Case Report

Epithelioid inflammatory myofibroblastic sarcoma harboring ROS1-TFG fusion; a case report

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Abstract: Epithelioid inflammatory myofibroblastic sarcoma (EIMS) is a rare and aggressive variant of inflammatory myofibroblastic tumor (IMT). ALK-RANBP2 fusion is one of the supposed mechanisms for the aggressive biological behavior of this tumor. To the best of our knowledge, we report the first case of EIMS carrying ROS1-TFG fusion which affected a 16 year-old male. The patient presented abdominal pain and nausea that had lasted for 3 weeks. CT scan revealed multiple masses in the abdominal cavity. Microscopically, the resected tumor showed growth of atypical round and epithelioid cells with large nucleoli infiltrated in myxoid stroma admixed with inflammatory cells. Mitotic figures were frequent. Immunohistochemically, the tumor cells were positive for vimentin, desmin, CD30, D2-40, focally positive for SMA, WT1, CD99 but negative for cytokeratin, EMA, or ALK. Ki-67 labeling index was around 30 %. FISH analysis revealed ROS1 rearrangement, but not of ALK. The partner gene fused with ROS1 was identified to be TFG by analysis of RT-PCR and Sanger sequence. Expression of ROS1 protein in the neoplastic cells was confirmed immunohistochemically. The patient did not respond to 3 cycles of chemotherapy, and died of perforative peritonitis due to progression of the tumor. EIMS is known to present typical clinical and immunohistological features with bleak prognosis. When ALK rearrangement is not demonstrated in such cases, ROS1 should be evaluated for the diagnosis and direction of treatment.

Keywords: Epithelioid inflammatory myofibroblastic sarcoma, ROS1-TFG fusion, ALK-RANBP2 fusion, ALK/ROS1 inhibitor

Introduction

Epithelioid inflammatory myofibroblastic sarcoma (EIMS) is a rare variant of inflammatory myofibroblastic tumor (IMT) mainly occurring in children and young adults, and it mostly involves in abdominal cavity. Genetically, EIMS possesses a characteristic gene fusion between ALK and RANBP2, which is associated with poor prognosis [1-8]. Herein, we present a case of EIMS harboring ROS1-TFG fusion gene which we believe is not hitherto known in cases with EIMS. The pathologic features and relevance of gene alteration to clinical and histological features will be discussed.

Case report

Clinical history

A 16 year-old male was admitted with complaints of abdominal pain and nausea that had lasted for about 3 weeks before admission. His mother died of soft tissue tumor (Allegedly, the tumor was difficult to diagnose and finally diagnosed as IMT, although the details are unknown). Computed tomography showed massive ascites and multiple tumor masses in the abdominal cavity. The tumor masses appeared to locate in the wall of small intestine (Figure 1). On laparotomy, tumor biopsy was performed, and tentative pathological diagnosis was undifferentiated sarcoma. The patient was subjected to 3 cycles of non-specific cancer chemotherapy of VIDE regimen (V: Vincristine, I: Ifosfamide, D: Doxorubicin, E: Etoposide) at a half dose in the second and third courses because of acute renal failure. The patient did not respond to the chemotherapy. Meanwhile, the final diagnosis was made as EIMS harboring ROS1-TFG fusion. Although molecular targeted therapy was being considered, the patient followed downhill course and died of perforative...
peritonitis because of the progression of the tumor 5 months after admission of this hospital.

Histological findings

Tumor cells showed a sheet and focally cord-like growth pattern (Figure 2A). They were mainly round and epithelioid (Figure 2B), focally spindle (Figure 2C), with prominent nucleoli and abundant eosinophilic cytoplasm (Figure 2B, 2C). Mitotic figures were focally prominent, and the highest mitotic rate was 5/10 HPF (Figure 2D). The stroma of the low cellularity areas was myxoid and admixed with lymphocytes and macrophages (Figure 2B-D).

