Virchows Archiv (2019) 475:245–249
https://doi.org/10.1007/s00428-019-02554-4

BRIEF REPORT

CROCC-mutated rhabdoid colorectal carcinoma showing in intercellular spaces lamellipodia and cellular projections revealed by electron microscopy

Andrea Remo[1] · M. P. Cecchini[2] · D. Benati[2] · P. Bernardi[2] · E. Manfrin[3] · G. Giordano[4] · F. Bonomi[3] · P. Parcesepe[3] · M. Fassan[5] · R. Colombari[1] · A. Sbarbati[2] · M. Pancione[6]

Received: 13 December 2018 / Revised: 16 February 2019 / Accepted: 28 February 2019 / Published online: 10 March 2019

Abstract

Background Rhabdoid colorectal carcinoma (RC) is a rare lesion localized to the proximal colon of patients with a mean age at diagnosis of around 70 years. This tumor shows an aggressive behavior with an overall survival period shorter than 12 months. The diagnostic hallmark is the presence of rhabdoid cells. Alterations in chromatin remodeling (*SMARCB1*) and in the centrosome structure (*CROCC*) are reported in RC usually $BRAF^{mut}$ and MSI-H. RKO intestinal neoplastic cells culture ($BRAF^{mut}$, $SMARCB1^{wt}$, MSI-H) with CROCC knockdown exhibit rhabdoid features and develop prominent projections from the edge of the cell.

Methods Here, we investigated two cases of $CROCC^{mut}SMARCB1^{wt}$ RC by scanning and transmission electron microscopy (SEM, TEM).

Results TEM confirmed the diagnostic presence of intermediate cytoplasmic filaments and nucleolar margination. SEM showed cellular protrusions (lamellipodia) in the intercellular spaces not evident at light microscopy.

Conclusions These protrusions *CROCC*-related might represent the pathogenetic mechanism underlying the rhabdoid aggressive behavior, independently of tumor staging. To our knowledge, the SEM technique was applied in the study of this neoplasm for the first time.

Keywords Ciliary Rootlet Coiled Coil · Rhabdoid colorectal carcinoma · *CROCC* · Lamellipodia · Scanning electron microscopy · Transmission electron microscopy · *SMARCB1* · *TAX1BP2* · *ROLT* · Nucleolar margination · Intermediate filaments · *SWI/SNF*

Introduction

Rhabdoid colorectal carcinoma (RC) is a rare lesion mainly localized to the proximal colon of patients with a mean age at diagnosis of around 70 years. This tumor shows an aggressive behavior and fatal outcome, displaying an overall survival period shorter than 12 months [1, 2]. The diagnostic hallmark of this neoplasm is the presence of rhabdoid cells characterized by an eccentrically located and large nucleus, prominent nucleoli (light microscopy) and cytosolic aggregates of intermediate filaments (ultrastructure) similar to the malignant extrarenal rhabdoid tumor [2]. The amount and distribution of the rhabdoid component in neoplasms are highly variable ranging from "composite," in which the rhabdoid elements are associated with adenocarcinoma, to the "pure" rhabdoid carcinoma without an evident epithelial component [1]. RC showed mainly $BRAF^{mut}$ and MSI-H molecular profile [2]. In this specific genotypic asset, alterations in chromatin

Andrea Remo and M. P. Cecchini contributed equally to this work.

✉ Andrea Remo
remino76@yahoo.it

[1] Pathology Unit, Service Department, ULSS9 "Scaligera", Via Valverde 42, 37122 Verona, Italy

[2] Department of Neurosciences, Biomedicine and Movement Sciences, Section of Anatomy and Histology, University of Verona, Verona, Italy

[3] Department of Diagnostic and Public Health, Section of Pathology, University of Verona, Verona, Italy

[4] Oncology Division IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo Foggia, Italy

