Int J Clin Exp Pathol 2016;9(10):10581-10586
www.ijcep.com /ISSN:1936-2625/IJCEP0037419

Original Article

The value of *ASPL-TFE3* dual-color single-fusion FISH assay in diagnosing alveolar soft part sarcoma

Xiancheng Chen[1*], Lei Xiong[2*], Baolei Miao[2], Ning Liu[2], Zhen Wang[2], Weidong Gan[2], Dongmei Li[3,4], Hongqian Guo[2]

Departments of [1]Critical Care Medicine, [2]Urology, Nanjing Drum Tower Hospital, Medical School of Nanjing University, Nanjing, Jiangsu Province, China; [3]Immunology and Reproductive Biology Laboratory & State Key Laboratory of Analytical Chemistry for Life Science, Medical School of Nanjing University, Nanjing, China; [4]Jiangsu Key Laboratory of Molecular Medicine, Nanjing University, Nanjing, China. [*]Equal contributors.

Received August 6, 2016; Accepted August 23, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Objectives: To improve the accuracy of the diagnosis of alveolar soft part sarcoma, *ASPL-TFE3* dual-color, single-fusion fluorescence in situ hybridization (FISH) probe was developed, especially for alveolar soft part sarcoma with atypical morphological features, unfamiliar positions or overlap of its pathological features with other tumors. Methods: *ASPL-TFE3* dual-color, single-fusion FISH probe was invented for the diagnosis of alveolar soft part sarcoma. The probe was performed on formalin-fixed, paraffin-embedded tissues from ten alveolar soft part sarcoma, five granular cell tumors, five rhabdomyosarcoma and five paraganglioma in our hospital. All the cases were restudied, including pathological morphology and immunohistochemistry, and TFE3 immunohistochemistry and FISH assay were taken for all the cases. Results: TFE3 Immunohistochemistry was positive in ten alveolar soft part sarcoma cases and one of paraganglioma. *ASPL-TFE3* single-fusion FISH assay was positive in all alveolar soft part sarcoma, and negative in granular cell tumor, rhabdomyosarcoma and paraganglioma cases. Conclusions: This FISH assay can precisely detect the *ASPL-TFE3* fusion gene, and it can be utilized as a useful adjunct way for the diagnosis of alveolar soft part sarcoma.

Keywords: Sarcoma, alveolar soft part, fluorescence in situ hybridization, TFE3, gene fusions, diagnosis

Introduction

Alveolar soft part sarcoma (ASPS) is a rare, malignant mesenchymal sarcoma accounting for less than 1% of all soft tissue tumors, mainly affecting children and young adults [1, 2]. Typically, patients with ASPS note a painless, slowly growing mass. Despite a relatively indolent clinical course, ASPS has a high propensity for metastasis to lung, bone and brain, which may precede the detection of the primary tumor [3, 4]. The prognoses of these patients are poor, with overall survival rates of 64% at 5 years and 48% at 10 years [5]. Portera et al [6] found that 5-year disease-free survival rate was 71% in patients with localized nidus, but 20% in patients with metastases. Treatment options for ASPS are extremely limited. For localized diseases, radical resection is the perfect treatment. But for metastasizing cases, ASPS is a generally chemo- and radio-resistant tumor, with no standardized treatment guideline [7-9]. These causations highlight the necessity of an early accurate identification of ASPS.

Up to now, the diagnosis of ASPS mainly relies on pathologic morphology [10]. Although diagnosis is straightforward in classical cases, ASPS with atypical morphological features may be difficult to be confirmed solely on the conventional histopathology, especially occurring in unusual locations [11].

ASPS has a cytogenetic profile involving an unbalanced der (17) t(X; 17) (p11; q25) translocation, which leads to the fusion of the *ASPL* gene and the *TFE3* gene. The part of *TFE3* gene is duplicated and then fused to chromosome 17, while the part of *ASPL* gene lost [12]. Thus, a genetic approach to confirm *TFE3* rearrangement can make a definite diagnosis. Genetic approaches, including reverse transcription polymerase chain reaction (RT-PCR), cytogenetic karyotypic analysis and fluorescence in situ

