Primary clear cell sarcoma of the femur: a unique case with RT-PCR and direct sequencing confirmation of EWSR1/ATF1 fusion gene

Yuta Kubota1, Kazuhiro Tanaka1*, Masanori Hisaoka2, Tsutomu Daa3, Tatsuya Iwasaki1, Masanori Kawano1, Ichiro Itonaga1 and Hiroshi Tsumura1

Abstract

Background: It is very rare for clear cell sarcomas (CCS) to arise in the bone. During diagnosis, it is important to distinguish primary CCS of bone from bone metastasis of melanoma because this difference fundamentally changes the therapeutic options. Recently, characteristic fusion genes of CCS have been detected using reverse transcription polymerase chain reaction (RT-PCR) or direct sequencing which allowed to distinguish CCS from melanoma. However, there was no study applying these analyses with positive results. In this case, we describe the use of fusion gene analysis to diagnose a primary CCS of the bone.

Case presentation: A 36-year-old male presented with a four-months history of left knee pain. Magnetic resonance imaging showed a lesion in the left femoral medial epicondyle. Histological examination of the biopsy specimen revealed proliferating oval or rounded cells. These cells had clear cytoplasm arranged in fascicles or compact nests with frequent deposits of brown pigment. Furthermore, immunohistochemistry analysis revealed that tumor cells were positive for S-100 protein, HMB-45, Melan-A, and SOX10. It stained negative for CD34 and BRAF v600e. Conclusively, detection of the EWSR1/ATF1 fusion gene using RT-PCR and direct sequencing confirmed that the lesion was a primary CCS of the bone. Wide-margin resection and reconstruction with a tumor endoprosthesis were performed.

Conclusions: Herein, we diagnosed a rare case of primary CCS of the bone by detecting EWSR1/ATF1 fusion gene using RT-PCR and direct sequencing. Since fluorescence-in situ hybridization (FISH) and RT-PCR could show false positive by mainly due to technical problems, it is better to perform direct sequencing to confidently diagnose the tumor as a primary CCS especially at very rare site such as bone.

Keywords: Clear cell sarcoma, Primary bone tumor, Melanoma, Fusion gene, Direct sequencing, Reverse transcription polymerase chain reaction
Background

Clear cell sarcoma (CCS) was first described by Enzinger in 1965 [1]. It is a malignant soft tissue tumor arising from tendons and aponeuroses. CCS has limited treatment options because effective radiotherapy and chemotherapy regimens have not been established for this type of tumor. The five-year survival rate of CCS is 47% and the 10-year survival rate is only 36%, [2] demonstrating the aggressive nature of this tumor. CCS is rare, and accounts for less than 1% of soft-tissue sarcomas [1]. It is very rare for CCS to be localized in the bone. To our knowledge, there are currently only 13 reports in English describing primary CCS of the bone (Table 1) [3–15]. The first primary CCS of the bone was reported in the right ulna by Yokoyama et al. [3]. They diagnosed the neoplasm as CCS on the basis of both histopathological and immunohistochemical features including the presence of S-100 protein, HMB-45, and vimentin. Their findings were consistent with those of both melanoma and CCS, but they seemed to be more closely related to CCS. However, their examination findings were as a result of techniques that did not involve cytogenetic analysis, which was not commonly used and the appropriate method for which had not been established until then; therefore, they could not definitely rule out melanoma as the diagnosis [3, 16]. Panagopoulos et al. [17] examined EWS/ATF1 fusion genes in CCS of soft tissue using reverse transcription polymerase chain reaction (RT-PCR) amplification and sequence analysis in 2002. Coindre et al. [18] detected EWS/ATF1 fusion transcripts in 38 paraffin-embedded CCS tissues out of 41 interpretable samples (93%) in 2006. This study showed that RT-PCR on paraffin-embedded tissues was useful for distinguishing CCS from melanoma. Furthermore, RT-PCR demonstrated that EWSR1/CREB1 fusion gene was another fusion gene of CCS [19]. The EWS/ATF1 fusion gene has also been detected using fluorescence in-situ hybridization (FISH) in bone CCS samples [10, 11, 15]. In contrast, there are no reports confirming whether the EWS/ATF1 or EWSR1/CREB1 fusion genes can be detected using direct sequencing. In this article, we report a case of CCS in the femur with the EWS/ATF1 fusion gene detected using direct sequencing and RT-PCR.