[5] Department of Medicine (DIMED), University of Padua, Padua, PD, Italy

[6] Department of Sciences and Technologies, University of Sannio, Benevento, Italy

remodeling (*SWI/SNF or SMARCB1*) complex [3–6] and in the centrosome structure (Ciliary Rootlet Coiled Coil; *CROCC*) have been reported as major genetic determinants of rhabdoid pathogenesis [7]. SWI/SNF chromatin remodeling complexes regulate critical cellular processes including cell cycle progression, programmed cell death, differentiation, genomic instability, and DNA repair [8]. Depending upon the configuration of the complex components, SWI/SNF complexes can carry out a variety of cellular functions including neural differentiation, embryonic stem cell differentiation, hepatic lipid, and glucose metabolism. The mechanism determining the types of cancers associated with inactivation of different complex members remains unraveled [8]. CROCC is a protein implicated in centrosome linker formation and function in association with multiple proteins. CROCC is able to maintain centrosome cohesion in part through inhibition of VHL-mediated Centrosomal protein 68 degradation. It has been proposed that a broad network of repeating CROCC units with C-Nap1 as ring organizer and Centrosomal protein 68 as filament modulator forms the centrosome linker structure. Genetic deletion in *CROCC* leads to centrosome anomalies resulting in tetraploid DNA segregation errors, providing insight into the mechanism by which genome instability contributes to lethal cancers [7].

To our knowledge, few studies investigated RC ultrastructural morphology [9]. In this regard, to our knowledge, no one examined RC ex vivo samples by means of scanning electron microscopy technique. For this purpose, we decided to investigate the ultrastructure appearance of two RC cases ($CROCC^{mut}SMARCB1^{wt}$) by means of scanning (SEM) and transmission electron microscopy (TEM). Then, we compared the results with our previous information obtained by in vitro experiments [7].

Materials and methods

Patients

Both cases have been previously published [1, 2, 6]. The first case represents a "composite" RC (woman, 78 years old, right colon) treated at the Legnago Hospital, Verona, Italy (Fig. 1a) [1]. The second case is a "pure" RC (woman, 71 years old, right colon) diagnosed at the Rummo Hospital, Benevento, Italy [2]. Both carcinomas showed $BRAF^{mut}$, $SMARCB1^{wt}$, $CROCC^{mut}$, and MSI-H genotypic profile. Both patients died within 8 months [1, 2, 7]. All investigations were carried out in accordance with the Helsinki Declaration and the protocol was approved by the Ethical Committee "Comitato Etico per la Sperimentazione Clinica—CESC" (Ethical Committee Approval PROT. n. CA 2207 2016, September 2016).

Electron microscopy

We investigated the ultrastructure appearance of both RC cases by means of SEM and TEM. For TEM, specimens were recovered from formalin-fixed samples. The tissue was cut into fragments with a razor blade. The fragments were fixed in 2% glutaraldehyde in Sorensen buffer with pH 7.4 for 2 h, post fixed in 1% Osmium tetroxide (OsO4) in aqueous solution for 2 h, dehydrated in graded concentrations of Acetone, and embedded in Epon-Araldite mixture (Electron Microscopy Sciences, Fort Washington, PA). The semithin sections (1-μm thickness) were examined by light microscopy and stained with toluidine blue. The ultrathin sections were cut at 70-nm thickness and placed on Cu/Rh grids with Ultracut E (Reichert, Wien, Austria). After staining with lead citrate, specimens were observed with Morgagni 268D Electron Microscope (Philips).

For SEM, histological sections previously stained with hematoxylin and eosin (H-E), were reused. After removal of the coverslip with xylene, the samples were brought to absolute alcohol, processed by critical point drying (CPD 030, Balzers, Vaduz, Liechtenstein), mounted on stubs with colloidal silver, sputtered with gold by means of an MED 010 coater (Balzers), and examined under an FEI XL30 scanning electron microscope (FEI Company, Eindhoven, NL).

Results

At TEM examination, the nuclear envelope of rhabdoid cells appears preserved and nuclear pores are often visible. Sometimes, nuclei present irregular contours. Nucleoli are often large; they may lie at the nuclear periphery (nucleolar margination) and show abnormal arrangements of their major components (Fig. 1b). Their large size, indicating rapid RNA turnover, perhaps reflects heightened synthetic activity necessary for cell replication [10, 11]. The cytoplasm, even if not well preserved by the recovering procedure from formalin-fixed samples, clearly shows abundant paranuclear aggregates of intermediate filaments, pathognomonic feature of the RC, often presenting focal densities (Fig. 1b,c) as reported in the literature [9].

Nuclear morphology and the large nucleoli often lying at the nuclear periphery are clearly visible also by means of SEM technique (Figs. 1d, 2c,d, and 3). In addition, the intercellular spaces, morphologically "empty" at light microscopy (Fig. 1a), show clear cellular protrusions (lamellipodia) from the cell edges (Figs. 1d, 2c,d, and 3).

