Pediatr Blood Cancer 2009;53:214–216

BRIEF REPORT
Clear Cell Sarcoma of the Stomach

Joanne P. Lagmay, MD,[1,2]* Mark Ranalli, MD,[1,2] Maria Arcila, MD,[3] and Peter Baker, MD[4]

Clear cell sarcoma (CCS) is a high grade soft tissue sarcoma with a distinct molecular profile. Gastrointestinal CCS is very rare and most reported cases are in adults. We describe a 10-year-old female with a 4-month history of anemia who later developed fever, weight loss and abdominal pain. She was subsequently found to have a large infiltrative gastric mass. A diagnosis of CCS was confirmed by molecular and cytogenetic studies. This case illustrates the necessity of a multimodal approach, particularly the use of molecular studies, in the diagnostic evaluation of rare tumors presenting in unusual sites. Pediatr Blood Cancer 2009;53:214–216. © 2009 Wiley-Liss, Inc.

Key words: clear cell sarcoma of soft tissues; cytogenetics; EWS-ATF1; gastrointestinal clear cell sarcoma; pediatric; visceral clear cell sarcoma

INTRODUCTION

Clear cell sarcoma of soft tissues (CCS) was first described by Enzinger in 1965 [1], most commonly presenting as a tumor of the extremities. Rarely, it has been reported as primary tumors of the gastrointestinal (GI) tract [2,3]. These tumors have many overlapping immunohistochemical and ultrastructural features with malignant melanoma (MM). Demonstration of t(12,22)(q13;q12) and the resulting fusion protein *EWS-ATF1,* a translocation that has never been shown in MM [4–6], greatly assists in the diagnosis of CCS. Recently, a distinct novel fusion gene transcript *EWS-CREB1* has been described in several GI CCS cases [7]. In light of the recent discovery of this rare and distinct group of tumors, we present the crucial role of molecular studies in establishing the correct diagnosis of this challenging pediatric case.

CASE

A 10-year-old Caucasian female presented with a 4-month history of easy fatigability, without weight loss, anorexia or bloody stools. Pertinent in her exam were pallor and the absence of significant adenopathy or a palpable abdominal mass. An extensive workup only revealed severe anemia of chronic inflammation with persistently elevated acute phase reactants (thromboscytosis of 1×10^6 /mm^3, ESR to 120 U/hr and ferritin to 900 ng/ml). Results of a comprehensive rheumatologic workup were normal. Numerous stool occult blood tests were negative. Two months after her initial evaluation, the patient developed daily high grade fevers, weight loss, early satiety and vomiting. An abdominal CT scan revealed a tumor extending intraluminally from the posterior wall of the body of the stomach (Fig. 1), with enlarged regional lymph nodes and a 1 cm liver nodule.

A partial gastrectomy and resection of the metastatic liver nodule and regional lymph nodes was done. The tumor was pale tan and rubbery without areas of necrosis and hemorrhage, measuring 7.8 cm × 4.4 cm × 4.8 cm. Histologic features are shown in Figure 2A,B. In contrast to GI CCS cases reported by Zambrano et al. [8], our case did not demonstrate osteoclast-like giant cells. Immunohistochemical stains (performed on the BondMax automated immunostainer Leica Microsystems, Bannockburn, IL) were positive for S-100 protein (Fig. 2B) and vimentin, and negative for CD117, CD34, smooth muscle actin, neuron specific enolase, synaptophysin, chromogranin, pankeratin, epithelial membrane antigen and pan-melanoma (HMB-45, tyrosinase and melan A). Metastasis was confirmed in one of fifteen regional lymph nodes and in the liver nodule. Cytogenetic analysis of the tumor showed t(12;22)(q13;q12)[1]/46,XX [19]), consistent with CCS. FISH analysis showed the EWS (22q12) gene rearrangement (Fig. 2C). Total RNA was extracted from frozen tissue using Trizol reagent (Life Technologies Inc., Gaithersburg, MD). Reverse transcription-PCR (RT-PCR) for the detection of both EWS-ATF1 and EWS-CREB1 fusion transcripts was performed as reported previously [7]