Case presentation

A 36-year-old male presented with a four-months history of pain in the left knee. His medical history was negative for injury, among others, as the underlying cause. Physical examination revealed a tenderness at the left femoral medial epicondyle but no swelling, redness or heat around the joint. Furthermore, there was no instability or joint contracture. Radiological examination showed an osteolytic lesion in the femoral medial epicondyle with a partially destructed cortex (Fig. 1). There was no sclerotic rim or periosteal reaction. Computed tomography (CT) revealed a 38 × 19 × 17 mm osteolytic lesion that partially destroyed and thinned the cortex (Fig. 2). There was no calcification in the mass. Magnetic resonance imaging (MRI) showed that the lesion had the most hypointense area, including both hyperintense and isointense areas heterogeneously on T1-weighted images and had hyperintense areas with septal walls on T2-weighted images. Hyperintense signal areas were observed at the femoral articular surface without extraosseous soft tissue signal change (Fig. 3). Considering all images, giant cell tumor (GCT), osteosarcoma or chondrosarcoma was suspected.

Open biopsy was performed for a definitive diagnosis. Histological examination revealed that oval or rounded cells were proliferating. These cells had clear cytoplasm arranged in fascicles or compact nests with frequent deposits of brown pigment (Fig. 4). For a more accurate evaluation of the tumor type, immunohistochemistry was performed using a panel of markers. This analysis revealed that tumor cells were positive for S-100 protein, HMB-45, Melan-A, and SOX10. It stained negative for CD34 and BRAF v600e. Considering these features [20], the main differential diagnoses were clear cell sarcoma and melanoma. RT-PCR and direct sequencing are the molecular techniques that help differentiate between the different EWS/ATF1 fusion types and breakpoints [19–21]; therefore, we used both the methods. We examined the tumor for EWSR1/ATFI transcripts using RT-PCR (Fig. 5) and direct sequencing on the paraffin-embedded tissue (Fig. 6). The tumor was found to be positive for the EWSR1/ATFI fusion gene. Thus, we diagnosed the patient with primary clear cell sarcoma of the bone.

As CCS of the bone is so rare, extensive investigations were conducted to search for other metastases or primary tumors. The patient’s skin was checked by a dermatologist, but no melanoma was found. Whole-body CT and positron emission tomography (PET)/CT were performed and showed no other metastatic dissemination.