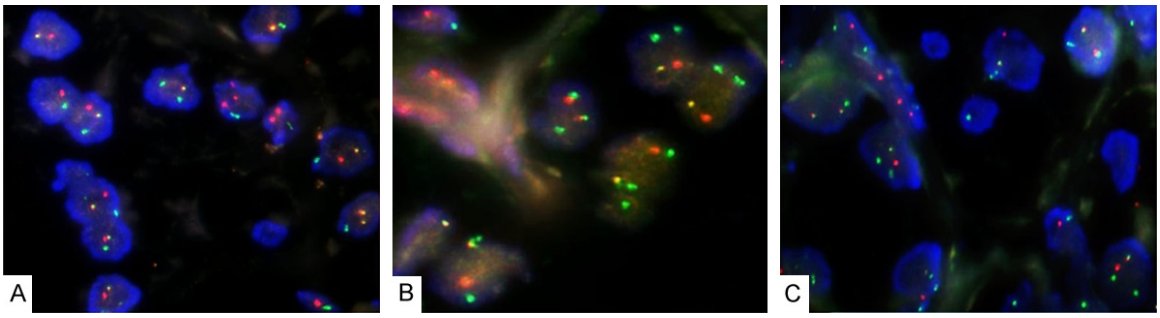

Figure 1. A: The typical FISH result of ASPS in male: one fusion signal in tumor cell nucleus and a couple of separated red and green signals in case 4, 1000× zoom; B: The typical FISH result of ASPS in female: one fusion signal, one red and two green signals in tumor cell nucleus, case 1, 1000× zoom; C: Part of nucleus had two fusion signals emerged simultaneously in case 3, 1000× zoom.

Table 1. Clinicpathologic features, TFE3 IHC, ASPL-TFE3 dual-fusion FISH of ASPS

Case No.	Age/sex	Site	CK	Vim	Des	CgA	MyoD1	NSE	PAS	TFE3	FISH
1	8/F	Axilla	-	+	+/-	-	++	-	+	++	+
2	13/F	Crus	N	N	-	-	N	N	+	++	+
3	29/M	Oxter	-	-	-	-	-	-	+	+	+
4	27/M	Retroperitoneum	-	N	+/-	+	-	-	+	+++	+
5	30/M	Thigh	-	N	+/-	+/-	-	N	+/-	++	+
6	11/M	Thigh	-	-	-	-	N	N	+	+	+
7	30/F	Acetabulum	-	-	-	+++	+	-	+	+++	+
8	27/F	Lung	-	-	-	++	-	++	+/-	++	+
9	38/M	Lung	-	+/-	-	N	-	-	+	+++	+
10	24/F	Thigh	-	-	-	N	-	N	+	++	+

Abbreviations: F, female; M, male; +/-: focal positive; N: did not test this immunohistochemistry.

hybridization (FISH) assay, are available tools to identify the type of genetic changes in tumor cells [13]. But fresh tumor tissues are not always available for RT-PCR, and karyotypic analysis is confined by availability of viable tumor cells.

FISH assay is a highly sensitive method for confirming the special gene translocation. It has been reported that FISH assay was used to diagnose Xp11.2 renal cell carcinoma, whose genetic change is similar to ASPS. Herein, we demonstrate an *ASPL-TFE3* dual-color, single-fusion probe for the diagnosis of ASPS.

Materials and methods

Patients

For this study, we selected all the ASPS cases that underwent resection in Nanjing Drum Tower Hospital during 2007 to 2014. All the formalin-fixed, paraffin-embedded tissues were reviewed by two experienced pathologists, and then ten cases of ASPS were selected for TFE3 immunohistochemistry (IHC) and FISH assay. Typical cases of five granular cell tumors, five rhabdomyosarcoma and five paraganglioma were chose as control groups. The IHC and FISH results of all the cases were reviewed and analyzed. This study was approved by the Institutional Review Board of Nanjing Drum Tower Hospital.

TFE3 IHC

All the selected cases were underwent the TFE3 IHC. Four μm paraffin section was prepared for TFE3 IHC. Deparaffinized sections were subjected to 0.3% H_2O_2 for 10 mins at room temperature to block endogenous peroxidase activity. The rabbit anti-TFE3 monoclonal antibody (prediluted, ZSGB-BIO, China) and biotinylated anti-rabbit IgG (ZSGB-BIO, China) were used for TFE3 IHC. This method has been reported [14].