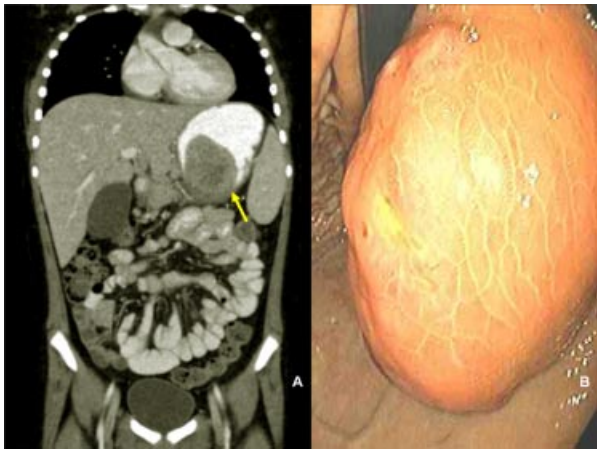

Fig. 1. Abdominal CT (**A**) and esophagoduodenoscopy (**B**) showing a large mass extending intraluminally from the posterior wall of the body of the stomach.

Additional Supporting Information may be found in the online version of this article.

[1]Department of Pediatrics, The Ohio State University College of Medicine, Columbus, Ohio; [2]Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, Columbus, Ohio; [3]Department of Pathology, Memorial Sloan Kettering Cancer Center, The Ohio State University, Columbus, Ohio; [4]Department of Pathology, Nationwide Children's Hospital, The Ohio State University, Columbus, Ohio

*Correspondence to: Joanne P. Lagmay, Division of Hematology/Oncology/BMT, Nationwide Children's Hospital, 700 Children's Drive, Columbus, OH. E-mail: lagmayj@chi.osu.edu

Received 31 October 2008; Accepted 12 February 2009

DOI 10.1002/pbc.22014
Published online 6 April 2009 in Wiley InterScience (www.interscience.wiley.com)