Based on our investigations, we concluded that this was a primary CCS localized to the bone. Because CCS does not usually respond to radiotherapy or chemotherapy [22], adjuvant therapy was not applied to this patient. A wide-margin resection and reconstruction with an endoprosthesis were performed. We presumed that the tumor invaded the intra-articular area as the CT image showed a partially destroyed femoral medial epicondyle cortex. Accordingly, we performed an extra-articular knee resection including the suprapatellar bursa and joint capsule. The resected specimen had a pathologically confirmed negative margin and the tumor spread extraskeletally at the femoral medial epicondyle but not into the soft tissue around the capsule. Nine months after surgery, no local recurrence or metastases were detected.
| Author          | Age/Sex | Location                        | General screening for primary lesion including melanoma                                                                 | Immunohistochemistry                                                                 | Genetic analysis                                                                 | Treatment                                                                 | Follow up              |
|-----------------|---------|---------------------------------|-------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------------------|
| Yokoyama et al. | 33/F    | Right ulna                      | Various radiograph                                                                                                       | Positive: S-100, HMB-45, vimentinNegative: desmin, keratin                          | Not performed                                                                  | Neoadjuvant: Ifosfamide, cisplatin and doxorubicinWide margin resection    | CDF, 65 months after surgery |
| Brekke et al.   | 62/F    | Right first metatarsal          | 99mTc MDP bone scan                                                                                                      | Positive: S-100 protein, vimentinNegative: HMB-45, cytokeratin (AE1/AE2)             | Not described                                                                  | Syme’s amputation                                                       | CDF, 15 months after surgery |
| Gczer et al.    | 18/M    | Left ninth rib                 | CT, 99mTc MDP bone scan, bone marrow aspiration                                                                      | Positive: S-100 protein, HMB-45                                                    | Not described                                                                  | Neoadjuvant: Performed but details were not describedWide resection      | CDF, 55 months after treatment  |
| Inacka et al.   | 55/M    | Right radius                    | CT, skin checked by dermatologist, ophthalmofundoscopy, upper gastrointestinal endoscopy, Bone scintigraphy with 99mTc-HMDP | Positive: S-100 protein, HMB-45, vimentinNegative (method not described)         | Negative (method not described)                                                | Neoadjuvant: cisplatin and doxorubicinTotal tumor resection              | CDF, 18 months after primary surgery  |
| Choi et al.     | 48/F    | Right first metatarsal          | CT, whole body radiosotope scan                                                                                         | Positive: S-100, HMB-45Positive: TFE3Negative: HMB-45, MART1, cytokeratin, epithelial membrane antigen | Not performed                                                                  | Below-knee amputation of the right leg                                       | DOD, 20 months after surgery |
| Hersekli et al. | 28/M    | Left ninth rib                 | CT, skin checked by dermatologist, Bone scintigraphy with 99mTc-HMDP and 67Ga                                            | Positive: S-100, HMB-45, NSE, EMA, cytokeratin, myosinNegative (method not described) | FISH: positive                                                                 | Total tumor resection adjunctive radiotherapy of 500 cGy                    | CDF, 33 months           |
| Kazakos et al.  | 61/M    | Left scapula                    | Bone scanning, CT                                                                                                       | Positive: S-100, HMB-45, TFE3Negative: HMB-45, MART1, cytokeratin, epithelial membrane antigen | FISH: positive                                                                 | Wide resectionAdjuvant: Ifosfamide, vincristine and epirubicin             | DOD, 15 months after adjuvant chemotherapy                        |
| Rocco et al.    | 53/M    | Sternum                         | CT, PET, bone scans                                                                                                      | Positive: S-100, TFE3Negative: HMB-45, MART1, cytokeratin, epithelial membrane antigen | FISH: positive                                                                 | Wide resection                                                          | Not described                        |
| Zhang et al.    | 25/M    | Sacrum                          | CT, MRI (thorax and abdomen), Bone scintigraphy with 99mTc-HMDP, skin, oral, anal fundus oculi checked                   | Positive: S-100, HMB-45, Melan-ANegative: EMA, CD117, CD34, MSA, GFAP, PGM-1, RCC, MB-1, AE1/AE3, CEA, Des, and HMB-1 | FISH: positive                                                                 | Curettage and debridement                                                   | AWD, 9 months after surgery                                    |
| Liu et al.      | 20/F    | Proximal right humerus          | Bone scintigraphy with 99mTc-HMDP                                                                                                | Positive: S-100, HMB-45                                                             | FISH: negative                                                                  | Neoadjuvant: cisplatin and doxorubicinTotal tumor excision-alkoholization-implantation Adjuvant: cisplatin, doxorubicin and methotrexate | CDF, 1 year after treatment    |
| Nakayama et al. | 81/M    | Left pubic bone                 | CT, PET/CT, Bone scintigraphy with 99mTc-HMDP, 99mTc-citrate-Bone scintigraphy, skin checked by dermatologist           | Positive: S-100, HMB-45, Melan-ANegative: cytokeratin, epithelial membrane antigen | FISH: negative, direct sequencing BRF mutation: negative                      | Dimethyl triazeno imidazole carboxamide, 1-(4-amino-2-methyl-5-pyrimidinyl)-methyl-3-[2-chloroethyl]-3-nitrosourea hydrochloride and vincristin, radiotherapy | DOD                      |
| Licata et al.   | 42/M    | Left third metatarsus           | Bone scan                                                                                                               | Positive: S-100, HMB-45, Melan-ANegative: cytokeratin, epithelial membrane antigen | Not described                                                                  | Transtibial amputation                                                     | Not described                        |
| Xu et al.       | 61/M    | Right calcaneus                 | Bone scintigraphy with 99mTc-HMDP                                                                                                | Positive: S-100, vimentin, melanANegative: HMB45, NSE, SMA, Negative (method not described) | Positive (method not described)                                                | Below-knee amputation                                                     | CDF, at the 6 months follow-up                  |
**Discussion**

The first report resembling primary malignant CCS of bone was presented by Yokoyama et al. [3] in 1996. In this first case, they did not collect material for genetic analysis and therefore, could not definitely diagnose CCS of bone without verification of the t(12;22) translocation; instead they suggested a diagnosis of either melanoma or CCS of the bone [3, 16]. It has been reported that t(12;22)(q13-14; q12) translocation was detected in 62.5 %-70 % of CCS cases, and that the tumors negative for it required histopathological diagnosis [17, 23].