Discussion

Over the past 20 years, only 34 cases of colorectal carcinomas with a rhabdoid phenotype have been reported [7]. This peculiar morphology stands for an independent adverse prognostic

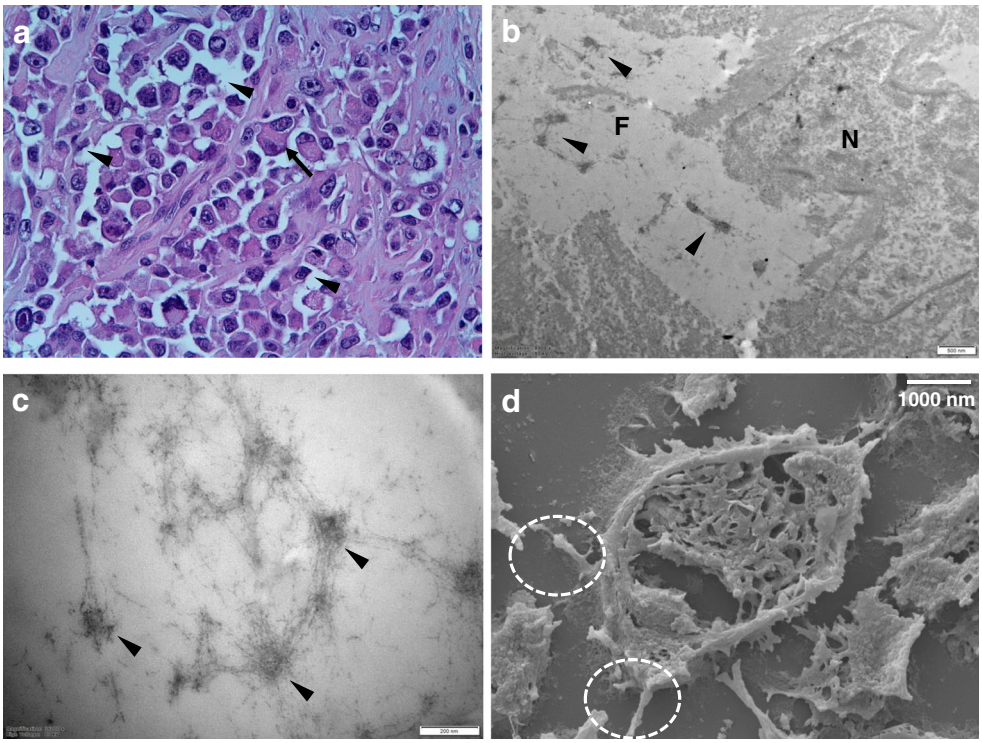

Fig. 1 Rhabdoid colorectal carcinoma light microscopy showing neoplastic cells with eccentrical nucleus and prominent nucleoli (rhabdoid). *Arrow heads* indicate the "empty" intercellular spaces; the *arrow* indicates a binucleated rhabdoid cell (panel A). TEM images show dense paranuclear aggregates of intermediate filaments (*arrow heads*) (panels B, C). SEM image shows some protrusions similar to lamellipodia in intercellular spaces (*circles*, panel D)

factor in colorectal carcinoma regardless of the tumor staging [2, 4, 7]. The main histological differential diagnosis is with the malignant extrarenal rhabdoid tumors, a neoplasm more common in childhood characterized by genetic inactivation of *SMARCB1* (SNF5, INI-1), a component of the SWI/SNF chromatin remodeling complex, or deletions at chromosome 22q [12]. It has been demonstrated that RC and malignant extrarenal rhabdoid tumor represent two distinct biologic entities that share rhabdoid morphology and sometimes INI1 immunohistochemical alterations [7]. Excluding malignant extrarenal rhabdoid tumors, some kind of neoplasms may lose INI1 immuno-expression but in these cases, the mechanism underlying the process consists mainly of

