CASE REPORT

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Primary clear cell sarcoma of the ileum: an uncommon and misleading site

Abstract A clear cell sarcoma, arising primarily in the ileum of a 35-year-old man, is reported. Histologically, the neoplasm infiltrated the full thickness of the intestinal wall. It consisted of strands and sheets of round to spindle-shaped cells with clear to eosinophilic cytoplasm, vesicular nuclei and prominent nucleoli. Vascular invasion was present at diagnosis. Tumour cells expressed S-100 protein, melan-A and tyrosinase. They were negative for HMB45, CD117, cytokeratins, epithelial membrane antigen, smooth muscle actin, desmin, CD31, CD34, chromogranin and synaptophysin. Reverse transcription–polymerase chain reaction analysis performed on paraffin-embedded tissue showed EWS–ATF1 fusion transcripts representative of the t(12;22) (q13;q12) clear cell sarcoma reciprocal translocation. The patient, who developed liver metastases 2 months after diagnosis, died of disease at 15 months. This case demonstrates that the gastrointestinal tract is a potential site for primary clear cell sarcoma of soft tissues, and, furthermore, that cytogenetics and/or molecular techniques play a central role in the diagnosis.

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Introduction

Clear cell sarcoma (also known as malignant melanoma of soft tissues) is a rare tumour that typically occurs in the tendon sheaths and aponeuroses of distal extremities of adolescents and young adults [3, 6, 10, 11]. Involvement of the gastrointestinal tract is exceedingly rare and, frequently, a source of diagnostic problems. Six cases of digestive tract clear cell sarcoma were heretofore reported in the literature, most of them very recently [1, 4, 5, 7, 13, 15], including one in the ileum [4]. A second ileal case is described below. It occurred in a 35-year-old man and caused the death of the patient 15 months after diagnosis.

Clinical history

A 35-year-old, HIV-negative man without any previous significant medical history was admitted in March 2003 for a 15-kg weight loss over a few months and symptoms of intestinal obstruction (i.e. colic pain, vomiting) without fever. CT scans showed a segmental thickening of the ileum, situated 80 cm above the ileo–caecal valve. Preoperative diagnoses included Meckel diverticulum, polyps and a malignant neoplasm. At laparotomy, a single circumferential, stenotic lesion of the ileum was found. There was neither other detectable tumour in the abdominal cavity nor any liver metastases. The ileal mass was resected along with 20 cm of ileum and one regional mesenteric lymph node. A pathologic diagnosis of unclassified non-epithelial malignant neoplasm was rendered. The mesenteric lymph node was devoid of tumour deposits, but the ascitic fluid, sampled at the time of surgery, contained clusters of malignant cells. A lymphoma, a GIST, a neuroendocrine neoplasm were excluded on the basis of immunohistochemical results. A melanoma (primary or metastatic) was strongly suspected owing to tumour cell reactivity for S-100 protein.

Careful examination of the patient, including extensive examination of skin, eyes, nasosinusal cavities, throat, testes and soft tissues failed to detect any other potential primary tumour. The metastatic work-up was negative, as was the PET scan. A colonoscopy showed several polyps in the ascending and sigmoid colon, which turned out to be adenomatous polyps with low-grade dysplasia at microscopic examination.

Two months post-operatively, several metastases were detected in the liver, along with multiple peritoneal implants and a malignant ascitis. The tumour regressed dramatically after six courses of a platinum-based chemotherapy, but a second look, performed in November 2003, showed that the malignant process extended microscopically to the whole peritoneal subserosa and to the omentum. Lymph node and liver biopsy specimens, taken during the second surgical procedure, were tumour-free. From then on, and despite various treatments, the tumour gradually progressed in the abdomen, leading to several episodes of intestinal obstruction. Patient's death occurred 15 months after disease onset, as a result of *Candida albicans* septicemia. No autopsy was performed.