Immunoprofiles

Diagnostic immunostainings were performed with Ventana GX System (Roche Diagnostics, K.K., Tokyo, Japan), using commercial antibodies. The tumor cells were positive for vimentin (Dako, Glostrup, Denmark; clone V9; dilution 1:200), desmin (Dako; clone D33; dilution 1:200) (Figure 3A), CD30 (Roche; clone Ber-H2; dilution 1:40) (Figure 3B), D2-40 (Nichirei, Tokyo, Japan; clone D2-40; prediluted) and INI1 (BD Biosciences, San Jose, CA, USA; clone BAF47; dilution 1:30), focally positive for WT1 (Dako; clone 6F-H2; dilution 1:50), SMA (Dako; clone 1A4; dilution 1:500), and CD99 (Dako; clone 12E7; dilution 1:200). ALK (Nichirei; clone 5A5; prediluted) was negative (Figure 3C), so was cytokeratin (Dako; clone AE1/3; prediluted), EMA (Dako; clone E29; dilution 1:100), S100 (Dako; clone S100; dilution 1:3000), chromogranin A (Dako; clone DAK-A3; dilution 1:300), synaptophysin (Thermo Fisher Scientific, Waltham, MA, USA; clone SP11; dilution 1:200), c-kit (Dako; polyclonal; dilution 1:300), Muscle actin (Dako; clone HHF35; dilution 1:100), MDM2 (Thermo Fisher Scientific; clone IF2; dilution 1:100), CDK4 (Thermo Fisher Scientific; clone DCS-31+DCS-35; dilution 1:100), calponin (Dako; clone calp; dilution 1:100), or calretinin (Nichirei; clone SP13; dilution 1:20).

Genetic findings

Fluorescence in situ hybridization (FISH): FISH analyses using dual color break-apart probe for ALK, ROS1 and PDGFRβ were performed (LSI Medience Corporation, Tokyo, Japan). ALK probe did not yield any split signals (Figure 4A). In contrast, ROS1 probe showed positive split signals, so that the tumor cells were found to bear ROS1 rearrangement (Figure 4B). FISH analysis for PDGFRβ rearrangement was negative (data not shown).

Reverse transcription and PCR (RT-PCR): RT-PCR was performed to find the partner gene with ROS1. Total RNA was extracted from paraffin sections with RNA easy FFPE kit (Qiagen, Hilden, Germany). Subsequently, RT-PCR was carried out with SuperScript® VILO cDNA Synthesis kit (Thermo Fisher Scientific), following manufacturers’ manuals. In the literature, TFG and YWHE were reported to be partner genes of ROS1 in cases with conventional IMT [9, 10]. Therefore, PCR with the primers to detect ROS1-TFG and ROS1-YWHE fusion was carried out. The sequence of primers used as follows:

TGF-ROS1: 5’-AGAACTTCAAAATAGTGAACGT-3’ and 5’-CACTGTCACCCCTTCCTTGG-3’ [9], YWHE: 5’-GCCACAGGAAACGACAGGAAGGAGGC-3’; ROS1: 5’-GAAGAAGGGTTCCACAGACCAAGG-3’ [10].

ROS1-TFG fusion transcripts showed as a single band around 150 bp (Figure 4C). With the use of Sanger sequence procedure, a fusion between ROS1 exon 35 and TFG exon 4 was confirmed (Figure 4D).
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Immunostaining of ROS1

Briefly, 3.5 µm-thick sections were deparaffinized, immersed in citrate buffer at 125°C for 5 minutes, treated with Background Sniper (Biocare Medical, Concord, CA, USA) for 10 minutes, and then incubated with the primary antibodies against ROS1 (Cell Signaling, Danvers, MA, USA; clone D4D6; dilution 1:250) at 37°C overnight [9]. Following elimination of the endogenous peroxidase in 3% H₂O₂ in distilled water for 10 minutes, sections were subjected to Envision+ System-HRP Labelled Polymer Anti-Rabbit (Dako). The reaction products were visualized as brown with Metal Enhanced DAB Substrate Kit (Thermo Fisher scientific), following counterstaining with hematoxylin. Cytoplasmic expression of ROS1 protein was detected by immunohistochemistry in tumor cells (Figure 4E) [11].

Final diagnosis

Based on the epidemiology, aggressive clinical course and the pathological features of round-epithelioid tumor cells with immunoreactivity for CD30, WT1, and D2-40, the final diagnosis was made as EIMS harboring ROS1-TFG fusion (T. S., C. I., K. H., S. Y., and H. M.).