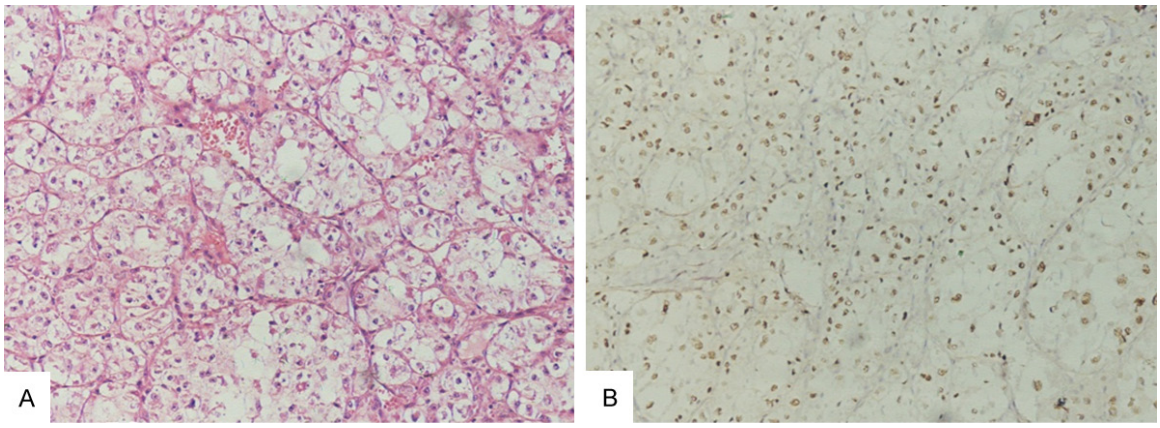

Figure 2. A: The typical pathology of ASPS: a clear voluminous cytoplasm and growth in a uniform, organoid nests, separated by fibrovascular septa, HE staining, 100× zoom; B: Nuclear TFE3 immunostaining of ASPS, showed strong nuclear TFE3 positivity, 100× zoom.

DNA probe design

Dual-color, single-fusion probe design: The bacterial artificial chromosomes (BAC) (Invitrogen, U.S.A.) which cover more than *ASPL* gene were labeled with tetramethylrhodamine-5-dUTP (Roche, Switzerland) as red fluorescein, including RP11-634L10, RP11-51H16, and RP11-475F12. The X chromosome probe, labeled with fluorescein-12-dUTP (Roche, Switzerland) as green fluorescein, consists of CTD-2311N12, RP11-416B14, CTD-2522M13, CTD-2516D6, CTD-2312C1, CTD-2248C21, and RP11-959H-17, covering the entire *TFE3* gene.

The probe was composed of purified DNA labeled with certain fluorochrome, human Cot-1 DNA and hybridization buffer on a proportional basis.

FISH assay

The process of this study was conventional, of which the main steps include deparaffinizing, hybridization, incubation, redyeing. This procedure was performed as previously described [15].

FISH result was analyzed by two experienced pathologists in our hospital. During the assessment, they were blinded to the result.

According to the FISH probe design, two typical signal patterns would be observed. Fusion signal (yellow or adjacent green-red signal) emerged when *ASPL-TFE3* fusion gene was existed, so the translocation of *TFE3* gene and *ASPL* gene can be identified. Split green or red signal was the indication of the location of *TFE3* gene or *ASPL* gene, respectively. Based on the genetic change in ASPS, one fusion signal is the typically positive pattern in ASPS (**Figure 1A**, **1B**). Clear FISH signals must be observed in >100 non-overlapping nucleus. Only the proportion of single-fusion signal is more than 10% that emerged in all observed tumor nucleus, positive result can be identified.

Results

Data regarding the clinic pathologic features, IHC, *ASPL-TFE3* dual-fusion FISH of ASPS in 10 cases of ASPS are summarized in **Table 1**. Of the 10 patients, five were male and five female and median age was 23.7 years (range from 8 to 38). Part of tumors located in limbs, also in crus, lung or even retroperitoneum.

About pathologic morphology of these ten ASPS, seven of them grew in typical alveolar or nest pattern, surrounded by fibrovascular septa (**Figure 2A**). Periodic acid-Schiff (PAS) stain was relatively specific for the diagnosis of ASPS. In the retrospective analysis of our patients, PAS was positive in most ASPS, but two of them were only focal positive. The pathological diagnoses of three patients (case 3, 4 and 8) were uncertain. Their morphology was indiscriminate from paraganglioma, and case 4 was confused with clear cell renal cell carcinoma. After all of the IHC had finished, these three cases were diagnosed as ASPS. However, because of atypi-

cal histological characteristics and confused IHC results, paraganglioma still couldn't be eliminated in case 8.