Materials and methods

Tumour samples were fixed in 4% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin and Fontana–Masson stains. Additional sections were studied with the following antibodies: S-100 protein (Dakopatts, Glostrup, Denmark, pre-diluted), melan-A (Dako, diluted 1:50), HMB45 (Dako, diluted 1:50), tyrosinase (Ludwig Institute, Lausanne, Switzerland, diluted 1:50, gift from Dr. D. Rimoldi), cytokeratins (clone C-11, diluted 1:40 and clone AE1/AE3, diluted 1:200, Novocastra Laboratories Inc., Newcastle upon Tyne, UK), CD31 (Dako, diluted 1:40), CD34 (Immunotech Beckam Coulter, Inc., Fullerton, CA, USA, diluted 1:160), CD45 (Dako, diluted 1:50), CD3 (Novocastra, pre-diluted), CD20 (clone L26, Dako, pre-diluted), CD56 (clone 1B6, Novocastra, diluted 1:50), smooth muscle actin (clone 1A4, Sigma Aldrich, St Louis, MI, USA, diluted 1:20,000), myeloperoxidase (Dako, diluted 1:2,000), synaptophysin (Dako, diluted 1:25), chromogranin-A (Dako, diluted 1:1,000), somatostatin (Bachem Peninsula Laboratories, Inc., San Carlos, CA, USA, diluted 1:800), serotonin (clone 5HT-H209, Dako, diluted 1:50) and CD117 (c-kit, Dako, polyclonal diluted 1:200). Immunostaining was performed according to the avidin biotin complex (ABC) method. Tissue sections were submitted to microwave oven heating prior to staining, except for CD117 staining. All steps were performed at room temperature, and diaminobenzidine was used as chromogen. Appropriate positive and negative controls were employed throughout.

Reverse transcription–polymerase chain reaction (RT-PCR) was used to detect the presence of EWS–ATF1 transcripts in paraffin-embedded tumour samples. RNA extraction and RT-PCR reaction were performed as previously described [9]. Briefly, PCR amplification was performed in duplicate using a 96-well plate (Applied Biosystems, Foster City, CA, USA) with a 50 µl final reaction mixture containing 300 nM each primer; 200 nM probe ATF1 or 50 nM probe beta2-microglobulin; 200 µM of an equal concentration of dATP, dCTP, dGTP, dTTP, dUTP; MgCl2 4 mM; 1.25 U Taq polymerase and 0.25 U Amperase UNG in a 1X Real-Time PCR Buffer containing a passive reference (Rox fluorochrome). Thermal cycling conditions were 2 min at 50°C for amperase activation, 10 min at 95°C for Taq polymerase activation, then 50 cycles of two PCR steps consisting of 15 s at 95°C and 60°C for 1 min. All reactions were performed in the ABI Prism 5700 Sequence Detection System (Applied Biosystems).

Primers and probe sequences were chosen with Primer Express software (Applied Biosystems). Primers were purchased from Eurobio (Les Ulis, France) and probes and QPCR Core kit containing Real-Time PCR Buffer, dNTP, MgCl2, amperase UNG and Taq polymerase were purchased from Eurogentec (Herstal, Belgium). Probes and primer sequences were as follows: forward primer EWS, 5′-CAT GAG CAG AGG TGG GCG-3′; reverse primer ATF1, 5′-CCC CGT GTA TCT TCA GAA GAT AAG TC-3′; probe ATF1, 5′-6-FAM-AGG AGG ACG CGG TGG AAT GGG-TAMRA-3′; forward primer beta2-microglobulin, 5′-TGA CTT TGT CAC AGC CCA AGA TA-3′; reverse primer beta 2-microglobulin, 5′-AAT CCA AAT GCG GCA TCT TC-3′; probe beta 2-microglobulin, 5′-6-FAM-TGA TGC TGC TTA CAT GTC TCG ATC CCA-TAMRA-3′.

Results

A 20-cm segment of ileum was resected, centered by a well-defined firm, circumferential mass, measuring 1.8 cm in maximal diameter. Cut section showed a whitish neoplasm that ulcerated the overlying mucosa and infiltrated the whole thickness of the intestinal wall, extending into the subserosa (Fig. 1.). The tumour cells, arranged in sheets and nests separated by fibrous septa of varying thickness, were polygonal, with a clear to eosinophilic cytoplasm. Tumour cell nuclei were oval, often vesicular with a single central prominent nucleolus (Fig. 2.). Neither multinucleate giant cells nor melanin pigment were observed. Mitoses were numerous (up to 32 mitoses per ten high-power fields; one high-power field, 0.174 mm^2). There was no necrosis, but vascular invasion was present. The mesenteric lymph node sampled was devoid of metastatic deposits. The peritoneal and omental biopsies taken in November 2003 showed the same neoplastic proliferation, although tumour cells were very scarce, embedded in an abundant collagenous matrix. These tumour cells could easily be overlooked in some samples.