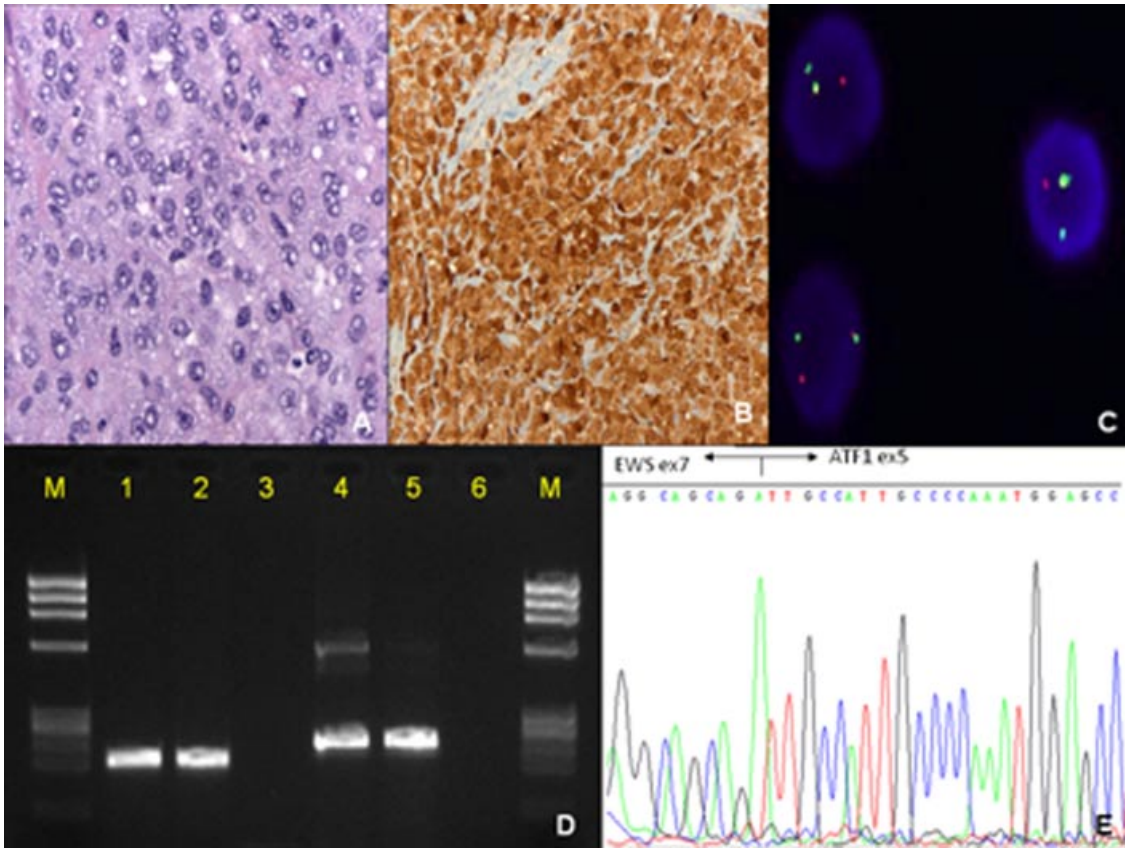

Fig. 2. (**A**) The tumor cells have foamy, amphophilic cytoplasm with vesicular nuclear chromatin and prominent nucleoli (Hematoxylin and eosin, original magnification 400×). (**B**) The tumor cells showed nuclear and cytoplasmic staining (Immunohistochemical stain for S-100 protein, original magnification 200×). (**C**) Fluorescent in situ hybridization (FISH) showing separate green and red signals, indicative of a rearrangement of one copy of the EWSR1 region. (**D**) Agarose gel of RT-PCR products using the forward primer EWSex7F1 and reverse primer CREB1ex7REVa (consensus primer for CREB1 and ATF1). EWS-ATF1 and EWS-CREB1 RT-PCR products using the consensus primer are of similar size and only distinguishable by direct sequencing: (1) patient RT-PCR product, (2) Positive control containing the EWS-CREB1 fusion product, (3) negative control lacking template, 4&5 PGK (RNA quality control) for patient (4) and positive control (5). The faint higher band in lane 4 represents a product derived from residual genomic DNA. (6) PGK negative control lacking template. (M) Size marker. (**E**) Electrophoretogram of direct sequencing of RT-PCR product showing junction point of EWS-ATF1 fusion transcript.

using the forward primer EWSEx7-F1 and the reverse consensus primer CREB1ex7-REVa (binds both CREB1 and ATF1; sequence: 5′-TCCATCAGTGGTCTGTGCATACTG-3′). The adequacy of the extracted RNA was assessed using primers for *PGK* (phosphoglycerate kinase) transcripts [9]. Direct sequencing of PCR products was done using the Big-Dye Terminator kit (Applied Biosystems, Foster City, CA) and run on an Applied Biosystems model 3730 DNA sequencer. RT-PCR showed a product of similar size as the positive controls for the EWS-CREB1 (Fig. 2D) and EWS-ATF1 (not shown) fusion products. Direct sequencing of the patient RT-PCR product revealed a chimeric transcript consisting of a junction between EWS exon 7 and ATF1 exon 5 (Fig. 2E). The reverse primer CREB1ex7-REVa primes both CREB1 and ATF1 due to the extensive similarity between the two sequences.

After two cycles of chemotherapy consisting of ifosfamide and doxorubicin, the patient's hemoglobin and inflammatory markers (ESR, ferritin and platelet count) normalized. She has since completed a total of seven cycles of the same chemotherapy and radiation therapy. At the time of this report, she has been off treatment for 4 months and remains in remission.

DISCUSSION

GI CCS is exceedingly rare [2–4,10,11–16], with only five pediatric cases reported [8,15,16]. GI CCS tend to have distinct immunohistologic and molecular markers compared to soft tissue CCS. Recently, three adult cases of GI CCS with novel *EWS-CREB1* fusion transcript have been described [7]. This case is quite intriguing in the sense that it shares characteristic absence of melanocytic differentiation (HMB45 negative) as in other GI CCS, but has the EWS-ATF1 fusion transcript instead of the variant EWS-CREB1.

All GI CCS cases encountered in the literature presented with chronic nonspecific symptoms followed by dramatic clinical deterioration with fever, weight loss, anorexia, abdominal pain, bloody stools and anemia [2–4,10,11–16]. Fever and iron deficiency anemia seem to be more pronounced among the pediatric cases [8,15,16]. Severe anemia in our patient was due to chronic inflammation, a laboratory abnormality that has not been reported before as a presenting symptom of GI CCS. Interestingly, two pediatric patients [8,16] had a prior history of acute lymphoblastic leukemia.

