Journal of
Carcinogenesis & Mutagenesis

Rekhi et al., J Carcinog Mutagen 2017, 8:1
DOI: 10.4172/2157-2518.1000280

Research Article Open Access

Extraskeletal Myxoid Chondrosarcomas are not Characterized by Loss of *INI1/SMARCB1*: Immunohistochemical Analysis of 16 Cases

Bharat Rekhi[1*], **Mukta Ramadwar**[1] **and Jyoti Bajpai**[2]

[1]*Department of Surgical Pathology, Tata Memorial Centre, Parel, Mumbai, India*

[2]*Department of Medical Oncology, Tata Memorial Centre, Parel, Mumbai, India*

[*]**Corresponding author:** Bharat Rekhi, Department of Surgical Pathology, Tata Memorial Hospital, Mumbai, India, Tel: 022-24177000; E-mail: rekhi.bharat@gmail.com

Received date: December 20, 2016; **Accepted date:** January 05, 2017; **Published date:** January 10, 2017

Abstract

Background: Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma, characterized by t (9; 22) translocation, including *EWSR1* and *NR4A3* gene rearrangements, observed in most cases. On histopathologic examination, an EMC has certain diagnostic mimics, such as myoepithelial tumors, epithelioid malignant peripheral nerve sheath tumor and epithelioid sarcomas. All these tumors are included in the category of *INI1/SMARCB1*-deficient tumors. Lately, few studies have shown loss of INI1 in certain EMCs. Considering there is preclinical data regarding role of *EZH2* inhibitor in "INI1 deficient" tumors, it is crucial to recognize such tumors.

Objectives: To evaluate immunohistochemical features, including *INI1/SMARCB1* immunostaining results in 16 prospectively diagnosed cases of EMC.

Methods: Immunohistochemical staining was performed on formalin-fixed paraffin embedded tissue sections by immunoperoxidase method using a MACH 2 Universal HRP-Polymer detection kit. Two cases were tested for *EWSR1* rearrangement by fluorescent *in-situ* hybridization (FISH) technique.

Results: Sixteen EMCs occurred in 13 males and 3 females, most commonly in lower extremities; followed by chest wall, pelvis, including iliac fossa, shoulder and parasapinal region; in patients within age-range of 17-72 years (median=47.5). On histopathologic examination, most tumors displayed round to polygonal cells arranged in cords, trabeculae and pseudoglandular pattern in an abundant myxoid stroma. Three tumors revealed "rhabdoid" cells. By immunohistochemistry, tumor cells were positive for NSE (13/13) (100%), S100 protein (10/15) (66.6%), EMA (2/12) (16.6%), AE1/AE3 (0/9), P63 (0/2) and SMA (2/3), the latter in tumors containing rhabdoid-like cells. INI1/SMARCB1 was diffusely retained in all 16 tumors (100%). Two cases tested for *EWSR1* rearrangement, were found to be positive for the same.

Conclusion: An optimal immunohistochemical panel for differentiating an EMC from its diagnostic mimics may include antibody markers, such as NSE, S100 protein, AE1/AE3, EMA and SMA. EMCs, including those containing rhabdoid cells do not seem to be in the category of INI1-deficient tumors.

Keywords: Extraskeletal myxoid chondrosarcoma; *INI1/SMARCB1*; *EWSR1*; Soft tissue sarcoma

Introduction

Extraskeletal myxoid chondrosarcoma (EMC) is a relatively uncommon, intermediate to high-grade soft tissue sarcoma of uncertain histogenesis, characterized by certain histopathologic features and specific t (9; 22) (q22; q12) translocation, or less commonly, t (9; 15) (q22; q11.2) translocation, resulting in the formation of *EWSR1*-TEC and *EWSR1*-CHN fusion genes, respectively. Clearly, an EMC is one of the sarcomas characterized by rearrangement of *EWSR1* gene, which is located at 22q12.2 and NR4A3 that is located on 9q31.1 [1-4]. It is one of the chemoresistant sarcomas [5]. In view of overlapping histopathologic features, various other soft tissue tumors constitute as its differential diagnoses [1].