Immunohistochemically, the tumour cells were diffusely positive for S-100 protein (Fig. 3.) and, focally, for tyrosinase and melan-A. They were negative for HMB45, keratins, EMA, smooth muscle actin, desmin, chromogranin-A, synaptophysin, CD117, CD31, CD34, CD45, CD20, CD3 and myeloperoxidase. Ultrastructurally, the tumour

Fig. 1 Diffuse infiltration of the mucosa and submucosa of the ileum by tumour cells. Hematoxylin and eosin stain ×50

cells showed rounded to oval nuclei with smooth or indented contours, open chromatin and one or two prominent nucleoli. The cytoplasm was moderately abundant and contained few organelles as well as immature melanosomes. EWS–ATF1 fusion transcripts representative of the t(12;22) (q13;q12) clear cell sarcoma translocation were detected using real-time RT-PCR (Fig. 4.). The fusion occurred between the exon 8 of EWS gene and the exon 4 of the ATF-1 gene. SYT-SSX1/2 fusion transcripts, representative of the t(X;18) synovial sarcoma translocation, were not detected.
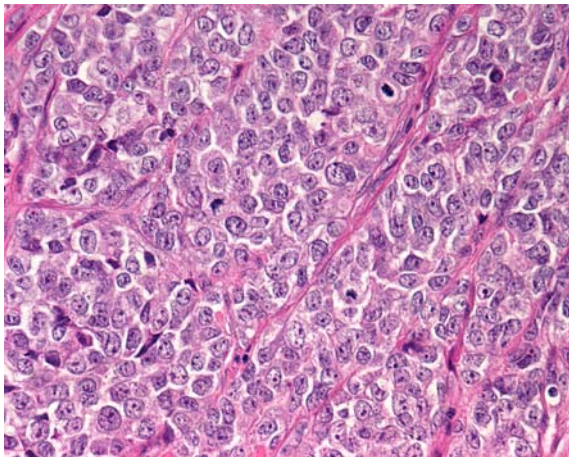

Fig. 2 Tumour cells are arranged in nests and fascicles separated by collagenous septa. Nuclei are round to oval, vesicular, with prominent nucleoli. Mitotic figures are numerous. Hematoxylin and eosin stain ×400

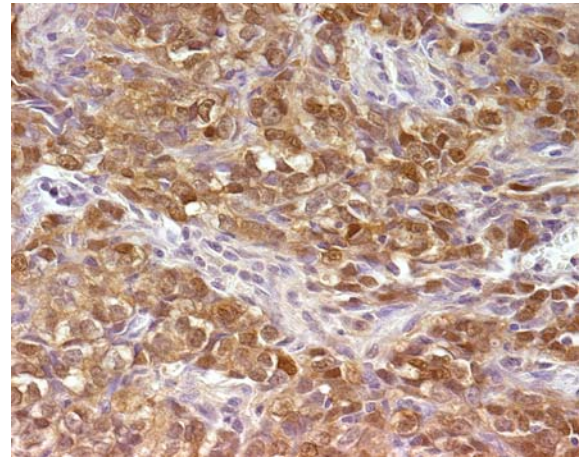**Fig. 3** Strong reactivity of clear cell sarcoma cells for S-100 protein ×400

Discussion

Described in 1965 by FM Enzinger [6], clear cell sarcoma of tendons and aponeuroses is a peculiar type of sarcoma which shows features of melanocytic differentiation. Clinically, it presents as a slow-growing tumour (median size, 2–5 cm) that mainly affects young adults (20–40 years). These tumours may be present for years before coming to clinical attention. The distal extremities, particularly the feet, ankles, knees, wrists and hands are the most common sites of tumour development [3, 6, 10, 11]. The lesions are almost always deep seated and intimately associated with tendons, aponeuroses and fascia.

Clear cell sarcoma is infrequent outside the limbs. Heretofore, six cases of clear cell sarcoma of the digestive tract have been reported [1, 4, 5, 7, 13, 15]. The salient clinicopathologic features of those cases, and the case under discussion, are summarized in Table 1. Males seem to be predominantly affected, in contrast to soft tissue lesions, which are divided equally between the sexes. Median age is 37 years (mean, 40 years), and median tumour size is 4 cm. Clear cell sarcoma of soft tissue has a poor prognosis, despite various treatment regimens [3, 6, 10, 11]. The tumour tends to metastasize to lungs but also to regional lymph nodes and bone. The 5-year survival rate is about 50–60%. Long term follow-up is mandatory because metastases can develop late in the course of the disease (10 years or more after diagnosis). Of the seven patients with a clear cell sarcoma of the gastrointestinal tract, two had metastatic disease at the time of diagnosis [13, 15], and an additional four developed liver metastases 2 (current case) to 24 [4] months post-operatively. Only two patients were disease-free at last follow-up, 11 [1] and 18 [5] months after diagnosis. Follow-up of patients 4 and 6 of Table 1 have been updated recently (see Acknowledgements).

