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ERG EXPRESSION IN EPITHELIOID SARCOMA – A DIAGNOSTIC PITFALL

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Abstract

ERG transcription factor is constitutively expressed in endothelial cells. Because benign and malignant vascular endothelia retain the ERG-expression, ERG is considered a useful marker for angiosarcomas and related tumors. ERG is also expressed in a subset of prostate carcinomas and Ewing sarcomas due to ERG-involving translocations, so that this marker is also of high interest for the study of these malignancies. In this study, we evaluated 109 epithelioid sarcomas for ERG expression, based on an initial observation of an ERG-positive case. We also studied expression of other endothelial antigens in epithelioid sarcoma. ERG was expressed in 38% of epithelioid sarcomas (41/109), usually with a uniform nuclear staining, similar to that seen in angiosarcomas. However, all epithelioid sarcomas were negative for ERG gene rearrangement indicating that that ERG expression is not likely related to ERG involving translocations in epithelioid sarcoma. Other endothelial markers, CD31, claudin 5, and Prox1 were absent in epithelioid sarcomas. The only exception was a pulmonary metastasis of epithelioid sarcoma showing focal CD31 expression, which was probably resulting from antigen adsorption onto tumor cell surfaces. However, podoplanin was commonly (7/9) expressed in epithelioid sarcoma, so that this marker is not useful in the distinction of epithelioid sarcoma and angiosarcoma. INI1/SMARCB1 gene product was absent in all epithelioid sarcomas (considered here a definitional feature) but was absent from only one epithelioid angiosarcoma, indicating its relative specificity for epithelioid sarcoma in this differential diagnostic setting. ERG expression is fairly common in epithelioid sarcoma, and should be recognized as a diagnostic pitfall in the differential diagnosis of epithelioid sarcoma and epithelioid angiosarcoma. General lack of endothelial cell specific markers in epithelioid sarcoma helps in this distinction.

Keywords

epithelioid sarcoma; angiosarcoma; ERG; INI1; SMARCB1; immunohistochemistry

INTRODUCTION

ERG, an ETS-family transcription factor is constitutively expressed in endothelial cells. It regulates endothelial cell differentiation, angiogenesis, and expression of several endothelial-specific antigens and is also required for embryonic stem cells to differentiate into endothelial cells.^{1–5}

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Availability of a highly specific monoclonal antibody to ERG developed at the Center for Prostate Disease Research opened ERG for immunohistochemical exploration.^{6–8} Nearly consistent expression of ERG in benign and malignant endothelial proliferations makes ERG a valuable marker in the diagnostic evaluation of endothelial neoplasms including hemangiomas, hemangioendotheliomas and angiosarcomas. ERG is also expressed in myeloid precursors, and this probably explains ERG expression in myeloid sarcomas.⁸

In addition, ERG is a known immunohistochemical marker for a subset of prostate carcinomas, in which ERG-expression correlates with ERG expression-activating oncogenic translocations, most commonly TMPRSS2-ERG fusions.^{7,9–11} ERG-involving translocations also occur in a small subset of Ewing sarcoma, and this largely correlates with ERG-expression in this sarcoma.¹²

Because we recently encountered ERG-expression in an epithelioid sarcoma, we examined ERG-expression in a large group of epithelioid sarcomas, and found nearly 40% of cases were positive. Herein, we describe these finding that have to be considered a diagnostic pitfall when using ERG immunohistochemistry in the diagnosis of vascular and other tumors.

MATERIAL AND METHODS

Study material

Well-documented epithelioid sarcomas (n = 109) were arranged in multitumor blocks containing 5–40 different cases. By definition, immunohistochemical negativity for INI1/SMARCB1 gene product was required for diagnosis. Angiosarcomas of skin and soft tissue sites (n = 99) were studied in comparison using similar multitumor blocks.

Antibodies and immunostaining

Primary mouse monoclonal antibody to ERG transcription factor (clone 9FY) was obtained from Biocare Medical (Concord, CA) and used in a dilution of 1:200. Other antibodies used for characterization of epithelioid sarcomas and angiosarcomas were: CD31, CD34, claudin 5, EMA, INI1/SMARCB1 gene product, keratins AE1/AE3, keratin 19, podoplanin (D240), and Prox1 (Table 1).

Immunostaining was performed in a Leica-Bond Max automated immunostainer (Leica, Bannockburn, IL). Primary antibody was incubated for 30 min, followed by Bond Max polymer detection reagent (15 min). Diaminobenzidine was used as a chromogen, followed by a light hematoxylin counterstain.