Integrase interactor 1 (INI1), also known as *hSNF5/SMARCB1/BAF47* is a tumor suppressor gene located on chromosome 22q11.2 is ubiquitously expressed in various cells [6]. Deletions, mutations or other somatic alterations in this gene have been classically demonstrated in malignant rhabdoid tumors [7,8]. By immunohistochemistry (IHC), loss of INI1 has been observed in malignant rhabdoid tumors and various other soft tissue sarcomas [9-12]. Lately, few studies have shown loss of *SMARCB1/INI1* in certain EMCs, while another study has shown contrasting results [9, 13-15]. Considering there is emerging preclinical data to show role of EZH2 inhibitor in tumors lacking INI1, it is important to correctly identify such tumors [16,17].

Herein, we present clinicopathologic and immunohistochemical features, including *INI1/SMARCB1* immunostaining results in 16 cases of EMC.

Materials and Methods

This was a prospective study of 16 EMCs that included consultation cases of two authors (BR and MR) and were included in the study after critical review by BR, as per established histopathologic criteria [1].

All, except one patient (referred from Bangladesh), were of Indian origin, from various states across the country.

Conventional Haematoxylin and Eosin (H&E) staining microsections were available in all cases.

Immunohistochemical staining was performed on formalin-fixed paraffin embedded (FFPE) tissue sections by immunoperoxidase method using a MACH 2 Universal HRP-Polymer detection kit (Biocare, CA, USA), including 3′-3′-diaminobenzidine tetrahydrochloride as the chromogen. Appropriate positive and negative controls were included. Various antibody markers are enlisted in Table 1.

Antibody	Company	Clone	Dilution	Retrieval
S100 Protein	Dako Cytomation (Glostrup, Denmark)	Polyclonal	1/1500	Heat, Tris-EDTA
Neuron specific enolase (NSE)	Dako	BsNcHI4	1/100	Pepsin(Enzymatic)
INI1/ SMARCB1	Acris, GMBH, Herford, Germany	3E10	1/600	Heat, Sodium citrate
Epithelial membrane antigen (EMA)	Dako	E29	1:1200	Pepsin(Enzymatic)
Pan cytokeratin(CK)	Dako	AE1/AE3	1:200	Heat, Sodium citrate
Smooth muscle actin (SMA)	Dako	SM1A4	1:400	Heat

Table 1: Antibodies used in the present study.

Two cases revealing were tested for *EWSR1* rearrangement by fluorescent *in-situ* hybridization (FISH) technique.

Fluorescent *in-situ* hybridization (FISH)

FISH was performed on 2 μm thick paraffin sections with Vysis LSI EWSR1 dual color break apart, probe (Abbott Molecular, Chicago, Illinois, USA) and Zytolight SPEC SS18 Dual color break-apart probes (Zytovision GmBH, Germany). The signals were viewed under an Olympus BX53F upright fluorescence microscope equipped with filters specific for 4'-6 Diamidino-2 phenylindole (DAPI), TRITC/ Spectrum Orange and FITC/ Spectrum Green, QI cam Olympus camera and Q Capture pro 7.0 image analyzer software. At least 100 non-overlapping tumour cell nuclei were counted.

A probe result was considered to be rearranged/ break-apart when a pair of orange and green signals was separated by a distance greater than the size of one signal. More than 20% tumor cells demonstrating split (break-apart) signals were classified as positive for EWSR1 gene rearrangement, while those with 'split' in less than 20% of cells were classified as negative.

Results

Sixteen EMCs occurred in 13 males and 3 females (M: F ratio = 4.3: 1), most commonly in the lower extremities; followed by chest wall, pelvis, including iliac fossa, shoulder and parasapinal region; in patients within age-range of 17-72 years (median=47.5) (average=48.5).

Gross examination in one of the available cases showed a tumor with a multilobulated and a conspicuously myxoid cut surface, including focal necrosis.

Microscopically, most tumors displayed lobules of round to polygonal cells, containing moderate to abundant, eosinophilic to vacuolated cytoplasm, arranged in interconnecting cords, trabeculae and clusters within an abundant myxoid matrix. Three tumors revealed "rhabdoid" cells, including intracytoplasmic inclusions.

By IHC, tumor cells were positive for NSE(13/13)(100%), S100 protein(10/15)(66.6%), EMA(2/12)(16.6%),AE1/AE3(0/9), P63(0/2) and SMA(2/3), the latter antibody marker positive in tumors containing "rhabdoid" cells. INI1/SMARCB1 was diffusely retained in all 16 tumors (100%).

By FISH, two tumors showed orange green 'split signals more than 20% tumor nuclei, and were reported as *EWSR1* positive (Table 2) (Figures 1-5).

