Case Report
Epithelioid inflammatory myofibroblastic sarcoma in abdominal cavity: a case report and review of literature

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Abstract: In this study, we present a rare and difficult case of epithelioid inflammatory myofibroblastic sarcoma (EIMS) in abdominal cavity. A 47-year-old female presented as left upper abdominal pain for 6 months and abdominal distention for 1 month. CT examination showed a solid mass in the left upper intra-abdomen. Grossly, the tumor was found in the mesenterium of colon with the size of 7.5 × 6.5 × 3.5 cm, and was solid and gray-yellowish in the cut surface. Focal myxomatous appearance was observed. Microscopically, stromal myxoid change together with prominent infiltrated lymphocytes, neutrophils and eosinophils were found in the tumor, and the tumor cells were round, epithelioid with vesicular nuclei, large prominent nucleoli and high mitotic rate. Immunohistochemically, strong diffused positive for vimentin, desmin, ALK (nuclear membrane staining pattern) and AAT, focally positive for CD99 and CD30, were showed, Ki67 index was about 20%; Especially, WT-1 and D240 were focally expressed in this tumor. FISH analysis showed rearrangement of ALK, and reverse-transcription polymerase chain reaction (RT-PCR) analysis was used to detect the fusion location of the RANBP2 and ALK gene. The diagnosis of EMIS was made based on its location, typical morphology, the immunohistochemical features especially the nuclear membranous immunostaining of ALK and rearrangement of RANBP2-ALK. The tumor showed higher aggressive behaviors and a poor prognosis. The differential diagnosis and other treatments of EMIS are also discussed in the present study. This finding may increase the case information of EMIS.

Keywords: Epithelioid inflammatory myofibroblastic sarcoma, ALK, retroperitoneal

Introduction

Inflammatory myofibroblastic tumor (IMT) is regarded as an intermediate-grade tumor in the World Health Organization classification, with potential for recurrence and rare metastasis [1]. Epithelioid inflammatory myofibroblastic sarcoma (EIMS) has been proposed to be a variant of IMT with malignant characteristics and consisting mainly of round-to-epithelioid cells [2], and with a nuclear membrane or perinuclear pattern of immunostaining for ALK. It has been reported that this unique nuclear membrane staining pattern is attribute to the rearrangement of RANBP2 with ALK, which is correlated with its poor prognosis [3]. EMIS is a rare disease, and so far, only 12 cases have been reported in English, and it is prefers to male patients. Herein, we report the occurrence of EMIS in adult women, and the specific character and relevant literature is reviewed. This may provide further information on EMIS.

Case report

Clinical history

A 47-year-old female who was hospitalized due to left upper abdominal pain for 6 months and abdominal distention for 1 month. CT examination showed a solid mass in the left upper intra-abdomen, which do not distinguish with the small intestine. When strengthen scan was performed, the mass showed non-uniform intensification (Figure 1A and 1B). Intraoperatively, the tumor was situated in the mesenterium region of the descending colon, and revealed a local invasion to the adjacent colon wall and adhesion with spleen, pancreas and diaphragm. The resection of the tumor together with, part of descending colon, pancreas, diaphragm and spleen was conducted. Two months after the operation, CT imaging displayed recurrent intra-abdominal tumor with the dimension of 9.5 × 5.4 cm, and the multiple metastasis were
observed in liver and lungs. The patient was subjected to further chemotherapy of liver and abdomen (3.5 Gy, five times), but the therapeutic efficacy was poor. Concurrently, the patient was died of the digestive tract hemorrhage, anemia and functional failure of multiple organs after maintenance treatment for 4 months.