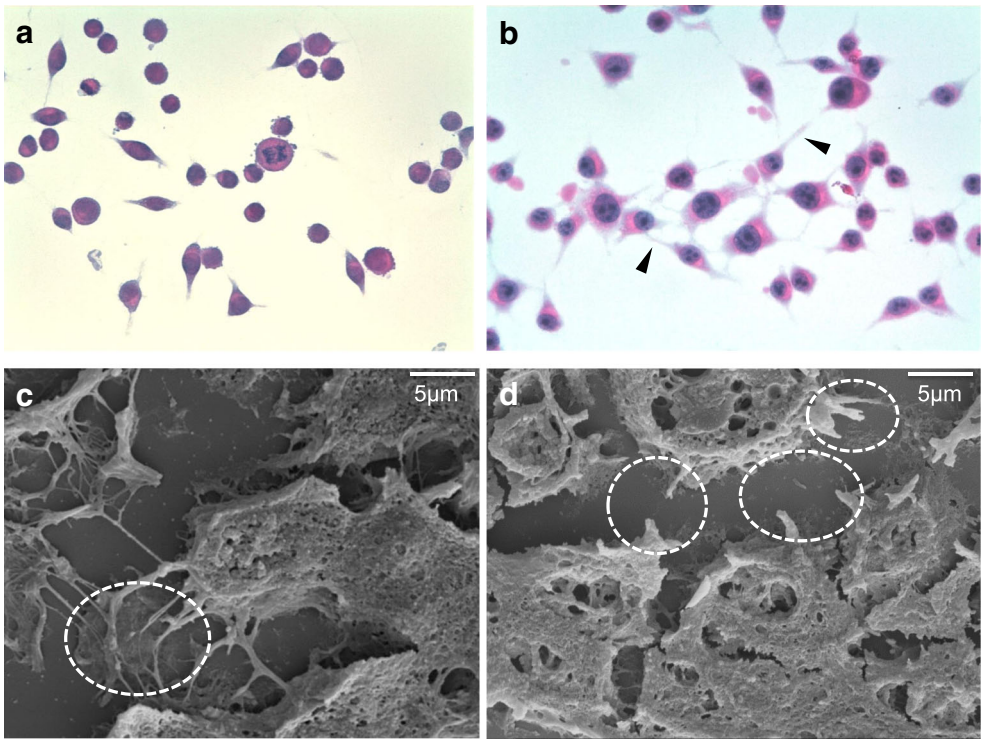

Fig. 2 In vitro RKO intestinal neoplastic cells (BRAF*mut*, CROCC*wt*, and MSI-H) with normal CROCC gene (panel A) and in which the CROCC gene was silenced (panel B). The CROCC-deficient cells (panel B) exhibit rhabdoid features and evident protrusions (*arrow heads*) similar to projections revealed in ex vivo samples by SEM (*circles*) (panels C, D)

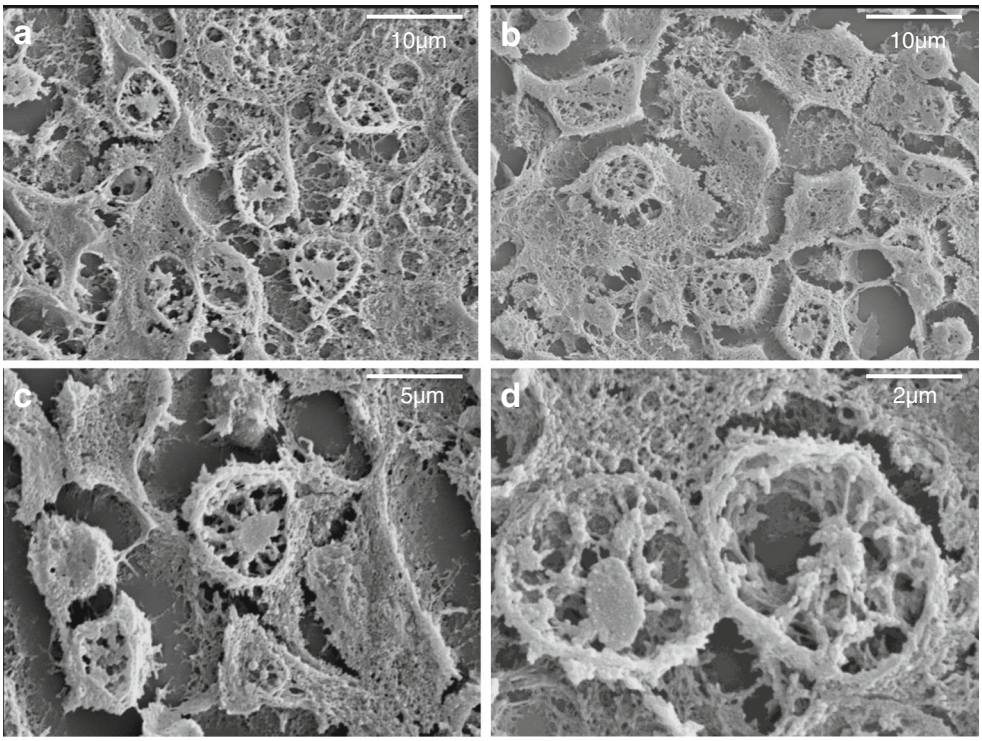

Fig. 3 Rhabdoid colorectal carcinoma ex vivo samples studied by SEM technique showing neoplastic nuclei with nucleoli and nuclear abnormal arrangements, beyond lamellipodia in intercellular spaces (panels A, B, C). Details on two nuclei with the aforementioned features (panel D)

the epigenetic events or a functional feedback, as hypothesized in RC [3, 7].

