (IHC stain; magnification, x100) and (E) Melan A (IHC stain; magnification, x100). Additionally, the tumor was positive for (F) CD56 (IHC stain; magnification, x100). IHC, immunohistochemical; SOX10, SRY-related HMG-box 10; HMB45, human melanin black-45.
EWSR1-ATF1 fusion gene and changed its morphology to a GNET-like one. However, this theory has a limitation. The recurrent MM sample (Sample 2), which had been resected nearly seven years earlier, may have been too old for the detection of the EWSR1-ATF1 fusion gene. Fusion gene detection may have been falsely negative due to a sampling error.

Therefore, the combination of BRAF mutation analysis and EWSR1 fusion gene detection has attracted a significant amount of attention as a means of differentiating CCS from MM (13). Our patient's GNET exhibited both a BRAF mutation and two subtypes of EWSR1-ATF1 fusion genes, and combination therapy with dabrafenib mesylate and trametinib dimethyl sulfoxide proved to be temporarily effective. This combination of tests for GNET may contribute to the diagnosis as well as the choice of treatment method.

In summary, we reported the case of a woman with a history of desmoplastic MM exhibiting a BRAF mutation, which later transformed into a GNET exhibiting both a BRAF mutation and two subtypes of EWSR1-ATF1 fusion genes. Combination therapy with dabrafenib mesylate and trametinib dimethyl sulfoxide proved to be temporarily effective for this type of tumor. Further accumulation of similar cases will be necessary to elucidate the pathological significance of this type of tumor.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TYag conceived the present study. SN pathologically diagnosed the patient and revised the manuscript. TYam performed...
the gene analysis. TW, YI, HT, HS, KY and ST collected the clinical data. All authors read and approved the final manuscript prior to submission.

Ethics approval and consent to participate

The soft-tissue mass sample was collected after obtaining written informed consent from the patient according to a protocol approved by the Osaka International Cancer Institute (Osaka, Japan).

Patient consent for publication

Written informed consent was obtained from the patient’s husband for the publication of her data and associated images.

Competing interests

The authors declare that they have no competing interests.

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