A total of twenty-five cases were evaluated for TFE3 IHC and FISH assay. For TFE3 IHC, all the ASPS and one of the five granular cell tumors were positive in TFE3 IHC (**Figure 2B**), but part of ASPS were slight to moderate positive. For FISH assay, the ASPS, including case 8, were all positive, with the others negative.

Discussion

In recent years, ASPS has attracted broad attention of clinicians and pathologists, owing to its unique histopathological features and unpredictable clinical manifestation. The most common onset age is 15 to 35 years old [2]. According to large series, as many as 60% of ASPS cases are seen in women [3], while our cases are composed of five male and five female patients. There is a wide variety in location, primarily occurring in the deep soft tissues of lower extremities, with a smaller number of cases at other soft-tissue locations such as the uterine cervix, axilla, retroperitoneal tissue, bone [3, 16]. When presented at these unusual sites, untypical ASPS may significantly cause diagnostic confusions. In our series, three cases locate in the thigh, two in the lung, two in the axilla and the other three cases' sites are acetabulum, crus and retroperitoneal tissue, respectively.

ASPS is characterized by uniform, organoid nests of polygonal tumor cells, separated by fibrovascular septa and delicate capillary-sized vascular channels [11]. So diagnosis of ASPS is usually straightforward by characteristic histology, but can be challenging when presenting at unusual sites or with atypical histological features. Such as in infants and children, the tumor may show a diffuse growth pattern without nested architecture or intervening vascular channels [17]. And furthermore, ASPS' histological features can be mimicked by a number of more common tumors such as granular cell tumor, rhabdomyosarcoma, paraganglioma and metastatic clear cell renal cell carcinoma [10].

Moreover, IHC is a useful tool for the diagnosis. In ASPS, cytological features include PAS+ crystalline cytoplasmic inclusions. But the typical cytoplasmic crystals are observed only in 22% to 80% of ASPS cases [18]. TFE3 IHC, identifying the carboxy terminal portion of the TFE3 protein, can develop strong nuclear staining in tumors with chromosome translocations involving the TFE3 gene. Because of its high sensitivity and specificity, TFE3 IHC has been used as an efficient auxiliary diagnostic method. However, TFE3 reactivity by IHC is not entirely specific, because it can be observed in some non-ASPS tumors, such as adrenal cortical carcinoma, granular cell tumor, Xp11.2 translocation renal cell carcinoma, perivascular epithelioid cell neoplasm [19, 20], which indicates that TFE3-positive findings do not always result in a confirmed diagnosis. Besides, a negative result cannot finally rule out ASPS. And TFE3 IHC has not rarely accompanied with strong background stain, or even with false positive and negative results, which will interfere with the diagnosis [19]. In our study, three cases show strong positive in TFE3 IHC, but the other seven ASPS cases are moderate and weak positive. Especially for weak positive result, misjudgment or false positive will be presented in many cases. Hence, further tests are necessary to consolidate the IHC results to obtain more accurate diagnosis.

Genetically, translocation of chromosomes X and 17 resulting in *ASPL-TFE3* fusion was reported in a balanced form in Xp11.2 renal cell carcinoma and in an unbalanced form in ASPS [21]. The *TFE3* break-apart and fusion FISH assays have been proven to be effective tools for the diagnosis of Xp11.2 renal cell carcinoma [21-23]. The novel *ASPL-TFE3* fusion probe was designed for Xp11.2 translocation RCC firstly, emerging dual-fusion signal [15]. For ASPS, the break-apart probe is unable to identify the fusion partner with this gene, but fusion signal pattern would occur when ASPS is detected by the *ASPL-TFE3* single-fusion FISH assay, verifying the formation of *ASPL-TFE3* fusion gene. According to the distinctive design, BACs cover the entire *ASPL* and *TFE3* gene, and two fusion signals emerge in tissue with reciprocal translocation of *ASPL* gene and *TFE3* gene, but the single fusion signal signify non-reciprocal translocation. This fusion probe can accurately distinguish the balanced and unbalanced translocation of the *TFE3* gene.

