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CASE REPORT

M. A. den Bakker · S. J. Flood · M. Kliffen

CD31 staining in epithelioid sarcoma

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Abstract We report an unusual case of epithelioid sarcoma. The tumour occurred in the finger of a 27-year-old female. The clinical history, histology and the electron microscopy of the lesion were typical for epithelioid sarcoma. However, immunohistochemical analysis showed strong membranous CD31 staining, a finding hitherto not described. All other robust vascular markers, including factor-VIII-related antigen (FVIIIrag) were negative. The findings were compared with the available literature data, leading us to conclude that there is insufficient evidence for endothelial derivation of epithelioid sarcoma, but in the differential diagnosis with vascular tumours CD31 may stain and to rule out angiosarcoma FVIIIrag is a useful antibody.

Keywords Epithelioid sarcoma · CD31 · Immunohistochemistry

Introduction

Epithelioid sarcoma (ES) is a rare but distinctive soft tissue tumour, which most frequently arises in the extremities of young individuals [3, 5]. The classical type of ES often presents as a cutaneous lesion and may be mistaken for a benign process. Histologically, ES is composed of nodular aggregates of monomorphic intermediate sized cells often with eccentrically placed nuclei and deeply eosinophilic cytoplasm, imparting a rhabdoid appearance to the tumour cells. Epithelioid sarcoma tumour nodules frequently form granulomatous structures, which may have central necrosis. The differential diagnosis of ES includes carcinoma and epithelioid mesenchymal tumours. Cutaneous epithelioid angiosarcoma and the recently described tumour epithelioid sarcoma-like hemangioendothelioma (ES-H) are often considered on morphological grounds. In particular ES-H, which shares considerable morphological and immunohistochemical overlap with classical ES needs to be differentiated [2]. In ES, immunoreactivity for cytokeratin and epithelial membrane antigen (EMA) is almost invariably present. CD34 staining, present in most cases of ES, is valuable in ruling out carcinoma. The CD31 marker is often quoted as useful when distinguishing between epithelioid sarcoma and epithelioid angiosarcoma but is positive in ES-H. Although CD31 expression has been described in ES, it is reported as focal and non-membranous. We report a case of epithelioid sarcoma with typical clinical and morphological features but with unusual immunohistochemical findings.

M. A. den Bakker (✉) · M. Kliffen
Department of Pathology, Erasmus Medical Center,
PO Box 1738, 3000 DR Rotterdam, Netherlands
e-mail: m.denbakker@erasmusmc.nl
Tel.: +31-10-4087901, Fax: +31-10-4089487

S. J. Flood
Department of Plastic and Reconstructive Surgery,
Erasmus MC, Rotterdam, Netherlands

Clinical history

A 27-year-old female presented with an ill-defined mass in the soft tissue of the middle phalanx of the fourth finger of the right hand, which had slowly increased in size in a period of 3 years. The clinical impression raised the possibility of a giant cell tumour. An excisional biopsy was initially diagnosed as an inflammatory granulomatous lesion. However, the lesion recurred shortly after removal with ulceration of the skin. Histological review of the original specimen revealed histological findings in keeping with an ES. The finger was subsequently amputated in continuity with the metacarpal bone and submitted for pathological evaluation.

Materials and methods

The amputation specimen was submitted fresh; tumour tissue was reserved for frozen sections and small samples were placed in formalin—glutaraldehyde for electron microscopy (EM). Routine histological haematoxylin and eosin (H&E) stained sections from formalin-fixed paraffin-embedded tissue were prepared and, in

Table 1 Immunohistochemical reagents and staining results. Tissue: *P* paraffin, *F* frozen. Pre-treatment: *w* wet heat antigen retrieval, *pr* protease digestion. Result: ++ strong universal staining, + focal staining, variable intensity, – no staining, *m* membranous, *c* cytoplasmic, *n* nuclear