Sr No.	Age	Gender	Site	Immunohistochemical Results
1	47	M	Popliteal Fossa	NSE+,AE1/AE3-, S100P-, CD34-, INI1+
2	60	M	Iliac Fossa	NSE+, S100P+,INI1+
3	40	M	Knee	S100P+, AE1/AE3-, EMA-, CD34-, INI1+
4	17	M	Shoulder	NSE+, S100P-, EMA-, Desmin-, GFAP-, INI1+
*5	48	M	Thigh	NSE+, S100+, SMA+, EMA-Focal+, INI1+
6	68	M	Foot	NSE+, S100P-, AE1/AE3-, EMA-,INI1+
7	28	M	Chest wall	S100P+, AE1/AE3-, INI1+
8	71	F	Parasp inal	NSE+, S100P+, SMA-Focal+, AE1/AE3-, EMA-, INI1+
9	42	F	Hand	NSE+, S100P-, AE1/AE3-, EMA-, P63-, INI1+
*10	41	M	Arm	NSE+, S100P+, EMA-, SMA-, P63-, INI1+
11	20	M	Chest wall	NSE+, S100P+, INI1+
12	60	F	Thigh	NSE+, S100P-, EMA-, AE1/AE3-N
13	45	F	Pelvis	NSE+, AE1/AE3-, EMA-,MIC2+, Calretinin-, Inhibin-, INI1+
14	72	M	Chest wall	S100P+, EMAAE1/AE3-
15	57	M	Gluteal region	S100P+, NSE+,EMA-,CD34-
16	61	M	Scapula	S100P+, NSE+,EMA-

Table 2: Clinicopathologic features and immunohistochemical results of 16 Extraskeletal Myxoid Chondrosarcomas.*: Tumors tested for *EWSR1* rearrangement and found to be positive, M: Male, F: Female,

+: Positive, -: Negative; NSE: Neuron specific enolase; S100P: S100 Protein; AE1/AE3: Pan Cytokeratin; EMA: Epithelial membrane antigen; SMA: Smooth muscle actin.

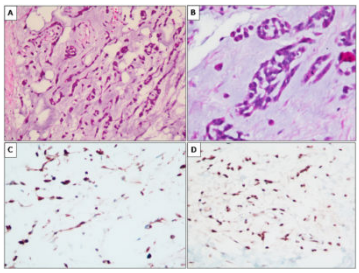

Figure 1: A. Cords and trabecular arrangement of tumor cells embedded in a rich myxoid matrix. **B.** Higher magnification showing round to polygonal shaped tumor cells arranged in cords, trabeculae and clusters, with interspersed mitotic figures, in a myxoid-rich stroma. H and E X400. **C-D.** Immunohistochemical staining results. **C.** Neuron specific enolase (NSE). Diaminobenzidine trihydrochloride (DAB) X400. **D.** Diffusely retained *INI1/SMARCB1* immunostaining. DAB X400.

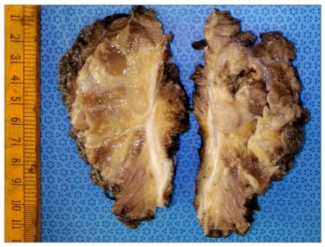

Figure 2: Gross examination showing a greyish-white tumor with a conspicuous myxoid cut surface, along with areas of haemorrhage and necrosis.

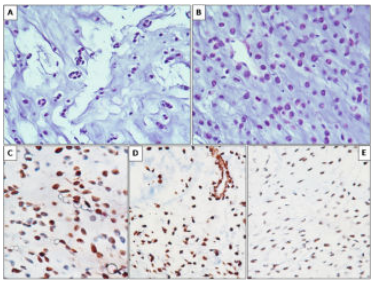

Figure 3: Microscopic features. **A.** Tumor cells arranged in cords, and pseudoglandular pattern in a mucoid to myxoid-rich stroma. H and E x 200. **B.** Tumor cells exhibiting "rhabdoid" morphology H and E X400. C-E. Immunohistochemical staining results. **C.** Tumor cells displaying S100 protein positivity. DAB x 400. **D.** Tumor cells displaying SMA positivity, especially within intracytoplasmic "rhabdoid" inclusions. DAB X400. **E.** Diffusely retained *INI1/SMARCB1* immunostaining. DAB X400.

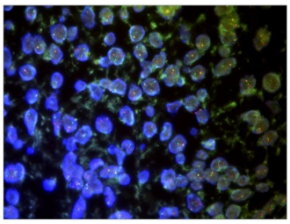

Figure 4: Tumor cells exhibiting *EWSR1* rearrangement. DAPI X1000.