In the digestive tract, clear cell sarcoma has the same histology and immunoprofile as in the soft tissues. The tumour presents as bundles or nests of pale staining to eosinophilic spindle cells separated by dense fibrous tissue septa. Tumour cells have prominent vesicular nuclei with

Fig. 4 Real-time PCR assay demonstrating the presence of EWS–ATF1 transcripts. *Graphs* show fluorescence emission data during each PCR cycle. This fluorescence is directly related to the quantity of PCR products. ATF1, graph of amplification of EWS–ATF1 transcripts. B2M, graph of amplification of beta-2-microglobulin transcripts, used as internal control
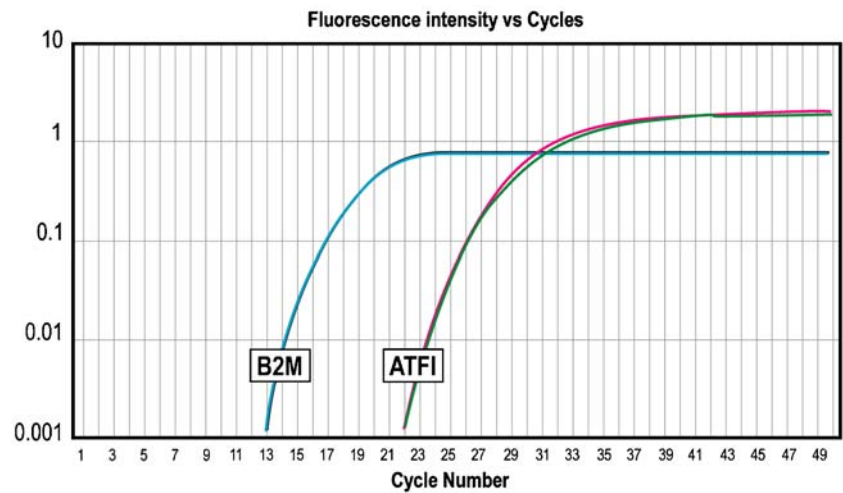
mostly a large, central, single nucleolus. Multinucleate giant tumour cells, a relatively common feature of clear cell sarcoma of soft tissues, seem to be rarer in the gastrointestinal tract (one of seven cases). Intracellular melanin accumulation is reported in half of the cases.

The tumour cells in clear cell sarcoma of soft tissues usually express melanocytic makers, including S-100 protein,

Table 1 Clear cell sarcoma of the gastrointestinal tract

Case	Author (reference)	Age (years)	Sex	Site	Size (cm)	Metastases at diagnosis	Molecular confirmation	Treatment	Adequacy of surgical resection	Outcome
1	Ekfors et al. [5]	38	M	Duodenum	3	No	No	Truncal vagotomy, pancreatico-duodenectomy, cholecystectomy	Complete	AWNED at 18 months
2	Donner et al. [4]	37	M	Ileum	6.5	No	Yes (karyotype)	Intestinal resection, along with tumour	Complete	Liver mets at 24 and 46 months, AWD at 46 months
3	Fukuda et al. [7]	74	M	Transverse colon	2	No	Yes (RT–PCR on liver met)	Partial colectomy+ regional lymphadenectomy	Complete	Single liver met at 9 months (resected), AWD at 15 months
4	Pauwels et al. [13]	30	M	Stomach	4	Yes (peritoneum, regional lymph nodes)	Yes (karyotype and FISH)	Palliative partial gastric resection	Complete	Pancreatic, liver and Douglas cul-de-sac mets at 4 months, AWD at 18 months
5	Zambrano et al. [15]	15	F	Jejunum	5	Yes (mesenteric lymph nodes)	Yes (karyotype)	Intestinal resection, along with tumour and enlarged lymph nodes + CT	Incomplete	Tumour progression at 12 months, DOD at 16 months
6	Achten et al. [1]	57	M	Jejunum	6.5	No	Yes (FISH)	Intestinal resection, along with tumour	Complete	AWNED at 11 months
7	Current case	35	M	Ileum	1.8	No	Yes (RT-PCR)	Intestinal resection, along with tumour	Complete	Liver mets at 2 months, DOD at 15 months