Antibody (clone)	Manufacturer	Tissue, pretreatment	Dilution	Result
Cytokeratin 8,18 (NCL5D3)	Biogenex, San Ramon, California, USA	P, pr	1:100	++ c
Pancytokeratin (AE1/AE3)	Biogenex	P, w	1:100	++ c
Cytokeratin 19 (RCK108)	Euro-diagnostica, Arnhem, Netherlands	P, w	1:20	++ c
EMA (E29)	Dako, Glostrup, Denmark	P,w	1:2000	++ c
Desmin (D33)	Dako	P, w	1:400	–
Muscle actin (HHF35)	Dako	P, w	1:400	+ c
Vimentin (V9)	Dako	P, w	1:3200	++ c
S100 (rabbit polyclonal)	Dako	P, –	1:2000	–
p53 (D-O7)	Dako	P, w	1:1000	+ n
CD99 (12E7)	Dako	P, w	1:100	++ m
CD31 (JC70A)	Dako	P, w	1:240	++ m
FVIIIrag (2C8)	Dako	P, pr	1:1000	–
CD34 (QBEnd10)	Dako	P, w	1:100	++ m
Fli-1 (polyclonal)	Santa Cruz Biotechnology Inc. Santa Cruz, California, USA	P, w	1:50	+n
EGF-R (H11)	Dako	F	1:80	++ m
VEGF-R1 (Flt-19)	Sigma, Saint Louis, Missouri, USA	F	1:80	–
VEGF-R2 (KDR-1)	Sigma	F	1:400	–
VEGF (JH121)	Oncogene Research Products, San Diego, California, USA	F	1:20	–
Thrombospondin (46.4)	Oncogene Research Products	F	1:320	–
CD146 (P1H12)	Chemicon International Inc. Temecula, California, USA	F	1:100	–
PAL-E	Monosan, Sanbio, Uden, Netherlands	F	1:80	–

addition, PAS and reticulin stains were prepared from selected blocks. Immunohistochemistochemistry was performed in an automated immunostainer on paraffin-embedded tissue and on acetone-fixed cryostat sections, using a polymer based system (Dako Envision, Dako, Glostrup, Denmark; Table 1)

Pathological findings

A nodular and ulcerated area was seen in soft tissues and skin of the middle phalanx, over an area of approximately 1.5×1 cm, extending over the dorsal, lateral and ventral regions (Fig. 1). Grey, shiny firm tissue was seen extending from the ulcerated surface down to the bone but without macroscopic invasion of the tendon or bone. The borders were ill defined; lesional tissue did not appear to involve the joint capsule.

The histology of the excisional biopsy and the amputation specimen were similar. A diffuse and nodular, focally granulomatous appearing lesion composed of intermediate sized cells was seen embedded in collagenous stroma and infiltrating diffusely in muscle and connective tissue. Angio-invasion was clearly present. The cells formed a monotonous population with many rhabdoid cells. Typical "granulomatous" structures with central necrosis were present (Fig. 2A). Fascicular areas were not observed and the cellular composition was exclusively epithelioid in nature.

Fig. 1 Amputation specimen with ulcero-nodular lesions over the mid-phalanx

Immunohistochemical staining

The immunohistochemical results are summarised in Table 1. The tumour cells showed intense staining with cytokeratin antibodies AE1/AE3, NCL5D3 (cytokeratins 8, 18) and cytokeratin 19 (Fig. 2B). Strong EMA staining was seen and the tumour cells expressed CD34 (Fig. 2C) and CD99 with a membranous pattern. Surprisingly, in the biopsy, all tumour cells stained strongly with CD31 antibodies. The staining pattern was membranous and consistent throughout the sample (Fig. 2D). In the amputation specimen, the staining was more variable. However, most tumour cells were positive again with membranous staining. Additional vascular markers were tested, including PAL-E, CD146, factor-VIII-related antigen (FVIIIrag), vascular endothelial growth factor (VEGF), VEGF receptors 1 and 2 and thrombomodulin, these were all negative. Nuclear staining of varying intensity was seen in Fli-1 immunostaining.

