Case Report

Absence of 19 known hotspot oncogenic mutations in soft tissue clear cell sarcoma: two cases report with review of the literature

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Abstract: Clear cell sarcoma (CCS) of the tendons and aponeuroses is a rare soft tissue sarcoma that morphologically resembles cutaneous malignant melanoma but exhibits a distinct molecular profile. Gastrointestinal (GI) CCS is extremely rare. In this study, two cases of CCS were presented: (1) left thumb and (2) jejunum. Case 1 manifested the characteristic CCS morphology. Case 2 was morphologically unusual and difficult to diagnose. Immunohistochemically, the two cases of tumor cells were diffusely positive for S100, vimentin, NSE protein, focal expression of CgA, and CAM2.5 protein. In case 1, the tumor cells were diffusely positive for HMB45, focal expression of CD56, and melan A antigen. Reverse transcriptase-polymerase chain reaction (RT-PCR) results confirmed the presence of the EWS/ATF1 translocation (type 1) in the two cases. Then, we detected 19 hotspot oncogenes in the two cases. To the best of our knowledge, this study is the first to apply a high-throughput OncoCarta panel 1.0 and MassARRAY system to detect 238 known mutations in 19 hotspot oncogenes in soft tissue clear cell sarcoma. In this study, no mutations were observed in these hotspot oncogenes in the two cases.

Keywords: Clear cell sarcoma, mutations, fuse gene

Introduction

Clear cell sarcoma (CCS) of the tendons and aponeuroses is a rare tumor that occurs mainly in the soft tissue of the extremities of adolescents and young adults. Poor prognosis of CCS is associated with local recurrence and metastasis. Cases of CCSs in other anatomical sites, such as the bone, penis, kidney, and gastrointestinal (GI) tract, have been reported [1-6]. Clear cell sarcoma of the GI (CCS GI) is extremely rare. Few cases have been described in the GI tract, mimicking metastatic melanoma, alveolar soft part sarcoma, and other similar conditions. CCS GI is usually presented as an ulcerated mass accompanied by one or more of the following symptoms: obstruction; anemia; and GI tract bleeding. The most frequently affected site is the ileum; the stomach and the colon are also commonly affected sites.

The present study described two cases of CCS: (1) a 29-year-old male with CCS of the left thumb and (2) a 76-year-old male with CCS GI of the jejunum. We focused on the clinicopathological and molecular cytogenetic features and differential diagnoses. In this study, a high-throughput OncoCarta panel 1.0 and MassARRAY system was applied for the first time to detect 238 known mutations in 19 hotspot genes in the two cases. The Sequenom MassARRAY technology employs a mass spectrometry-based genotyping approach, which allows a more sensitive mutational analysis than a traditional Sanger sequencing technique [7]. The platform also efficiently employs the DNA extracted from archived formalin-fixed, paraffin-embedded (FFPE) samples. Furthermore, multiplexed PCR assays allow an efficient high-throughput screening of large tumor sample sets [8, 9]. To the best of our knowledge, this study is the first to apply a high-throughput OncoCarta panel 1.0 and MassARRAY system to detect 238 known mutations in 19 hotspot oncogenes in soft tissue clear cell sarcoma. Herein, we describe two soft tissue clear sarcomas and review the related literature.
Absence of mutations in soft tissue clear cell sarcoma

Two cases report

Case 1

A 29-year-old male exhibited a tumor in the left thumb. The patient observed a painless mass for one month after a three-year history of thumb injury. The patient was otherwise fit and well. Subsequent staging investigations showed no evidence of metastatic spread.

The results of macroscopic examination showed a tumor (2.2 cm×1.7 cm×0.9 cm) with a solid gray-white cut surface. Microscopy further revealed a lesion with a uniform appearance composed of packed nests of round to spindle cells with clear or eosinophilic cytoplasm surrounded by a fibrous framework (Figure 1A). The neoplastic cells contained centrally located round to ovoid vesicular nuclei that show prominent basophilic nucleoli. Some nucleated, large Touton-like tumor cells were also present (Figure 1B).

Case 2

A 76-year-old male suffered from bowel obstruction. Colonoscopy revealed a mass in the jejunum.

The results of macroscopic examination showed a tumor (2.5 cm×2.2 cm×1.5 cm) with a whitish-grey surface. Microscopically, the tumor revealed sheets and nests of medium-sized pale eosinophilic or clear cytoplasmic cells separated by fibrous septa (Figure 1C). The cells contained vesicular nuclei with small nucleoli (Figure 1D).