It is very important to distinguish primary CCS of the bone from bone metastasis of melanoma as they share many common histopathological features; however CCS of the bone is very rare. Compared to melanoma, CCS typically lacks significant nuclear pleomorphism [24]. CCS is also usually strongly positive for HMB-45, S-100, Melan-A, MITF, and negative for smooth muscle actin, desmin and keratin [8, 19, 24, 25]. Melanoma, on the other hand, is typically positive for c-kit, CD68, S-100, HMB-45, Melan-A, throsinase, and vimentin, and negative for smooth muscle actin, desmin, chromogranin, and epithelial membrane antigen [24, 25]. However, each case of CCS varies, and the overlapping staining profiles between CCS and melanoma suggest that immunohistochemical examination alone cannot discriminate between these tumors. We used S-100 protein, HMB-45, Melan-A, SOX10, CD34, and BRAF v600e for histopathological examination. As mentioned above, S-100 protein, HMB-45, and Melan-A are usually positive in CCS and melanoma [24]. Positive staining for SOX10 is suggestive of CCS because EWS/ATF1 activates melanocyte-specific MITF-SOX10 expression [26]. BRAF was reported to be rare in CCS [27], but positive in clear cell melanoma and melanoma [28]. Similarly, CCS is negative for CD34 [19]. CD34 is useful to distinguish CCS from Epithelioid neoplasms with SMARCB1 and SMARCA4 deficiency or Mesenchymal tumors with NTRK fusions which are positive for CD34 [29].

Since 1996, diagnoses of primary CCS of the bone have been supported by further evidence including: (1)
whole body screening tests such as PET-CT that showed no melanoma, [10, 13] (2) no previous history of melanoma, [7, 9] and (3) patients survived much longer than those with bone metastasis of melanoma [3, 6].

Meanwhile, EWSR1/ATF1 and EWSR1/CREB1 transcript fusions have been identified in CCS. [30, 31] Hisaoka et al. [19] reported that 33 CCSs analyzed using RT-PCR were positive for transcripts of either EWSR1/ATF1 type 1,2,3,4, or EWSR1/CREB1. The case we have presented here was positive for the type 1 fusion transcript of EWSR1/ATF1, consisting of the forward EWSR1/exon 8 and reverse ATF1-exon 4.

To our knowledge, 13 cases of primary CCS of the bone have been reported in the literature, as shown in Table 1. The first genetic analysis was conducted by Rocco et al. in 2009 [10]. Although they confirmed rearrangement of the EWS gene localized on chromosome 22q12 using FISH, fusion transcripts were not detected. When seven of the 13 known cases of primary CCS of the bone were assessed for chromosomal translocation by cytogenetic analysis including FISH, only three cases were positive [10, 11, 15]. Of the four negative cases, the one reported by Inaoka et al. [6] was deemed a primary CCS of bone rather than melanoma as their patient survived for more than 18 months, which is significantly longer than the mean survival of 4.7 months for patients with melanoma [32]. Two other cases by Herskli et al. [8] and Liu et al. [12] concluded on primary CCS of the bone based on morphological and immunoenzymatic features only. The last case described by Nakayama et al. [13] reported that whole body screening (CT, PET/CT), bone scintigraphy, and a skin check by a dermatologist were all negative for primary melanoma; therefore, their case was diagnosed as primary CCS of the bone. All cases describing the method of cytogenetic analysis used FISH. Nakayama et al. [13] also performed BRAF (exons 11 and 15) mutation analysis using direct sequencing.

However, there were possibilities that RT-PCR and FISH produce false positive results due to technical problems although it might be rare, [33–38] and that not all the best probes and primers for known fusion genes with optimal conditions were used in all 13 cases. Thus, we are still unable to definitively conclude that all thirteen reported cases were primary CCS of the bone.

Furthermore, dual-color, break-apart FISH using break-apart rearrangement specific for EWSR1 gene on 22q13 is usually used for distinguishes clear cell sarcoma of soft tissue from melanoma, [39] but using the probe does not suggest fusion types or breakpoints of EWS gene rearrangement [19–21]. Moreover, CCS cases with EWS/ATF1 fusion gene but not translocation t(12;22)(q13;q12-13) have been reported [17]. FISH test might not be appropriate for these cases. On the other hand, RT-PCR amplification carryover contamination leads to false-positive PCR reactions [40], and positive and negative controls for all fusion types should be prepared for reliable results. Nevertheless, there was no report using such controls in the
Therefore, it is better to perform direct sequencing to confidently diagnose the tumor as a primary CCS especially at very rare site such as bone.