DOD Dead of disease, *AWD* Alive with disease, *AWNED* Alive with no evidence of disease, *mets* metastases, *CT* Chemotherapy, *RT–PCR* Reverse transcriptase–polymerase chain reaction, *FISH* Fluorescence in situ hybridization

HMB45, melan-A and the melanoma isoform of microphthalmia transcription factor (MITF-M) [2]. In the gastrointestinal tract, clear cell sarcoma cells consistently expressed vimentin and S-100 protein. HMB45 was expressed in three of seven cases only, and melan-A in two of four cases with available data. They were consistently negative for EMA, cytokeratins, muscle-specific actin, smooth muscle actin, desmin, CD34, chromogranin, synaptophysin, CD45 and CD117. Melanosomes at varying stages of development were visible ultrastructurally in all (6/6) cases with available data.

The diagnosis of clear cell sarcoma is a challenging one, especially if the tumour occurs in an unexpected site such as the gastrointestinal tract. In our case, the diagnosis was considered only at the time of the peritoneal tumour regrowth 8 months after the primary tumour had been removed. A metastatic melanoma was initially suspected, but an extensive work-up failed to find any primary elsewhere. The other differential diagnoses, successively contemplated and ruled out on the basis of tumour immunoprofile, included a poorly-differentiated neuroendocrine carcinoma (tumour cells were negative for keratins, EMA, chromogranin and synaptophysin), a malignant gastrointestinal stromal tumour (negativity for CD117 and CD34), a clear cell myomelanocytic tumour/PEComa (negativity for smooth muscle actin, strong positivity for S-100 protein), a paraganglioma (negativity for chromogranin and synaptophysin, diffuse positivity for S-100 protein), some sort of germ cell neoplasia (negativity for epithelial markers and placental alkaline phosphatase), a synovial sarcoma (negativity for epithelial markers, absence of detectable SYT-SSX fusion transcripts), a leiomyosarcoma (negativity for muscle markers) and a lymphoma (negativity for lymphoid markers). Although initially considered as improbable, the other diagnostic hypotheses that could not be definitely ruled out included epithelioid malignant peripheral nerve sheath tumour, sclerosing epithelioid fibrosarcoma and clear cell sarcoma. Ruling out the diagnosis of malignant peripheral nerve sheath tumour was effectively difficult on account of the negativity of the HMB45 marker. Nevertheless, the focal positivity of the melan-A marker was a helpful indication of the melanocytic differentiation of the tumour. The detection of EWS–ATF1 fusion transcripts by RT-PCR finally demonstrated that this lesion was a clear cell sarcoma. The lack of primary tumour elsewhere strongly suggests that this tumour arose primarily in the ileum and was not a metastatic deposit from a soft tissue tumour, as has sometimes been reported [12].

The relationship between clear cell sarcoma and melanoma has been controversial for a long time. Both neoplasms show morphologic features of melanocytic differentiation (melanin pigment), as well as immunohistochemical and ultrastructural features (melanosomes), and some authors refer to clear cell sarcoma as malignant melanoma of soft parts [3, 5]. Clear cell sarcoma differs, however, from cutaneous melanoma in several respects: it is almost always deep-seated, associated with tendons, tendon sheaths and aponeuroses, seldom involves the epidermis and behaves differently, metastasizing predominantly to the lungs. In addition, clear cell sarcoma bears the t(12;22) (q13;q12) translocation which is specific for this tumour type and had never been identified in cutaneous melanoma [2, 8]. This translocation, present in 75 to 90% of cases, fuses the activating transcription factor 1 (ATF1) gene on chromosome 12 with the EWS gene on chromosome 22 [2, 8].

Based on these differences, clear cell sarcoma was considered heretofore as a clinicopathologic entity distinct from melanoma. However, recent gene expression profiling data would suggest that this is not really the case. While examining a series of sarcomas including clear cell sarcomas, Segal et al. [14] found that clear cell sarcomas do not 'cluster' with other soft tissue sarcomas and may rather represent a distinct genomic subtype of melanoma. In addition to genes of melanocytic differentiation, this tumour was also shown to express fibroblast growth factor receptor 1 (FGFR1), a tyrosine kinase receptor involved in angiogenesis and tumour cell growth and migration, suggesting that the use of tyrosine kinase inhibitors might have some value in the treatment of patients with uncontrolled disease.

In conclusion, this case shows that clear cell sarcoma of soft tissues may occur as a primary neoplasm of the gastrointestinal tract. It also underlines the pivotal role of molecular techniques in the diagnostic approach when dealing with 'unusual' locations.

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