More in-depth studies found that translocation of ASPS is present as type 1 and 2 variants,

involving the fusion of the 1-7 exons of the *ASPL* gene to *TFE3* exon 6 in type 1 and to exon 5 in type 2 transcripts (GenBank NM_006521) [12]. Williams showed no differences in terms of clinical setting, morphology or behavior correlating to the fusion transcript types [20].

In our study, specific single-fusion signals were observed in ASPS, and the ratio was conformed to the diagnostic criteria, so the FISH assay can be a useful tool to the diagnosis of ASPS. But during the analysis the results, part of nucleus in ASPS emerged two fusion signals simultaneously. The ratio of this phenomenon was seldom but need to pay attention (**Figure 1C**). And minority ASPS cases with balance *TFE3* translocation had been reported [12, 20, 24]. We need additional study, such as PCR or gene sequencing, to identify these phenomena. The relevant works are prepared in our laboratory.

In conclusion, we have performed the *ASPL-TFE3* dual-color, single-fusion probe to identify *ASPL-TFE3* gene fusion in formalin-fixed, paraffin-embedded ASPS tissues. Besides, these FISH assays are also adjunctive and powerful tools to clarify a diagnosis of *ASPL-TFE3* renal cell carcinoma, perivascular epithelioid cell tumors (PEComas) and other *ASPL-TFE3*-associated neoplasms.

Acknowledgements

This work was supported by National Natural Science Foundation of China (ID: 81572512) and Natural Science Foundation of Jiangsu Province (ID: BK20131281).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Weidong Gan, Department of Urology, Nanjing Drum Tower Hospital, Medical School of Nanjing University, 321 Zhongshan Road, Nanjing 210093, Jiangsu Province, China. Tel: +86-13305186699; Fax: +86-25-833-07115; E-mail: gwd@nju.edu.cn; dr.gwd@yeah.net; Dr. Dongmei Li, Immunology and Reproductive Biology Laboratory & State Key Laboratory of Analytical Chemistry for Life Science, Medical School of Nanjing University; Jiangsu Key Laboratory of Molecular Medicine, Nanjing University, 22 Hankou Road, Nanjing 210093, China. Tel: +86-25-83686497; Fax: +86-25-83686497; E-mail: lidm@nju.edu.cn

References

[1] Agarwal S, Gupta R, Iyer VK, Mathur SR, Ray R. Cytopathological diagnosis of alveolar soft part sarcoma, a rare soft tissue neoplasm. Cytopathology 2011; 22: 318-22.

[2] Zarrin-Khameh N, Kaye KS. Alveolar soft part sarcoma. Arch Pathol Lab Med 2007; 131: 488-91.

[3] Lieberman PH, Brennan MF, Kimmel M, Erlandson RA, Garin-Chesa P, Flehinger BY. Alveolar soft-part sarcoma. A clinico-pathologic study of half a century. Cancer 1989; 63: 1-13.

[4] Fanburg-Smith JC, Miettinen M, Folpe AL, Weiss SW, Childers EL. Lingual alveolar soft part sarcoma; 14 cases: novel clinical and morphological observations. Histopathology 2004; 45: 526-37.

[5] Ogura K, Beppu Y, Chuman H, Yoshida A, Yamamoto N, Sumi M, Kawano H, Kawai A. Alveolar soft part sarcoma: a single-center 26-patient case series and review of the literature. Sarcoma 2012; 2012: 907179.

[6] Portera CA, Ho V, Patel SR, Hunt KK, Feig BW, Respondek PM, Yasko AW, Benjamin RS, Pollock RE, Pisters PWT. Alveolar soft part sarcoma-Clinical course and patterns of metastasis in 70 patients treated at a single institution. Cancer 2001; 91: 585-91.

[7] Orbach D, Brennan B, Casanova M, Bergeron C, Mosseri V, Francotte N, Van Noesel M, Rey A, Bisogno G, Pierron G, Ferrari A. Paediatric and adolescent alveolar soft part sarcoma: A joint series from European cooperative groups. Pediatr Blood Cancer 2013; 60: 1826-32.

[8] Kayton ML, Meyers P, Wexler LH, Gerald WL, LaQuaglia MP. Clinical presentation, treatment, and outcome of alveolar soft part sarcoma in children, adolescents, and young adults. J Pediatr Surg 2006; 41: 187-93.