Here, we have reported the first case of primary CCS of the bone diagnosed by detection of the fusion gene using RT-PCR and direct sequencing.

In this case, we confirmed there were no other primary tumors using MRI and whole-body CT scans. According to Gonzaga et al., [41] 13 of the 489 cases of CCS of soft tissue (3%) had bone metastasis at diagnosis. These 13 cases were classified as American Joint Committee on Cancer (AJCC) stage IV and their probability of 5-year survival was 15%, and median overall survival was 8.9 months. Kawai et al. [2] showed that the cases which first metastasis site was bone were three out of the 52 cases of CCS (5.8%) and the median time to metastasis was 13 months. Tumors > 5 cm had a
significantly higher rate of metastases (79%) than smaller tumors (48%). Large CCS primary tumors consistently lead to metastases. Lucas et al. [42] reported that all 12 cases with tumors larger than 5 cm developed metastases. As metastases are usually derived from larger tumors (greater than 5 cm) and bone metastases from CCS are rare, it is unlikely that a primary CCS would have been missed. Additionally, our patient had no local recurrence or metastasis for nine months after surgery. Together this allowed us to obtain a diagnosis of primary CCS of the bone.

In conclusion, to our best knowledge, this is the first case of primary CCS of the bone definitively diagnosed by detecting the fusion gene using RT-PCR and direct sequencing, and the first primary CCS of the bone arising in the femur. Because primary CCS of bone is exceedingly rare, it is important for definitive diagnosis to perform the most sensitive and accurate tests to confirm the presence of the characteristic fusion genes in order to obtain a definitive diagnosis.

Abbreviations
CCS: Clear cell sarcoma; CT: Computed tomography; FISH: Fluorescence in situ hybridization; MRI: Magnetic resonance imaging; PET: Positron emission tomography; RT-PCR: Reverse transcription polymerase chain reaction; CDF: Continuous disease free; DOD: Died of disease; AWD: Alive with disease
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Author contributions

The conception and design of the study: YK, KT and HT. Drafting the manuscript: YK and KT. Analysis and interpretation of data: YK, KT, MK, MH and TD. Critical revision of the manuscript for important intellectual content: MH, TD and HT. Surgery performance: KT, MK, IT and II. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures performed in this case report involving the patient were in accordance with the ethical standards of the local ethics committee.

Consent for publication

Written informed consent was obtained from the patient for publication of the case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal. The design of the work conforms to standards currently applied in the country of origin.

Competing interests

The authors declare that they have no competing interests.

Author details

1. Department of Orthopaedic Surgery, Faculty of Medicine, Oita University, 1-1 Iidaigaoka Hasama, 879-5593 Yufu City, Oita, Japan.
2. Department of Pathology and Oncology, School of Medicine, University of Occupational and Environmental Health, 1-1 Ieigaoka, Yahatanishi-ku, 807-8555 Kitakyushu, Japan.
3. Department of Diagnostic Pathology, Faculty of Medicine, Oita University, 1-1 Iidaigaoka Hasama, 879-5593 Yufu City, Oita, Japan.