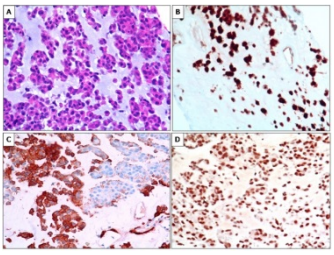

Figure 5: Tumor composed of epithelioid to "rhabdoid" cells. H and E X200. **B-D.** Immunohistochemical staining results. **B.** Tumor cells displaying NSE positivity. DAB X400. **C.** Tumor cells displaying SMA positivity. DAB X400. **D.** Diffusely retained *INI1/SMARCB1* immunostaining. DAB X400.

Discussion

The present study describes clinicopathologic and immunohistochemical features of 16 EMCs that most commonly occurred in the lower extremities of male patients, over a wide age-range [15]. Characteristic histopathologic features, such cords, trabeculae and clusters of polygonal-shaped tumor cells, embedded in a myxoid-rich matrix, noted in the present series, have been previously documented [1,18,19]. Unusual features included cellular variants and "rhabdoid" cells, noted in 3 tumors of the present study [1].

Diagnosis of an EMC can be challenging as a result of its various differential diagnoses, such as myoepithelial tumors, epithelioid sarcoma, epithelioid MPNST and myxoid/round cell liposarcoma. To some extent, immunohistochemical antibody markers are useful in differentiating an EMC from its diagnostic mimics [1,18,19].

Among various immunohistochemical antibody markers performed in the present study, NSE was consistently positive in all 13 tumors(100%), where it was tested, followed by S100 protein in 10 of 15 tumors (66.6%); focal EMA in mere 2 of 12 cases and SMA in 2 out of 3 tumors, containing "rhabdoid" cells, where it was tested. Previously, S100 protein positivity was observed in 20% cases [1]. Few cases of EMC with neuroendocrine differentiation have also been reported [20]. Subsequently, in a larger study of 23 cases, Oliveira et al. [21] observed synaptophysin positivity in 72% cases of EMC and S100 protein positivity in only 17% cases. In another study comprising 18 cases of EMC, Okamoto et al. [22] observed S100 protein positivity in 50% cases and NSE positivity in 89% cases, similar to our results. Infrequent expression of epithelial IHC markers and positive expression of neuroendocrine markers is useful in differentiating an

EMC from myoepithelial tumors that invariably display epithelial antibody IHC markers, apart from S100 protein positivity, in most cases [23]. At times, on a limited biopsy, it might be difficult to differentiate an EMC from a myxoid/round cell liposarcoma. In such cases, it is ideal to confirm the diagnosis with molecular techniques, wherein *CHOP* gene rearrangement would confirm a diagnosis of a myxoid/round cell liposarcoma and *NRA43* gene rearrangement or *EWSR1* rearrangement, as demonstrated in two cases of the present study, would be useful in confirming a diagnosis of EMC. It is noteworthy that *EWSR1* gene rearrangement is observed in certain myoepithelial tumors that constitute as diagnostic mimics of an EMC [23,24]. However, the underlying fusion genes in myoepithelial tumors are different from EMCs [2-4,23].

Besides various aforementioned immunohistochemical antibody markers, INI1/SMARCB1 was diffusely retained in all 16 tumors (100%), including 3 tumors containing rhabdoid cells, in the present study. Immunohistochemically, INI1 is ubiquitously expressed in various cells. Complete loss of INI1 is consistently observed in 100% malignant rhabdoid tumors and in significant percentage within various soft tissue sarcomas, for example in 80%-100% cases of epithelioid sarcomas, 20-40% myoepithelial tumors and in up to 67% cases of epithelioid MPNSTs, all which constitute as differential diagnoses of an EMC [9-12,25,26]. Similar to the present study, recently, in two different studies, Agaram et al. [14] and Huang et al. [15], respectively, did not observe loss of INI1 in any of their cases of EMC, including 4 out of 5 tumors containing "rhabdoid cells", in the former published study [14]. Earlier, Siguake et al. [9] noted retained INI1 immunoexpression in both cases of EMC, where this was tested. Contrastingly, Kohashi et al. [13] observed retained INI1 immunoexpression in 20 out of 24 cases of EMC, including three tumors with rhabdoid cells, where its expression was lost. Furthermore, they observed homozygous deletion and frameshift mutation of the *SMARCB1/INI1* gene in 2 of the 4 tumors revealing loss of immunohistochemical expression of IN11. Although their cases resembled EMC, as per the diagnostic criteria, none of those cases revealed underlying, common specific fusion transcripts of an EMC. Agaram, et al. [4] observed that EMCs with rhabdoid phenotype and high-grade morphology were more commonly associated with non-*EWSR1*-R4A3 variant fusions. One of the 2 cases in the present study, showing *EWSR1* rearrangement displayed "rhabdoid" cells.