**Histopathological and genetic findings**

Grossly, the tumor was detected in the mesenterium of colon with the size of 7.5 × 6.5 × 3.5 cm, and was solid and gray-yellowish in the cut surface. Focal myxomatous appearance was observed too. The tumor infiltrated the adjacent small bowel wall, adhered with spleen, pancreas and some diaphragm (Figure 1). Microscopically, the tumor showed a sheet and nodular growth pattern and stromal myxoid was changed together with prominent infiltrated lymphocytes, neutrophils and eosinophils (Figure 3A-C). The tumor cells were round, epithelioid cells with vesicular nuclei, large prominent nucleoli and abundant of amphophilic or eosinophilic cytoplasm. Mitotic figures were frequent (average of 2-3 per 10 high power fields) (Figure 3D and 3E). The tumor was predominantly observed in the mesenterium, invaded into the adjacent muscular tissues and submucosa of intestinal wall. Although the mucosa was intact, the tumor revealed part invasion to the surrounding fat tissues, pancreas and some diaphragm, and adhesion with spleen by lamina fibrous tissue (Figure 3A, 3B, 3G and 3H). The tumor emboli were still found in the vascular spaces (Figure 3F).

Immunohistochemically, the tumor cells showed diffuse strong staining for Vimentin (1:500, Clone V9, Leica, Germany), Desmin (1:250, Clone DE-R-11, Leica, Germany) (Figure 4A), distinctive nuclear membrane staining for ALK (1:100, Clone 5A4, Maixin Biotechnology, China) (Figure 4B). Similarly, positive staining for CD30 (Ready to use, Clone IR602, Dako, Denmark) (Figure 4C), WT-1 (1:100, Clone WT49, Maixin Biotechnology, China) (Figure 4D), and D240 (1:100, Clone D2-40, Maixin Biotechnology, China) (Figure 4E) was also observed in some tumor cells. The Ki67 index was approximately 20% (Figure 4F). In addition, the tumor cells showed strong diffused staining for CD99 (1:200, Clone EP8, Zhongshan Biotechnology, China) and AAT (1:100, Clone HFF35, Zhongshan Biotechnology, China) (Figure 4G and 4H). The tumor cells were non-reactive to AE1/AE3 (1:200, Clone AE1/AE3, Zhongshan Biotechnology, China), cytokeratin 7 (1:200, Clone OV-TL12/30, Maixin Biotechnology, China), cytokeratin 20 (1:100, Clone PW31, Leica, Germany), CEA (Leica; 1:200, Clone 12-140-10, Germany), AFP (1:200, clone...
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Fluorescence in situ hybridization (FISH) was carried out on 4 μm-thick paraffin sections according to the manufacturer’s instructions. The presence of ALK rearrangement was determined using the LSI ALK dual color break-apart probe (Abbott Molecular, Vysis, Des Plaines, IL). FISH analysis showed the rearrangement of ALK through the identification from a set of separate green and red signals and a fused signal in tumor cell nuclei or only one red signal and a fused signal that was tested (Figure 4I).

Reverse-transcription polymerase chain reaction (RT-PCR) analysis was conducted to explore the fusion location of the RANBP2 and ALK gene. Total RNA was extracted from 20 μm-thick paraffin sections using the RNeasy® FFPE kit (QIAGEN, Germany), and reverse transcription was conducted using random hexamer primers. PCR was performed using the primer sequence of RANBP2-ALK was: (forward) 5’-CAGACTCGTGCGTGGATGTA-3’ and (reverse) 5’-CTAGGCTTGCTCAGGATGTA-3’, for 45 cycles as follows: 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. An expected 139-bp amplified product was detected in the Case (Figure 5A). Positively amplified results (268-bp) of beta-actin as a house-keeping gene were present (Figure 5B). That the RANBP2-ALK fusion point was composed of exon 18 of RANBP2 to exon 20 of ALK (Figure 5C) was confirmed by direct sequencing of the chimeric cDNA product.