Although very few are reported, pediatric GI CCS appears to behave more aggressively when compared to adult counterparts. One explanation may be that all pediatric cases were at least five centimeters at presentation. Metastases at diagnosis were reported in four out of five patients. Even with complete resection, with or without adjuvant chemotherapy, four patients died of disease. The only patient with localized disease at diagnosis, progressed shortly after resection and died of disease. Of the two gastric CCS cases encountered in the literature, one is a 30-year-old male [2] (tumor size 4cm), who at the time of report was alive with disease after complete resection, and the other case was a 13-year-old male [8] (tumor size 6.7cm) who recurred despite extensive surgery and chemotherapy.

Diagnosis was challenging in this patient since she presented with nonspecific symptoms that did not immediately lead us to suspect a primary gastrointestinal pathology. This case shows that CCS may occur as a primary gastric neoplasm in children. Without confirmatory cytogenetic and molecular pathology studies, correct diagnosis of rare tumors, tumors occurring at unusual sites or tumors mimicking other entities such as in this case, would be very difficult.

ACKNOWLEDGMENT

We would like to thank Dr. Marc Ladanyi, Chief of Molecular Diagnostics Service, Department of Pathology, Memorial Sloan-Kettering Cancer Center, for reviewing the molecular diagnostic results of this case.

REFERENCES

1. Enzinger FM. Clear cell sarcoma of tendons and aponeurosis: An analysis of 21 cases. Cancer 1965;18:1163–1174.
2. Pauwels P, Debiec-Rychter M, Sciot R, et al. Clear cell sarcoma of the stomach. Histopathology 2002;41:526–530.
3. Taminelli L, Zaman K, Gengler C, et al. Primary clear cell sarcoma of the ileum: An uncommon and misleading site. Virchows Arch 2005;447:772–777.
4. Lyle PL, Amato CM, Fitzpatrick JE, et al. Gastrointestinal melanoma or clear cell sarcoma? Molecular evaluation of 7 cases previously diagnosed as malignant melanoma. Am J Surg Pathol 2008;32:858–866.
5. Covinsky M, Gong S, Rajaram V, et al. EWS-ATF1 fusion transcripts in gastrointestinal tumors previously diagnosed as malignant melanoma. Hum Pathol 2005;36:74–81.
6. Sandberg AA, Bridge JA. Updates on the cytogenetics and molecular genetics of bone and soft tissue tumors: Clear cell sarcoma (malignant melanoma of soft parts). Cancer Genet Cytogenet 2001;130:1–7.
7. Antonescu C, Nafa K, Segal N et al. EWS-CRE B1: A recurrent variant fusion in clear cell sarcoma- association with gastro-intestinal location and absence of melanocytic differentiation. Clin Cancer Res 2006;12:5356–5362.
8. Zambrano E, Reyes-Mugica M, Franchi A, et al. An osteoclast-rich tumor of the gastrointestinal tract with features resembling clear cell sarcoma of soft parts: Reports of 6 cases of a GIST stimulator. Int J Surg Pathol 2003;11:75–81.
9. Argani P, Perez-Ordonez B, Xiao H, et al. Olfactory neuroblastoma is not related to the Ewing family of tumors: Absence of EWS/FLI1 gene fusion and MIC2 expression. Am J Surg Pathol 1998;22:391–398.
10. Ekfors TO, Kujari H, Isomaki M. Clear cell sarcoma of tendons and aponeurosis: The first visceral case. Histopathology 1993;22:255–259.
11. Donner LR, Trompler RA, Dobin S. Clear cell sarcoma of the ileum: The crucial role of cytogenetics for the diagnosis. Am J Surg Pathol 1998;22:121–124.
12. Achten R, Debiec-Rychter M, De Wever I, et al. An unusual case of clear cell sarcoma arising in the jejunum highlights the diagnostic value of molecular genetic techniques in establishing the correct diagnosis. Histopathology 2005;46:472–474.
13. Friedrichs N, Testi M, Moiraghi L, et al. Clear cell sarcoma-like tumor with osteoclast-like giant cells in the small bowel: Further evidence of a new tumor entity. Int J Surg Pathol 2005;13:313–318.
14. Fukuda T, Kakihara T, Baba K, et al. Clear cell sarcoma arising in the transverse colon. Pathol Int 2000;50:412–416.
15. Granville L, Hicks J, Popek E, et al. Visceral clear cell sarcoma of the soft tissue with confirmation by EWTS-ATF1 fusion detection. Ultrastruct Pathol 2006;30:111–118.
16. Venkataraman G, Quinn A, Williams J, et al. Clear cell sarcoma of the small bowel: A potential pitfall. APMIS 2005;113:716–719.