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References

1. Enzinger FM. Clear-cell sarcoma of tendons and aponeuroses. An analysis of 21 cases. Cancer. 1965;18:1163–74.
2. Kawai A, Hosono A, Nakayama R, Matsumine A, Matsumoto S, Ueda T, et al. Clear cell sarcoma of tendons and aponeuroses: A study of 75 patients. Cancer. 2007;109:10–16.
3. Yokoyama R, Mukai K, Hirota T, Beppu Y, Fukuma H. Primary malignant melanoma (clear cell sarcoma) of bone: report of a case arising in the ulna. Cancer. 1996;77:2471–5.
4. Brekke MK, Northcote K, Temple WE. Clear cell sarcoma in the first metatarsal. An unusual case. J Am Podiatr Med Assoc. 1998;88:457–61.
5. Gelcer RK, Wenger DE, Wold LE. Primary clear cell sarcoma of bone: a unique site of origin. Skeletal Radiol. 1999;28:240–3.
6. Inaoka T, Takahashi K, Tandai S, Miyokawa N, Abe Y, Matsuno T, et al. Primary clear cell sarcoma of malignant melanoma in the right radius. Skeletal Radiol. 2003;32:594–7.
7. Choi JH, Gu MJ, Kim MJ, Bae YK, Choi WH, Shin DS, et al. Primary clear cell sarcoma of bone. Skeletal Radiol. 2003;32:598–602.
8. Herseki MA, Ozkoc G, Bircan S, Alpinar S, Ozalay M, Tuncer I, et al. Primary clear cell sarcoma of rib. Skeletal Radiol. 2005;34:167–70.
9. Kazakos CJ, Galanis VG, Giatromanolaki A, Verettes DA, Sviridis E. Clear cell sarcoma of the scapula. A case report and review of the literature. World J Surg Oncol. 2006;4:48.
10. Rocco G, de Chiara AR, Fazio F, Scognamiglio F, La Rocca A, Apice G, et al. Primary giant clear cell sarcoma (soft tissue malignant melanoma) of the sternum. Ann Thorac Surg. 2009;87:1927–8.
11. Zhang W, Shen Y, Wan R, Zhu Y. Primary clear cell sarcoma of the sacrum: a case report. Skeletal Radiol. 2010;40:533–9.
12. Liu X, Zhang H, Dong Y. Primary clear cell sarcoma of humerus: case report. World J Surg Oncol. 2011;9:163.
13. Nakayama S, Yokote T, Iwaki K, Aioki T, Miyoshi T, Hirata Y, et al. A rare case of primary clear cell sarcoma of the pubic bone resembling small round cell tumor: an unusual morphological variant. BMC Cancer. 2012;12:538.
14. Licitra L, Fenga D, Speciale G, Rosa MA. Clear cell sarcoma of metatarsus. Folia Medica. 2014;56:271–4.
15. Xu Z, Suo H, Zhang Y, Feng W. Primary clear cell sarcoma of the calcaneus: Report of a rare case and review of the literature. Orthopade. 2019;48:232–8.
16. Yokoyama R. Primary clear cell sarcoma of bone. Skeletal Radiol. 2000;29:302.
17. Panagopoulos L, Mertens F, Débiec-Rychter M, Isaksson M, Limon J, Kardas I, et al. Molecular genetic characterization of the EWS/ATF1 fusion gene in clear cell sarcoma of tendons and aponeuroses. Int J Cancer. 2002;99:560–7.
18. Coindre JM, Hostein I, Terrier P, Bouvier-Labat C, Collin F, Michels JJ, et al. Diagnosis of clear cell sarcoma by real-time reverse transcription-polymerase chain reaction analysis of paraffin embedded tissues: clinicopathologic and molecular analysis of 44 patients from the French sarcoma group. Cancer. 2006;107:1055–64.
19. Hisaoa M, Ishida T, Kuo TT, Matsuyama A, Inamura T, Nishida K, et al. Clear cell sarcoma of soft tissue: a clinicopathologic, immunohistochemical, and molecular analysis of 33 cases. Am J Surg Pathol. 2008;32:452–60.
20. Curry CV, Dishop MK, Hicks MJ, Naem R, Reed JA, Lopez-Terrada DH. Clear cell sarcoma of soft tissue: Diagnostick utility of fluorescence in situ hybridization and reverse transcriptase polymerase chain reaction. J Cutan Pathol. 2008;35:411–7.
21. Antonescu CR, Tschemyanovsky SJ, Wodruff JM, Jungbluth AA, Brennan MF, Ladanyi M. Molecular diagnosis of clear cell sarcoma: detection of EWS-ATF1 and MITF-M transcripts and histopathological and ultrastructural analysis of 12 cases. J Mol Diagn. 2002;4:44–52.
22. Ferrari A, Casanova M, Bisogno G, Mattke A, Mezza C, Gandola L, et al. Clear cell sarcoma of tendons and aponeuroses in pediatric patients: a report from the Italian and German Soft Tissue Sarcoma Cooperative Group. Cancer. 2002;94:3269–76.