[9] Reichardt P, Lindner T, Pink D, Thuss-Patience PC, Kretzschmar A, Dorken B. Chemotherapy in alveolar soft part sarcomas. What do we know? Eur J Cancer (Oxford, England: 1990) 2003; 39: 1511-6.

[10] Ordonez NG. Alveolar soft part sarcoma: A review and update. Adv Anat Pathol 1999; 6: 125-39.

[11] Folpe AL, Deyrup AT. Alveolar soft-part sarcoma: a review and update. J Clin Pathol 2006; 59: 1127-32.

[12] Ladanyi M, Lui MY, Antonescu CR, Krause-Boehm A, Meindl A, Argani P, Healey JH, Ueda T, Yoshikawa H, Meloni-Ehrig A, Sorensen PH, Mertens F, Mandahl N, van den Berghe H, Sciot R, Dal Cin P, Bridge J. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene 2001; 20: 48-57.

[13] Zhong M, De Angelo P, Osborne L, Paniz-Mondolfi AE, Geller M, Yang Y, Linehan WM, Cordon-Cardo C, Cai D. Translocation renal cell carcinomas in adults: a single-institution experience. Am J Surg Pathol 2012; 36: 654-62.

[14] Klatte T, Streubel B, Wrba F, Remzi M, Krammer B, De Martino M, Waldert M, Marberger M, Susani M, Haitel A. Renal cell carcinoma associated with transcription factor E3 expression and Xp11.2 translocation: incidence, characteristics, and prognosis. Am J Clin Pathol 2012; 137: 761-8.

[15] Chen X, Yang Y, Gan W, Xu L, Ye Q, Guo H. Newly designed break-apart and ASPL-TFE3 dual-fusion FISH assay are useful in diagnosing Xp11.2 translocation renal cell carcinoma and ASPL-TFE3 renal cell carcinoma: a STARD-compliant article. Medicine 2015; 94: e873.

[16] Evans HL. Alveolar soft-part sarcoma. A study of 13 typical examples and one with a histologically atypical component. Cancer 1985; 55: 912-7.

[17] Jaber OI, Kirby PA. Alveolar Soft Part Sarcoma. Arch Pathol Lab Med 2015; 139: 1459-62.

[18] Tsuji K, Ishikawa Y, Imamura T. Technique for differentiating alveolar soft part sarcoma from other tumors in paraffin-embedded tissue: comparison of immunohistochemistry for TFE3 and CD147 and of reverse transcription polymerase chain reaction for ASPSCR1-TFE3 fusion transcript. Hum Pathol 2012; 43: 356-63.

[19] Argani P, Lal P, Hutchinson B, Lui MY, Reuter VE, Ladanyi M. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions-A sensitive and specific immunohistochemical assay. Am J Surg Pathol 2003; 27: 750-61.

[20] Williams A, Bartle G, Sumathi VP, Meis JM, Mangham DC, Grimer RJ, Kindblom LG. Detection of ASPL/TFE3 fusion transcripts and the TFE3 antigen in formalin-fixed, paraffin-embedded tissue in a series of 18 cases of alveolar soft part sarcoma: useful diagnostic tools in cases with unusual histological features. Virchows Arch 2011; 458: 291-300.

[21] Hodge JC, Pearce KE, Wang X, Wiktor AE, Oliveira AM, Greipp PT. Molecular cytogenetic analysis for TFE3 rearrangement in Xp11.2 renal cell carcinoma and alveolar soft part sarcoma: validation and clinical experience with 75 cases. Mod Pathol Inc 2014; 27: 113-27.

[22] Aulmann S, Longerich T, Schirmacher P, Mechtersheimer G, Penzel R. Detection of the ASPSCR1-TFE3 gene fusion in paraffin-embedded alveolar soft part sarcomas. Histopathology 2007; 50: 881-6.

[23] Pradhan D, Roy S, Quiroga-Garza G, Cieply K, Mahaffey AL, Bastacky S, Dhir R, Parwani AV. Validation and utilization of a TFE3 break-apart FISH assay for Xp11.2 translocation renal cell carcinoma and alveolar soft part sarcoma. Diagn Pathol 2015; 10: 179.

[24] Uppal S, Aviv H, Patterson F, Cohen S, Benevenia J, Aisner S, Hameed M. Alveolar soft part sarcoma--reciprocal translocation between chromosome 17q25 and Xp11. Report of a case with metastases at presentation and review of the literature. Acta Orthop Belg 2003; 69: 182-7.