Discussion

Marino-Enriquez et al. first described EIMS as a variant of IMT with round, epithelioid tumor cells and poor prognosis [5]. Most cases of EIMS share common clinical features; it predominates males, adolescents or young adults, locates in abdominal cavity, and develops aggressive course. Histologically, the tumor cells are round and epithelioid, with foci of myxoid stroma and lymphocyte infiltration. Immunohistologically, they were positive for
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CD30, ALK and myofibroblastic markers [1-8, 12, 13]. Genetically, EIMS is characterized by ALK expression and ALK-RANBP2 fusion [4]. Except for rearrangement and immunostaining of ALK, the present case appeared to be compatible with EIMS in terms of clinical, histological, and immunohistological features.

Immunohistological expression and rearrangement of ROS1 were previously demonstrated in IMT cases [9-11, 14], and ROS1 immunopositivity was considered to correlate with ROS1 rearrangement [14]. Lovely et al. reported that ROS1 fusion was detected in 4 of 11 cases among which 2 cases (10.8%) were positive for ROS1-YWHAE fusion and 2 cases for ROS1-TFG fusion in absence of ALK expression. A possibility that those cases might have been EIMS cannot be ruled out, because detailed clinical and histological profiles other than ALK expression were not described [10]. In the other reports, tumor cells were simply described as morphologically spindle cells, implicating conventional IMT tumors [9, 11, 14]. It is hence likely that, the present case is the first to demonstrate that ALK-negative EIMS may harbor ROS1 rearrangement. It may also be conceivable that the germline mutation underlies the tumor development in this case in view of the patient maternal history.

Tyrosine kinase fusions including ALK and ROS1 are considered as oncogenic drivers in preclinical models of epithelial malignancies [15]. Aberrant expression of fusion proteins leads the constitutive activation of downstream signal pathway, resulting in promotion of cellular proliferation and survival. In this setting, suppression of ALK and ROS1 may be plausible targets of chemotherapy oriented to EIMS. Although the efficacy of ALK/ROS1 inhibitor such as Crizotinib (a common inhibitor of ALK and ROS1) on EIMS is still under investigation, preliminary use of ALK/ROS1 inhibitor showed a dramatic response in some cases of EIMS with ALK rearrangement [1, 2, 5].

The present case showed that ALK expression is not requisite for diagnosis of EIMS but other

Figure 4. Genetic analysis and ROS1 protein expression. FISH with a break-apart/split-signal strategy analysis using (A). ALK probe, (B) ROS1 probe. ROS1 probe showed split signals. The arrows indicate isolated 5’ signals (red) and 3’ signals (green). (C) RT-PCR analysis. Lane M: 100 bp ladder marker, Lane 1-3: Negative control of GAPDH, ROS1-YWHAE, and ROS1-TFG primers in this order. Lane 4: GAPDH, 5: ROS1-YWHAE, 6: ROS1-TFG. ROS1-TFG fusion transcripts showed a single band around 150 base pairs (arrow). (D) Sanger sequencing analysis of the transcripts, demonstrating a fusion of TFG exon 4 with ROS1 exon 35. (E) Cytoplasmic, weak to moderate expression of ROS1 protein in tumor cells was confirmed by immunohistochemistry.
fusion genes might also be involved in this tumor. Poor prognosis of EIMS does not necessarily result from ALK-RANBP2 fusion as previously proposed [4, 5, 7, 8]. ALK-RANBP2 fusion gene was suggested to exert autonomous cellular proliferation [5, 16], but precise mechanism how ALK-RANBP2 fusion, different from the other fusion genes that activate tyrosine kinase, causes an aggressive behavior in EIMS is still unknown [16, 17]. Likewise, the role of ROS1 fusion gene encountered in our case is open to question and merits further studies.

In conclusion, we present the first EIMS case with ROS1-TFG fusion. If EIMS is suspected, possibility of ROS1 rearrangement should be considered for diagnosis and therapeutic indication.

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Disclosure of conflict of interest

None.

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References


[14] Hornick JL, Sholl LM, Dal Cin P, Childress MA and Lovly CM. Expression of ROS1 predicts ROS1 gene rearrangement in inflammatory...