Recently, a series of 7 RC (including the current cases) has been associated with alterations in the centrosome structure and in particular in the gene called *CROCC* (*Ciliary Rootlet coiled coil*; or *ROLT*, *TAX1BP2*) [7]. *CROCC* alterations are not fully unraveled yet and have been reported in human in: RC [7], adult T cell leukemia [13], and hepatocellular carcinoma [14]. Further studies are necessary to define its role in human diseases. Our previous experiments in vitro by means of cultured cells [7] showed that RKO intestinal neoplastic cells with *CROCC* silencing have abnormal chromosome segregation and higher frequency of monopolar spindles as compared with control cell line (RKO—$BRAF^{mut}$, $SMARCB1^{wt}CROCC^{wt}$, and MSI-H). These RKO *CROCC*-deficient cells exhibit all cardinal signs of rhabdoid features at light microscopy, displaying huge nuclei pushed to the periphery of the cells, with single or multiple large nucleoli associated with eosinophilic cytoplasmic inclusions, resembling the morphology we observed here in RC ultrastructural ex vivo specimens. At light microscopy, cellular protrusions (lamellipodia) from the cell edge are evident with higher frequency in RKO $CROCC^{mut}$ lacking in RKO $CROCC^{wt}$ cells [Fig. 2a,b]. Here, SEM evaluation of $CROCC^{mut}$ RC ex vivo specimens showed, for the first time, similar projections. For this reason, we hypothesize that these projections are not determined by the absence of cell contacts in a free environment but rather by genetic alterations.

These cellular protrusions usually promote diverse processes of tumor cell migration and invasion determining neoplastic metastatic capacities [15] and may represent a pathogenetic determinant of RC aggressiveness. TEM evaluation showed rhabdoid features consisting of dense paranuclear aggregates of intermediate filaments and highlights a new feature as the nucleolar margination.

In conclusion, this RC study by transmission electron microscopy confirms the diagnostic presence of intermediate cytoplasmic filaments distinctive of this kind of neoplasm, in association with nucleolar margination. In addition, the presence of cellular projections in the intercellular spaces visible at SEM technique for the first time and not clear at light microscopy allows us to consider an interesting morphological feature in RC $CROCC^{mut}$ to investigate in the future. The presence of these protrusions *CROCC*-related may be involved in the pathogenetic mechanism of the aggressive rhabdoid behavior.

Author contribution Remo A. conceptualization, investigation, writing—original draft, and writing, review and editing, Project administration; Cecchini MP. conceptualization, investigation, writing—original draft, and writing, review and editing; Benati D. Technical support TEM-Methodology and image interpretation; Bernardi P. Technical support SEM—methodology; Manfrin E. review and editing; Giordano G. molecular profile—investigation; Bonomi F. TEM—SEM image—investigation; Parcesepe P. review and editing; Fassan M. Molecular approach—methodology; Colombari R. histology in vitro *and* ex vivo—methodology; Sbarbati A. Image ultrastructural interpretation—methodology; Pancione M. cellular investigation.

Compliance with ethical standards

The study was approved by the Hospital Ethical Committee "CESC" (Ethical Committee Approval PROT. n. CA 2207 2016, September 2016) and conducted in accordance with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