An EMC is invariably not a chemosensitive soft tissue sarcoma [5]. Considering there is emerging evidence regarding role of EZH2 inhibitor in "INI1 deficient" tumors, such as epithelioid sarcomas, atypical teratoid rhabdoid tumors, synovial sarcoma and others, it is crucial to correctly recognize such tumors. *EZH2* is an epigenetic gene silencing protein. Loss of INI1 causes dysfunction of chromatin-remodelling complexes which normally oppose EZH2. Overexpression of EZH2 is known to cause ATRT oncogenesis. Using novel patient-derived orthotopic xenograft (PDOX) models, Lindsay et al. [17] investigated the pre-clinical utility of therapeutic EZH2 inhibition in ATRT. They observed that EZH2 inhibitor DZNep was effective in killing ATRT tumor cells *in vitro*. Further opportunities for therapeutic intervention with catalytic inhibitors of EZH2 are being considered in other solid tumors showing loss or reduced INI1 expression, such as synovial sarcomas [27].

To conclude, an EMC does not seem to be a tumor that can included in the category of "INI1 deficient" tumors, in contrast to its other diagnostic mimics. Optimal IHC panel for differentiating an EMC from its diagnostic mimics may be composed of NSE, S100 protein, AE1/AE3, EMA and SMA. This study reinforces that EMCs are associated with *EWSR1* rearrangement, including those showing "rhabdoid" cells. Surgical resection remains the treatment mainstay. In view of a specific underlying molecular 'signature', future options of targeted therapy might be explored in this tumor.

References

1. Lucas DR, Stenman G (2013) Extraskeletal myxoid chondrosarcoma. World Health Organization (WHO) classification of tumours of soft tissue and bone (4th edn), IARC Press, Lyon 223-224.
2. Sciot R, Dal Cin P, Fletcher C, Samson I, Smith M, et al. (1995) t(9;22)(q22-31;q11-12) is a consistent marker of extraskeletal myxoid chondrosarcoma: evaluation of three cases. Mod Pathol 8: 765-768.
3. Labelle Y, Bussières J, Courjal F, Goldring MB (1999) The EWS/TEC fusion protein encoded by the t(9;22) chromosomal translocation in human chondrosarcomas is a highly potent transcriptional activator. Oncogene 18: 3303-3308.
4. Attwooll C, Tariq M, Harris M, Coyne JD, Telford N, Varley JM (1999) Identification of a novel fusion gene involving hTAFII68 and CHN from a t(9;17)(q22;q11.2) translocation in an extraskeletal myxoid chondrosarcoma. Oncogene 18:7599-7601.
5. Grimer R, Judson I, Peake D, Seddon B (2010) Guidelines for the management of soft tissue sarcomas. Sarcoma 2010: 506182.
6. Roberts CW, Orkin SH (2004) The SWI/SNF complex--chromatin and cancer. Nat Rev Cancer 4: 133-142.
7. Versteege I, Sévenet N, Lange J, Rousseau-Merck MF, Ambros P, et al. (1998) Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature 394: 203-206.
8. Rousseau-Merck MF, Versteege I, Legrand I, Couturier J, Mairal A, et al. (1999) hSNF5/INI1 inactivation is mainly associated with homozygous deletions and mitotic recombinations in rhabdoid tumors. Cancer Res 59: 3152-3156.
9. Sigauke E, Rakheja D, Maddox DL, Hladik CL, White CL, et al. (2006) Absence of expression of SMARCB1/INI1 in malignant rhabdoid tumors of the central nervous system, kidneys and soft tissue: an immunohistochemical study with implications for diagnosis. Mod Pathol 19: 717-725.
10. Hollmann TJ, Hornick JL (2011) INI1-deficient tumors: diagnostic features and molecular genetics. Am J Surg Pathol 35: e47-63.
11. Hornick JL, Dal Cin P, Fletcher CD (2009) Loss of INI1 expression is characteristic of both conventional and proximal-type epithelioid sarcoma. Am J Surg Pathol 33: 542-550.
12. Rekhi B, Jambhekar NA (2013) Immunohistochemical validation of INI1/SMARCB1 in a spectrum of musculoskeletal tumors: an experience at a Tertiary Cancer Referral Centre. Pathol Res Pract 209: 758-766.
13. Kohashi K, Oda Y, Yamamoto H, Tamiya S, Oshiro Y, et al. (2008) SMARCB1/INI1 protein expression in round cell soft tissue sarcomas associated with chromosomal translocations involving EWS: a special reference to SMARCB1/INI1 negative variant extraskeletal myxoid chondrosarcoma. Am J Surg Pathol 32: 1168-1174.
14. Agaram NP, Zhang L, Sung YS, Singer S, Antonescu CR (2014) Extraskeletal myxoid chondrosarcoma with non-EWSR1-NR4A3 variant fusions correlate with rhabdoid phenotype and high-grade morphology. Hum Pathol 45: 1084-1091.
15. Huang H, Chen X, Zheng Z (2016) Extraskeletal myxoid chondrosarcoma: a clinicopathologic analysis of seven cases. Zhonghua Bing Li Xue Za Zhi 45: 25-30.
16. Italiano A (2015) A Phase 1 Study of EPZ-6438 (E7438), an Enhancer of Zeste-Homolog 2 (EZH2) Inhibitor: Preliminary Activity in INI1-negative Tumors. European Society of Medical Oncology.
17. Lindsay H, Kogiso M, Qi L, Murray J, Perlaky L, et al. (2015) AT-01. Therapeutic targeting of INI1 deficiency in peditaric ATRT: A pre-clinical