Immunohistochemically, the two cases of tumor cells were diffusely positive for vimentin (Figure 2A), S100 (Figure 2B), NSE protein, focal...
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expression of CgA, and CAM2.5 protein. By comparison, these cells were negative for cytokeratins (AE1/AE3), CD34, CD117, and desmin protein. In case 1, the tumor cells were diffuse-ly positive for HMB45 (Figure 2C), focal expression of melan A (Figure 2D), and CD56 antigen. In case 2, the tumor cells were negative for HMB-45, synaptophysin, hepatocyte, and TFE3 antigen.

Fusion gene expression was detected by one-step reverse transcriptase-polymerase chain reaction (RT-PCR) detection technology. The primer sequences were performed according to Cristina et al [10]. RT-PCR results confirmed the presence of the EWS/ATF1 translocation (type 1) in the two cases and case 2 was not diagnosed as alveolar soft part sarcoma (Figure 3).

To detect mutation, we used the MassARRAY system (Sequenom), which is a platform based on matrix-assisted laser desorption/ionization time-of-flight/mass spectrometry, and the OncoCarta mutation panel (Sequenom). The OncoCarta™ Panel v1.0 consists of 24 pools of primer pairs and 24 pools of extension primers. This system can detect 238 mutations in 19 genes, including ABL1, JAK-2, CDK4, KRAS, HRAS, NRAS, AKT1, AKT2, KIT, EGFR, MET, ERBB2, PDGFA, BRAF, FGFR1, FGFR3, PIK3CA, RET, and FLT3 (Table 1). The procedures were based on the manufacturer’s protocols with minor modifications. In the two cases, none of the 238 mutations was detected in the 19 genes.

Discussion

Clear cell sarcoma (CCS) of the tendons and aponeuroses is rare and comprises <1% of all soft tissue tumors [11]. CCS, first described by Enzinger in 1965, was originally termed “clear
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Cell sarcoma of tendon and aponeuroses based on the histological appearance and close association of CCS with other anatomical structures [12]. CCS has also been considered as the malignant melanoma of soft tissues because of the morphological, ultrastructural, and immunohistochemical similarities between the two types of tumors. Molecular genetic studies have identified biomarkers that differentiate CSS from malignant melanoma.

CCS commonly occurs in young to middle-aged adults of both genders, and this tumor involves the foot and ankle; less common sites affected by CCS include the knee, thigh, hand, penis, bone, kidney, GI tract, and retroperitoneum [13]. Many tumors are relatively small (<5 cm) at the time of diagnosis. Soft tissue-type CCS of the GI tract are extremely rare. To the best of our knowledge, 36 cases of CCS GI (including our cases) have been reported. These tumors occur more frequently in young adults (median age=46 years; range=13 years to 85 years). Common signs and symptoms include anemia, intestinal obstruction, or vague pain. In this type of tumor, lesions are developed usually in the ileum.

CCS is composed of compact nests and fascicles of pale fusiform or epithelioid cells; these structures are also surrounded by a delicate framework of fibrocollagenous tissue contiguous with the adjacent tendons and aponeurosis; the overall structure forms a vaguely organoid pattern and occasionally shows a pseudoalveolar pattern, reminiscent of an alveolar soft part sarcoma [12]. The cells of CCS range from epithelioid to spindle; many cells contain eosinophilic to amphophilic cytoplasm. The cytoplasm usually contains clear cells, which compose a neoplastic cell population. Such cells contain indistinct cell borders and uniform round nuclei with prominent macronucleoli. Multinucleated, large Touton-like tumor cells are frequently present and may be a valuable diagnostic clue. Mitotic figures are few in number and necrosis is uncommon. Case 1 is a typical morphological characteristic, and Touton-like tumor large cells were identified. CCS GI has a slightly different histology from its soft tissue counterpart. CCS GI cases demonstrate a high mitotic rate, necrosis, and pleomorphism [14]. Case 2 was morphologically atypical, with less prominent, clear, and medium-sized cells. These unusual features impede diagnostic protocols, but molecular studies helped reach the correct diagnosis. Case 2 which identified at first as an alveolar soft part sarcoma was later diagnosed as a CCS GI by one-step RT-PCR because of the presence of EWS-ATF1 fuse gene.