Disclaimer Each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Remo A, Zanella C, Molinari E, Talamini A, Tollini F, Piacentini P, Battaglia P, Baritono E, Bonetti A, Lanza F, Fasolin A, Manfrin E, Vendraminelli R (2012) Rhabdoid carcinoma of the colon: a distinct entity with a very aggressive behavior: a case report associated with a polyposis coli and review of the literature. Int J Surg Pathol 20(2): 185–190. https://doi.org/10.1177/1066896911415405
2. Pancione M, Remo A, Sabatino L, Zanella C, Votino C, Fucci A, Di Blasi A, Lepore G, Daniele B, Fenizia F, Molinari E, Normanno N, Manfrin E, Vendraminelli R, Colantuoni V (2013) Right-sided rhabdoid colorectal tumors might be related to the serrated pathway. Diagn Pathol Feb 20(8):31. https://doi.org/10.1186/1746-1596-8-31
3. Agaimy A, Rau TT, Hartmann A, Stoehr R (2014 Jul) SMARCB1 (INI1)-negative rhabdoid carcinomas of the gastrointestinal tract: clinicopathologic and molecular study of a highly aggressive variant with literature review. Am J Surg Pathol 38(7):910–920
4. Agaimy A, Daum O, Markl B, Lichtmannegger I, Michal M, Hartmann A (2016) SWI/SNF complex–deficient undifferentiated/rhabdoid carcinomas of the gastrointestinal tract A series of 13 cases highlighting mutually exclusive loss of SMARCA4 and SMARCA2 and frequent co-inactivation of SMARCB1 and SMARCA2. Am J Surg Pathol 40(4):544–553
5. Remo A, Pancione M, Zanella C, Vendraminelli R (2012) Molecular pathology of colorectal carcinoma. A systematic review centred on the new role of the pathologist. Pathologica 104(6):432–441 Review
6. Pancione M, Remo A, Zanella C, Sabatino L, Di Blasi A, Laudanna C, Astati L, Rocco M, Bifano D, Piacentini P, Pavan L, Purgato A, Greco F, Talamini A, Bonetti A, Ceccarelli M, Vendraminelli R, Manfrin E, Colantuoni V (2013) The chromatin remodelling component SMARCB1/INI1 influences the metastatic behavior of colorectal cancer through a gene signature mapping to chromosome 22. J Transl Med 28(11):297. https://doi.org/10.1186/1479-5876-11-297
7. Remo A, Manfrin E, Parcesepe P, Ferrarini A, Han HS, Ugnius M, Laudanna C, Simbolo M, Malanga D, Mendes Oliveira D, Baritono E, Colangelo T, Sabatino L, Giuliani J, Molinari E, Garonzi M, Xumerle L, Delledonne M, Giordano G, Ghimenton C, Lonardo F, D'angelo F, Grillo F, Mastracci L, Viglietto G, Ceccarelli M, Colantuoni V, Scarpa A, Pancione M (2018) Centrosome linker-induced tetraploid segregation errors link rhabdoid phenotypes and lethal colorectal cancers. M Mol Cancer Res. https://doi.org/10.1158/1541-7786.MCR-18-0062
8. Biegel JA, Busse BT, Weissman BE (2014) SWI/SNF chromatin remodeling complexes and cancer. Am J Med Genet 166C:350–366
9. Kono T, Imai Y, Imura J, Ono Y, Hagiwara S, Taira K, Fujita M, Tsubaki M, Sunagawa M, Fujimori T (2007) Cecal adenocarcinoma with prominent rhabdoid feature: report of a case with immunohistochemical, ultrastructural, and molecular analyses. Int J Surg Pathol 15(4):414–420
10. Henderson DW, Papadimitriou JM, Coleman M (1986) Ultrastructural appearance of tumours. Diagnosis and classification of human neoplasia by electron microscopy, 2nd edn. Churchill Livingstone, Edimburgh
11. Ghadially FN (1988) Ultrastructural pathology of the cell and matrix. A text and atlas of physiological and pathological alterations in the fine structure of cellular and extracellular components, 3rd edn. Butterworths, London
12. Fletcher C, Bridge J, Hogendoornn P, Mertens F (2012) WHO classifications of tumours of soft tissue and bone. IARC Press, Lyon
13. Pumfery A, de la Fuente C, Kashanchi F (2006) HTLV-1 tax: centrosome amplification and cancer. Retrovirology 9(3):50
14. Lai WL, Hung WY, Wong LL, Zhou Y, Leong VY, Lee JM, Ng IO, Jin DY, Ching YP (2012) The centrosomal protein Tax1 binding protein 2 is a novel tumor suppressor in hepatocellular carcinoma regulated by cyclin-dependent kinase 2. Hepatology 56(5):1770–1781. https://doi.org/10.1002/hep.25851
15. Alblazi KM, Siar CH (2015) Cellular protrusions–lamellipodia, filopodia, invadopodia and podosomes–and their roles in progression of orofacial tumours: current understanding. Asian Pac J Cancer Prev 16(6):2187–2191