23. Nedoszytko B, Mrózek K, Roszkiewicz A, Kopacz A, Swierbłewski M, Limon J. Clear cell sarcoma of tendons and aponeuroses with t(12;22)(q13;q12). diagnosed initially as malignant melanoma. Cancer Genet Cytogenet. 1996;91:37–9.
24. Pletneva MA, Andeya A, Palanisamy N, Betz BL, Carskadon S, Wang M, et al. Clear cell melanoma: a cutaneous clear cell malignancy. Arch Pathol Lab Med. 2014;138:1328–36.
25. Ozuguz P, Kocak M, Atasoy P, Yargil I, Cavusoglu T. Clear cell sarcoma. Indian Dermatol Online J. 2014;5:488–90.
26. Davis II, Kim JJ, Oszolak F, Widlund HR, Rozenblatt-Rosen O, Granter SR, et al. Oncogenic MITF dysregulation in clear cell sarcoma: defining the MIT family of human cancers. Cancer Cell. 2006;9:473–84.
27. Hocar O, Le Cesne A, Berissi S, Terrier P, Bonvalot S, Vanel D, et al. Clear cell sarcoma (malignant melanoma) of soft parts: a clinicopathologic study of 52 cases. Dermatol Res Pract. 2012;2012:984096.
28. Ciftlik JA, Ross JS, Slominski AJ. New techniques in dermatopathology that help to diagnose and prognosticate melanoma. Clin Dermatol. 2009;27:75–102.
29. Thway K, Folpe AL. Update on selected advances in the immunohistochecmical and molecular genetic analysis of soft tissue tumors. Virchows Arch. 2020;476:13–15.
30. Zucman J, Delattre O, Desmaze C, Epstein AL, Stenman G, Speleman F, et al. EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat Genet. 1993;4:341–5.
31. Antonescu CR, Nafa K, Segal NH, Dal Cin P, Ladanyi M. EWS-CREB1: a recurrent variant fusion in clear cell sarcoma – association with gastrointestinal location and absence of melanocytic differentiation. Clin Cancer Res. 2006;12:5336–62.
32. Forl GT, Wong WS, Gold RH, Kaiser LR. Skeletal metastasis of melanoma radiographic, scintigraphic, and clinical review. AJR Am J Roentgenol. 1981;137:103–8.
33. Marti NB, Del pozo ES, Casals AA, Garrote MJ, Masferrer NM. False-positive results obtained by following a commonly used reverse transcription-PCR protocol for detection of influenza A virus. J Clin Microbiol. 2006;44:3845.
34. Kim HJ. Improved diagnosis of spring viremia of carp by nested reverse-transcription PCR: development of a chimeric positive control for prevention of false-positive diagnosis. J Virol Methods. 2012;185:39–42.
35. Lin L, Carlquist J, Sinclair W, Hall T, Lopansri BK, Bennett ST. Experience with false positive test results on the TagPath real-time reverse transcription-PCR COVID-19 testing platform. Arch Pathol Lab Med. 2020. https://doi.org/10.5858/arpa.2020-0612-LE.
36. Kusk MS, Lausten-Thomsen U, Andersen MK, Olsen M, Hjalgrim H, Schmiegelow K. False positivity of ETV6/RUNX1 detected by FISH in healthy newborns and adults. Pediatr Blood Cancer. 2014;61:1704–6.
37. Alnahhas I, Ray A, Thomas D, Ding S, Giglio P, Puduvalli V. False-positive 1p/19q testing results in gliomas: clinical and research consequences. Am J Clin Oncol. 2020;43:802–5.
38. Ball MK, Kollmeyer TM, Praska CE, McKenna ML, Giannini C, Raghunathan A, et al. Frequency of false-positive FISH 1p/19q codeletion in adult diffuse astrocytic gliomas. Neurooncol Adv. 2020;2:eva109.
39. Patel RM, Downs-kelly E, Weiss SW, Folpe AL, Tubbs RR, Tuthill RJ, et al. Dual-color, break-apart fluorescence in situ hybridization for EWS gene rearrangement distinguishes clear cell sarcoma of soft tissue from malignant melanoma. Mod Pathol. 2005;18:1585–90.
40. Aslanzadeh J. Preventing PCR amplification carryover contamination in a clinical laboratory. Ann Clin Lab Sci. 2004;34:389–96.
41. Gonzaga MI, Grant L, Curtin C, Gootee J, Silberstein P, Voth E. The epidemiology and survivorship of clear cell sarcoma: a National Cancer Database (NCDB) review. J Cancer Res Clin Oncol. 2018;144:1711–6.
42. Lucas DR, Nascimento AG, Sim FH. Clear cell sarcoma of soft tissues. Mayo Clinic experience with 35 cases. Am J Surg Pathol. 1992;16:1197–204.

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