Figure 3. EWS-ATF1 chimeric transcripts were detectable in paraffin-embedded CCS tumors. A: RT-PCR products from the actin gene used as internal control. B: Lane 1 is from alveolar soft part sarcoma obtained by one-step RT-PCR. PCR products of 138 bp correspond to type 1 ASPL-TFE3 fusion gene. Lane 2, case 2, lane 3, synovial sarcoma tissue. C: Lanes 1 to 3 are from CCS tumors obtained by one-step RT-PCR. PCR products of 185 bp correspond to type 1/2 EWS-ATF1 fusion gene. Lane 4, synovial sarcoma tissue; M, 50 bp DNA ladder; N, negative control. (Line 1: case 1; lines 2 to 3: case 2 was added EWS-ATF1 Type 1/2 primer pair and EWS-ATF1 Type 3 primer pair, respectively).
**Table 1. 238 mutation across all 19 genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Reference sequence</th>
<th>Chromosome region</th>
<th>Mutation type</th>
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<tr>
<td>ABL1</td>
<td>NM_005157</td>
<td>9q34.1</td>
<td>G250E, Q252H, Y253F, E255V, D276G, F317L, M381T, E355G, F359V, H396R</td>
</tr>
<tr>
<td>AKT1</td>
<td>NM_005163</td>
<td>1q42.32</td>
<td>V461L, P388T, L357T, E319G, V167A, Q43X, E17del</td>
</tr>
<tr>
<td>AKT2</td>
<td>NM_001626</td>
<td>9q13.1-q13.2</td>
<td>S302G, R371H</td>
</tr>
<tr>
<td>CDK4</td>
<td>NM_000075</td>
<td>12q14</td>
<td>R24C, R24H</td>
</tr>
<tr>
<td>FGFR1</td>
<td>NM_023110</td>
<td>5p11.2-p11.1</td>
<td>S125L, P252T</td>
</tr>
<tr>
<td>FGFR3</td>
<td>NM_000142</td>
<td>4p16.3</td>
<td>G370C, Y373C, A391E, K650Q/E, K650T/M</td>
</tr>
<tr>
<td>FLT3</td>
<td>NM_004119</td>
<td>13q12</td>
<td>i836del, D835H/Y</td>
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<tr>
<td>HRAS</td>
<td>NM_003424</td>
<td>11p15.5</td>
<td>G12V/D, G13C/R/S, Q61E/K, Q61L/R/P, Q61H/H</td>
</tr>
<tr>
<td>JAK2</td>
<td>NM_004972</td>
<td>9p24</td>
<td>V617F</td>
</tr>
<tr>
<td>MET</td>
<td>NM_000245</td>
<td>7q31</td>
<td>R970C, T992I, Y1230C, Y1235D, M1250T</td>
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<tr>
<td>NRAS</td>
<td>NM_002524</td>
<td>1p13.2</td>
<td>G12V/A/D, G12C/R/S, G13C/R/S, A18T, Q61E/K, Q61L/R/P, Q61H/H</td>
</tr>
<tr>
<td>PDGFR</td>
<td>NM_006206</td>
<td>7p22</td>
<td>V561D, T674I, F808L, D846Y, H876D, D1071N, D842_H845del, I843_D846del,</td>
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<tr>
<td>PIK3CA</td>
<td>NM_006218</td>
<td>3q26.3</td>
<td>R88Q, N345K, C420R, P538R, E542K, E545K, Q546K, H701P, H1047R/L,</td>
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<tr>
<td>RET</td>
<td>NM_020975</td>
<td>10q11.2</td>
<td>C634R, C634W, C634Y, E632_L633del, M918T, A664D</td>
</tr>
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</table>

Immunohistochemical study has shown a strong S100 protein expression in 100% of the cases, with expression of HMB-45, Melan-A, and MiTF in 97%, 71%, and 81% of the cases, respectively [15]. CCS is negative for myogenic differentiation markers and shows a negative expression of the epithelial markers, CD34 and CD117 are generally negative. All of the cases of CCS GI are S100 protein positive, but other specific melanocytic markers (HMB45 and melan-A) are negative in previously reported cases; this result is similar to our case, which was positive for S100 and negative for HMB45 and melan-A.