Discussion

Rare IMT cases show malignant progression with relapse and metastatic capability. More aggressive tumors are often characterized by a morphological change known as round-cell transformation, which is regarded as an aggressive variant of IMT, called as the EIMS. EMIS is a rare disease. To date, 12 EMIS have been reported. 11 cases were occurred in the abdominopelvic area [2], and only 1 case presented in the pleural cavity [4]. Furthermore, it is detected in man commonly, Marino-Enriquez et al [2] had reported that ten patients were male and one patient was female with the age range of 7 months to 63 years (median year, 39 years). All are intra-abdominal tumors, and most cases are mesentery or omentum. The most common presenting symptoms include abdominal pain with the duration of 1-6 months before surgical excision. On the basis of histological results, the tumors are dominated by epithelioid-to-round cells with prominent inflammatory infiltrate. The stroma is predominantly myxoid, collagenous or mixed. The compositions of the inflammatory infiltrate are prominent neutrophils and small lymphocytes; some showed the eosinophils and plasma cells. According to the immunohistochemical analysis, the tumors were positive for ALK showing a nuclear membrane or perinuclear staining pattern. In addition, the tumors are positive for desmin, weak-to-moderate CD30 and focal SMA [1, 4].
In the present study, we reported a 47-year-old female who was hospitalized due to left upper abdominal pain for 6 months and abdominal distention for 1 month. The tumor was found in the mesenterium of colon with the size of $7.5 \times 6.5 \times 3.5$ cm, and was solid, gray-yellowish and myxoid in the cut surface. Microscopically, the tumor cells were round, epithelioid cells with vesicular nuclei, large prominent nucleoli and high mitotic rate, stromal myxoid change together with prominent infiltrated lymphocytes, neutrophils and eosinophils. The tumor was strongly positive for vimentin, desmin and CD30, and the nuclear membrane staining pattern of ALK was positive. All of these pathologic features and immunophenotypes were coincidence with the reported diagnosis of EMIS. It is worthy of attention that WT-1 and D240 were expressed specifically in EMIS of the present case. Commonly, WT-1, D240, CK5/6 and MC were the specific marker of mesothelial cells, only WT-1 and D240, but not CK5/6 and MC were expressed in EMIS, and the expression of WT-1 and D240 was also reported in the previous study of IMT [5]. All of this suggested that WT-1 and D240 may be the markers of EMIS. Furthermore, the difference between our case and previous reports was a uniformed expression of CD99 and AAT, suggesting that this may be correlated with the high malignancy of the tumor, which may require the further investigation.

Genetic studies have disclosed the rearrangement of...
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RANBP2-ALK in EMIS [2, 4, 6]. ALK is a receptor tyrosine kinase first identified as a component of NPM-ALK fusion oncoprotein aberrantly expressed in anaplastic large cell lymphomas (ALCL) harboring a t (2; 5) translocation [7]. Similar to ALCL, a variety of gene partners can be fused to ALK in IMT as a result of diverse chromosomal rearrangements [8-10]. The expression of ALK fusion proteins in IMT can be detected by immunohistochemistry, which shows an ALK staining pattern that seems to be determined by the fusion partner. The diffused cytoplasmic staining indicates that the fusion involves in cytoplasmic proteins such as TPM3, TPM4, CARS, ATIC, and SEC31L1 [11], and nuclear membrane staining with RANBP2, which is located at the nuclear pores [7].

Limited data originating from isolated case reports suggest that IMTs with the RANBP2-ALK fusion usually show epithelioid/round cell morphology and a more aggressive clinical course that may be regarded as a marker of diagnosis and prognosis [1, 11, 12]. In our study, we have demonstrated a round-cell transformation of the tumor, and the particular nuclear membrane staining pattern of ALK, which is consistent with the morphology and immune features of EMIS. Furthermore, fluorescence in situ hybridization was positive for ALK gene rearrangement, and a RANBP2-ALK fusion was detected by RT-PCR, which may provide more proves to the diagnosis of EMIS.