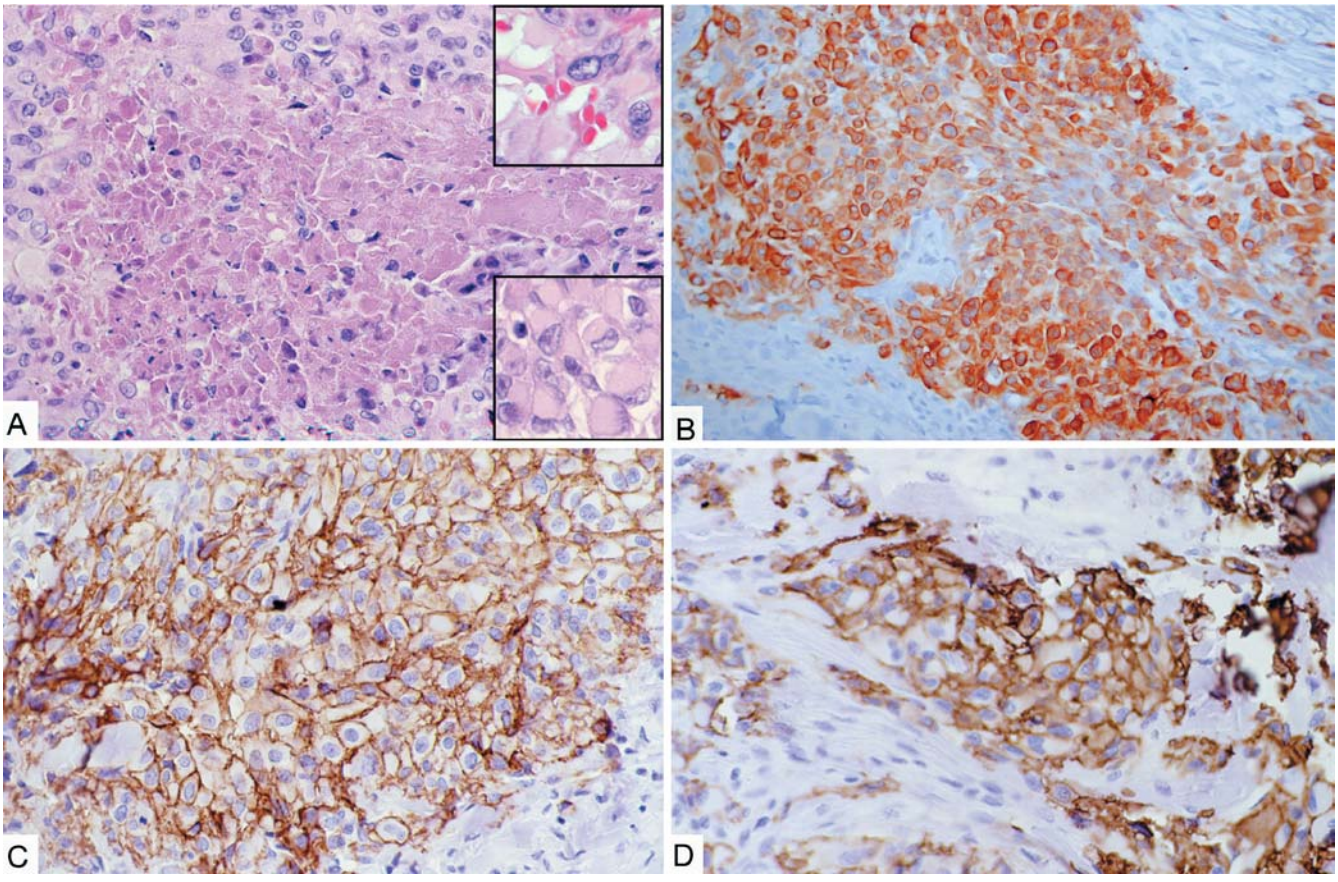

Fig 2 Light microscopy (amputation specimen). **A** Granulomatous structure with central necrosis formed by rhabdoid tumour cells (*lower inset*), occasionally with cytoplasmic vacuolisation (*upper inset*) (H&E staining, ×100, *insets* ×400 magnification). **B** Immunohistochemical staining for cytokeratin 19, cytoplasmic staining is seen (×100 magnification). **C** CD34 staining. Intense membranous staining is present (×100 magnification). **D** Immunohistochemical staining for CD31 shows distinct membranous staining (×100 magnification)

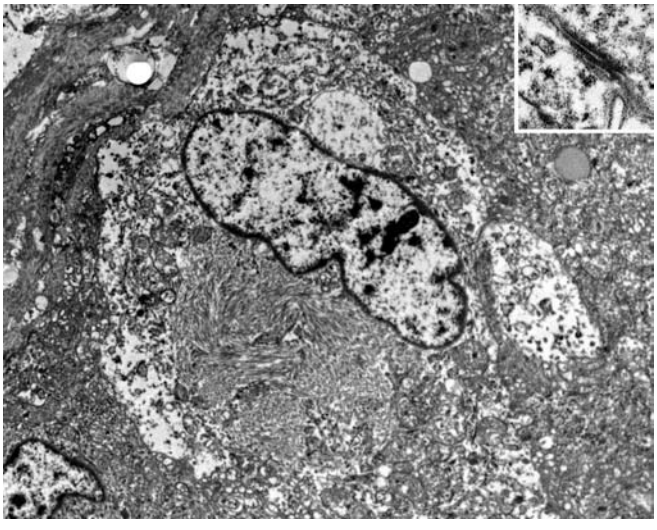

Fig. 3 Electron microscopy. Compact peri-nuclear whorls of intermediate filaments are seen. *Inset* Desmosomes were easily identified in the tumour

Electron microscopy

Compact perinuclear whorled aggregates of filaments were seen in many cells. Well-formed desmosomes were easily identified (Fig. 3). Weibel-Palade bodies were not observed.