study utilizing patient derived orthotopic xenograft (PDX) models. Neuro Oncol 17: iii1.

18. Enzinger FM, Shiraki M (1972) Extraskeletal myxoid chondrosarcoma. An analysis of 34 cases. Hum Pathol 3: 421-435.
19. Meis-Kindblom JM, Bergh P, Gunterberg B, Kindblom LG (1999) Extraskeletal myxoid chondrosarcoma: a reappraisal of its morphologic spectrum and prognostic factors based on 117 cases. Am J Surg Pathol 23: 636-650.
20. Harris M, Coyne J, Tariq M, Eyden BP, Atkinson M, et al. (2000) Extraskeletal myxoid chondrosarcoma with neuroendocrine differentiation: a pathologic, cytogenetic, and molecular study of a case with a novel translocation t (9;17)(q22;q11.2). Am J Surg Pathol 24: 1020-1026.
21. Oliveira AM, Sebo TJ, McGrory JE, Gaffey TA, Rock MG, et al. (2000) Extraskeletal myxoid chondrosarcoma: a clinicopathologic, immunohistochemical, and ploidy analysis of 23 cases. Mod Pathol 13: 900-908.
22. Okamoto S, Hisaoka M, Ishida T, Imamura T, Kanda H, et al. (2001) Extraskeletal myxoid chondrosarcoma: a clinicopathologic, immunohistochemical, and molecular analysis of 18 cases. Hum Pathol 32: 1116-1124.
23. Antonescu CR, Zhang L, Chang NE, Pawel BR, Travis W, et al. (2010) EWSR1-POU5F1 fusion in soft tissue myoepithelial tumors. A molecular analysis of sixty-six cases, including soft tissue, bone, and visceral lesions, showing common involvement of the EWSR1 gene. Genes Chromosomes Cancer 49: 1114-1124.
24. Rekhi B, Sable M, Jambhekar NA (2012) Histopathological, immunohistochemical and molecular spectrum of myoepithelial tumours of soft tissues. Virchows Arch 461: 687-697.
25. Rekhi B, Vogel U (2015) Utility of characteristic "Weak to Absent INI1/SMARCB1/BAF47 Expression in Diagnosis of Synovial Sarcomas. APMIS 123: 618-628.
26. Jo VY, Fletcher CD (2015) Epithelioid malignant peripheral nerve sheath tumor: clinicopathologic analysis of 63 cases. Am J Surg Pathol 39: 673-682.
27. Barsotti AM, Ryskin M, Kung PP, Verhelle D, Rollins RA (2015) EZH2 as a therapeutic target in solid tumors. Can Cell Microenviron 2: e1024.