The diagnosis of EMIS can be very challenging due to the unusual epithelioid-to-round cell morphology and the markedly atypical nuclear

Figure 4. Immunohistochemical and FISH images. A. Cytoplasmic desmin immunostaining, 200 ×; B. Nuclear membrane staining for ALK, 200 ×, inserted panel, (400 ×); C. Cytoplasmic CD30 immunostaining, 200 ×; D. Nuclear WT-1 immunostaining, 200 ×; E. Cytoplasmic D240 immunostaining, 200 ×; F. Ki67 staining was 20%. 200 ×; G. Strong diffused cytoplasmic CD99 immunostaining, 200 ×; H. Strong diffused cytoplasmic AAT immunostaining, 200 ×; I. Fluorescence in situ hybridization (FISH) showing split apart of the 2 signals of ALK, confirming the presence of an ALK rearrangement (arrow). Scattered cells showed only one red signal with a fused signal (arrowhead). 1000 ×.
features. The EMIS should be differentially diagnosed with disease as follows: (1) ALCL, especially the rare sarcomatoid variant of ALCL, which can show spindle cell morphology and an overlapped immunophenotype, including the reactivity for CD30, ALK, and SMA and negative staining for EMA. However the RANBP2-ALK has not been reported in ALCL, and strong expression of desmin staining is not observed in ALCL. (2) High-grade leiomyosarcoma, usually contains at least focal areas with typical histological features, namely, the fascicles of spindle cells with abundant bright eosinophilic cytoplasm and cigar-shaped nuclei, but ALK staining is not observed. (3) The solid variant of alveolar rhabdomyosarcoma is quite often ALK positive, and can usually be excluded on histological examination. Alveolar rhabdomyosarcoma is generally cytologically more uniform with less cytoplasm and lacks myxoid stroma and prominent neutrophils. Nuclear immunoreactivity for myogenin (MYF4) is helpful to confirm the diagnosis of rhabdomyosarcoma. (4) Dedifferentiated liposarcoma, showing the “inflammatory MFH” pattern, can also be difficult. In such cases, extensive sampling usually shows the areas of well-differentiated liposarcoma or the characteristic striking intratumoral heterogeneity of dedifferentiated liposarcoma. Furthermore, nuclear membrane staining for ALK is not observed in liposarcoma. (5) Gastrointestinal stromal tumor (GIST), the position and the epithelioid morphology maybe confused. But the GIST is positive for CD117, DOG1 and CD34, and mutation of C-kit and PDGFα are also found in GIST. In the present study, no mutation of C-kit and PDGFα were found (data not shown). (6) Tumors are usually originated in epithelium, such as hepatocellular carcinoma, cholangio carcinoma and pancreatic cancer. The morphology and immunophenotype may be helpful for the differentiation, such as the strongly positive for AFP, CKAЕ1/AE3, CK7, CEA and CA199.

At present, most of the reported EMIS are treated by surgical resection combined with chemotherapy and radiotherapy. However, EMIS may be recurrence and/or death early, and with poor prognosis, when compared with the conventional IMTs [12] Furthermore, EMIS arising in the abdomino-pelvic region is easy for recurrence and metastasis [2]. Follow-up data reported by Marino-Enriquez for 8 patients that all 8 patients developed local recurrences for 1-8 months after initial resection, 5 patients were multifocal and multiple recurrence, and 2 patients developed distant metastases. Thus far, 5 patients have died of disease after 3-36 months, and 2 patients are alive with recurrent intra-abdominal disease after 13 months. One patient is alive without the evidence of disease after 40 months (the patient treated with the experimental ALK inhibitor). In the present study, the patient was recurrence and metastasis after the reception, and was died of func-
tional failure of multiple organs after maintenance treatment for 4 months. It has been reported that the rearrangement of RANBP2-ALK is possibly associated with a poor prognosis [13]. Some other studies have suggested that the chimeric RANBP2-ALK gene can promote cellular proliferation, which may be a potential mechanism for the rapid re-growth and recurrence of EMIS. However, there is still a lack of direct experimental evidence associated with the functions of this fusion gene with the increased aggressiveness of the disease.

In summary, we present an EMIS in woman with an unusual RANBP2-ALK fusion gene, associated with peculiar round cell morphology, and a unique nuclear membranous pattern of ALK expression. This may provide more information of EMIS cases. An in-depth study should be the experimental investigation of RANBP2-ALK with poor prognosis and the effective ALK inhibitor treatment schedules.

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Disclosure of conflict of interest

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