Molecular genetic studies have shown that CCS is associated with the reciprocal translocation t (12; 22) (q13; q12) in > 90% of the cases, resulting in the fusion of the EWSR1 gene located at 22q12 and the ATF1 gene located at 12q13 [16-18]. EWSR1-ATF1 fuse gene was observed in our cases. To date, four types of EWSR1-ATF1 fusion transcripts have been identified, in which the fusions between exon 8 of EWSR1 and exon 4 of ATF1 represent the most common types [19]. Davis et al. showed that the EWSR1-ATF1 fusion protein can bind to and activate melanocyte-specific MiTF; in the presence of SOX10, this process results in the growth/survival of CCS cells [20]. Segal et al. conducted gene expression profiling studies and demonstrated that CCS can form clusters with melanomas, inducing the expression of various genes associated with melanocytic differentiation, including MiTF, SOX10, ERBB3, and FGFR1 [21]. Antonescu et al. identified EWSR1-CREB1 gene fusions in three CCS of the GI tract, and this same gene fusion has been observed at EWSR1-ATF1.
identified in a small number of otherwise conventional CCS of soft tissues [22]. These authors found a lack of melanocytic differentiation in all three EWS-CREB1-positive CCS cases. This result suggested that these tumors may have lost the ability to differentiate along the melanocytic lineages. EWS-CREB1-positive CCS cases in the peripheral soft tissue have been reported, suggesting that the EWS-CREB1 translocation is not specific to the GI tract site [19, 23].

Previous studies showed a lack of BRAF mutation, which is commonly observed in melanoma. Robert et al. showed that one of the 22 CCS harbored a BRAF mutation and two mutations of NRAS do not overlap with the BRAF mutation [24]. Panagopoulos et al. showed that none of the eight CCS harbors any mutation in exon 11 or 15 of the BRAF gene [25]. Gambichler et al. reported for the first time a female patient with CCS exhibiting both EWSR1-ATF1 fusion transcripts and hereditary homozygous point mutations in introns 11 and 16 of the KIT gene [26]. In the present study, the mutation of the 19 oncogenes in CCS was not detected.

The differential diagnoses of CCS of the tendons and aponeuroses as well as CCS GI include paraganglioma-like dermal melanocytic tumor, alveolar soft part sarcoma, clear cell myomelanocytic tumor, malignant melanoma, malignant peripheral nerve sheath tumor, synovial sarcoma, GI stromal tumor, and PEComa. Case 2 had considered as alveolar soft part sarcoma, but the result do not support it by RT-PCR. Studies have further suggested that malignant melanoma in unusual anatomical sites, most notably the GI tract, may represent CCS. For instance, Covinsky et al. investigated a series of 20 patients with GI tumors diagnosed as malignant melanoma and determined the EWS-ATF1 fusion transcript [27]. Results showed that two cases (10%) harbored the EWS-ATF1 fusion transcript and fluorescence in situ hybridization confirmed the presence of t(12; 22) in both cases. One of the two positive tumors developed in a patient who did not have a history of cutaneous melanoma; the other positive tumor developed in a patient with a remote history of vulvar melanoma. Therefore, a primary visceral CCS should not be automatically ruled out of the differential diagnosis even in patients with a history of malignant melanoma. Lyle et al. used cytogenetic studies in seven cases primarily diagnosed as melanoma of the GI tract. Four cases had no prior history of melanoma but revealed the EWS-ATF1 fusion transcript, suggesting a diagnosis of CCS rather than malignant melanoma [14]. Such difficulties in diagnosis have been resolved with molecular studies.

The therapy administered to patients with CCS includes wide surgical excision and adjuvant radiotherapy. However, approximately 30% of patients with CCS in some series suffered from metastases. Metastases of CCS commonly occur in the lungs, bones, and lymph nodes. Some series of CCS have reported 5-, 10-, and 20-year survival rates of 47% to 67%, 33%, and 10%, respectively [18, 28]. Surgery remains the standard treatment for CCS GI. This tumor has a worse prognosis than the soft-tissue tumor. Many patients die within two years of diagnosis [14].

In summary, CCS is a rare soft tissue tumor with distinctive morphological features and melanocytic differentiation. However, CCS GI can often be misdiagnosed. Molecular techniques exhibit an important function in dealing with such tumors at unusual locations. CCS is caused by fusions of the EWS gene with either the ATF1 gene or the CREB1 gene. Furthermore, mutation of the genes involved in CCS is rare.

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Disclosure of conflict of interest

None.

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