Discussion

Epithelioid sarcoma is a unique soft tissue tumour of which the origin is not clear. Two distinct subtypes have been described. The classical form typically occurs in the superficial soft tissues of the distal parts of the extremities [3, 5]. A recently described second form, designated the proximal type of ES, tends to occur in deeper soft tissue of the limb girdles [9, 10]. The long-term prognosis of ES is poor, with local recurrences preceding distant metastases [3]. The typical histological appearance of ES consists of aggregates of monomorphic cells with well-defined cell boundaries and little nuclear pleomorphism. The cytoplasm is eosinophilic and often imparts a rhabdoid appearance to the cells, which on the ultrastruc-

tural level is caused by peri-nuclear aggregates of filaments. A characteristic feature of ES is areas mimicking granulomas, often with central necrosis. Cytoplasmic vacuoles and pseudo-vascular spaces may be seen in ES raising the differential diagnostic possibility of an (epithelioid) vascular lesion sometimes referred to as "angiomatoid ES" [3, 5, 9, 20, 21]. Immunohistochemical findings in ES include staining with epithelial markers such as EMA or cytokeratins and CD34. The latter marker is very useful to rule out metastatic carcinoma. However, in differentiating ES from angiosarcoma, CD34 staining cannot discriminate and, in addition, cytokeratin staining is well recognised in epithelioid angiosarcoma. Although EMA staining is unusual in angiosarcoma, this too has been described [7, 12]. Antibodies to the CD31 antigen PECAM (platelet endothelial cell adhesion molecule) are deemed to be highly specific vascular markers and are often used to mark endothelial derived tumours [4, 14]. In keeping with the nature of PECAM as an adhesion molecule, specific staining by CD31 is membranous. In the case we describe here, strong and consistent membranous CD31 staining was seen in the biopsy specimen and the resection specimen. Weak CD31 immunoreactivity was observed in five of 70 (7%) typical ESs and two of eight angiomatoid ESs in a large series by Miettinen et al. [15]. Other workers have also described positive CD31 staining in peripheral ES in a small percentage of cases [17, 18]. However, in the proximal type CD31 is reported as negative [9, 10]. In all published reports with positive CD31 staining in ES it has been focal and cytoplasmic, not membranous. Because of this unusual finding, we extended our panel of antibodies to include other recognised vascular markers (Table 1). With the exception of Fli-1, a recently described marker of vascular endothelium, no staining was observed [8]. In particular FVIIIrag staining was completely negative. This is consistent with data from the literature where FVIIIrag staining in ES has not been described [1, 3, 9, 11, 15, 18, 19, 21]. A close mimic of ES was recently described by Billings et al. [2]. These authors report on seven cases of a novel vascular tumour entity, which show markedly overlapping features with ES. Due to the absence of metastases indicating low-grade behaviour, it is suggested that this tumour is a new form of hemangioendothelioma for which the name epithelioid sarcoma-like hemangioendothelioma (ES-H) is coined. The clinical presentation and architecture of ES-H is similar to classical ES, although a fascicular and sheet-like growth pattern was also noted, which is not typical for ES. Although the authors note that the cells of ES-H have an epithelioid shape with prominent eosinophilic cytoplasm, they do not describe the typical rhabdoid inclusions, which characterise classical ES, caused by the perinuclear condensation of intermediate filaments. Similar to true vascular neoplasms, ES often has intracytoplasmic lumina, as we describe in the case presented here. In the cases of ES-H, this was an infrequent finding and may be a useful morphological clue when distinguishing these entities. Remarkably, all six cases of ES-H that were analysed immunohistochemically were positive for CD31 (membranous pattern) and for Fli-1 (nuclear) but did not stain for CD34. These observations support an endothelial derivation of this tumour and are similar to the results of the ES case presented here. The Fli-1 antibody is highly specific for Ewing sarcoma and in a single study was found to be a specific marker for endothelium-derived tumours. However, it also stains a small number of non-vascular tumours. At this time, there is not enough experience with this antibody to comment on its use in epithelioid sarcoma [8]. Although the initial series of ES-H is small, CD34 may still serve as a distinguishing immunostain as all cases of ES-H were negative. Unfortunately, EM was not performed on the ES-H cases. The presence of Weibel-Palade bodies and pinocytic vesicles would have strengthened the case for a vascular origin of ES-H. In our case presented here, electron microscopy did not reveal features suggesting endothelial differentiation and were otherwise consistent with ES with perinuclear whorls of intermediate filaments and desmosomes [6, 9, 13, 16]. It is noted that immunohistochemistry has a critical role in distinguishing ES- and ES-H and that CD31 and Fli-1 are important discriminators [2]. We thus conclude that the case of ES described here is highly unusual in its immunohistochemical profile and because of its distinctive CD31 staining and Fli-1 positivity could be mistaken for an angiosarcoma or for the newly described ES-H [2]. However, the morphological features, EM findings and CD34 positivity permit a confident diagnosis of ES. In cases where the differential diagnosis includes ES-H and angiosarcoma, careful attention should be paid to distinguishing morphological features. Addition of FVIIIrag to an immunohistochemical panel in cases where the differential diagnosis includes angiosarcoma is indicated.

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