FISH analysis of the ERG gene translocation on paraffin-embedded tissue sections and tissue microarrays

ERG gene rearrangement was examined by interphase FISH in 16 epithelioid sarcomas and 4 angiosarcomas. For tumor interphase FISH analysis, the TMPRSS2-ERG DNA probe was purchased from Empire Genomics (Buffalo, NY, Cat. # CAT-00271). FISH assays were performed on 5 micron Formalin-Fixed-Paraffin-Embedded (FFPE) tumor sections using laboratory standardized protocol with slight modifications.¹³ In brief, 5 µm thick sections of FFPE tissue blocks were deparaffinized and rehydrated. Antigen retrieval was performed with IHC-Tek Epitope Retrieval Solution (IHC World, Woodstock, MD) with steaming for 25 min. After cooling, slides were subjected to 50 µg/ml pepsin treatment at 37°C, rinsed in PBS solution followed by dehydration in ethanol series. Co-denaturation of the probe and target DNA at 73°C in HYBrite (Abbott Molecular, Chicago, IL) for 5 min was followed by overnight hybridization at 37°C. The next day, slides were washed at 72°C in 0.4X SSC/

0.3% Tween-20 for 2 min and then in 2X SSC/0.1% Tween-20 at room temperature for 1 min. The slides were counterstained, mounted with DAPI/Antifade (Vector Laboratories, Burlingame, CA) and analyzed on the BioView Duet-3 fluorescent scanning station using 63X-oil objective and DAPI/FITC/Rhodamine single band pass filters (Semrock, Rochester, NY). At least, 100 tumor cell nuclei were scored for each specimen. A total of minimum 100 nuclei were scored for each specimen. Cut-off value of 10% was used for the assessment of the ERG gene rearrangement FISH results.

RESULTS

Epithelioid sarcomas

The 109 epithelioid sarcomas studied here occurred in 71 males and 33 females with a median age of 28 years (range: 4–74 years). Data were not available of five patients. The tumors occurred in the upper extremity (n = 57), lower extremity (n = 21), genital region (n = 11), and trunk wall (n = 7). There were also 5 metastases (2 lungs, and one each in the scalp, meninges, and retroperitoneum). The location was not specified for 8 tumors. At least some keratin-positivity was detected in 106 cases (91/98 positive for AE1/AE3, and 73/79 for keratin 8). Positivity for EMA was present in 84/86 cases, typically extensively. CD34 was detected in 51/98 epithelioid sarcomas. All 91 epithelioid sarcomas studied were negative for claudin 5, and all 85 cases studied were negative for Prox1. Most cases studied (7/9) showed extensive podoplanin immunostaining.

Nuclear positivity for ERG was detected in 41/109 cases (38%) (Figs. 1, 2). The estimated percentage of positive tumor cells varied 5–100% (median, 30%). Among the ERG-positive examples were 2/5 of rhabdoid large cell variants (“proximal type epithelioid sarcoma”), and 1 of 3 pseudoangiomatoid variants. All epithelioid sarcomas studied here lacked tumor cell nuclear positivity for INI1/SMARCB1 gene product (Fig. 1B, Fig. 2B). One ERG-positive epithelioid sarcoma, a hemorrhagic lung metastasis, showed focal CD31-immunostaining, whereas all other epithelioid sarcomas were negative.

In-situ hybridization studies with an ERG break-apart probe showed no evidence for ERG gene rearrangement in any of the 16 epithelioid sarcomas examined (Fig. 3).

The ERG-positive and negative subcohorts of epithelioid sarcoma are compared in Table 2. The comparison shows no major differences in patient age or site distribution, but ERG-positive cases had a greater male predominance (3:1 vs. 2:1). Patterns of expression of keratins and EMA were similar, but CD34-positivity was more common among the ERG-positive cases (60 vs. 48%).

Angiosarcomas

In comparison, 83/86 (97%) cutaneous or soft tissue angiosarcomas were positive for ERG. Only 1/60 angiosarcomas (1.7%) had loss of INI1 nuclear expression (Fig. 4). This tumor was a poorly differentiated epithelioid angiosarcoma of the buttock that also expressed CD31, ERG, keratins AE1/AE3, and claudin 5, but was negative for Prox1. A variable majority of angiosarcomas expressed CD31 (88/89, 99%), Claudin 5 (65/67, 97%), Podoplanin (49/66, 74%), Prox1 (40/67, 60%), and CD34 (38/67, 57%). In situ hybridization showed no ERG gene rearrangement in any of the studied 4 ERG-positive cases.

DISCUSSION

In this study we found ERG-expression in 38% of epithelioid sarcomas, and most of the positive cases had extensive nuclear immunoreactivity. This finding expands the number of

known ERG-positive mesenchymal tumors from endothelial and myeloid ones⁸ and rare Ewing sarcoma variants¹² and has to be considered when applying ERG as a marker for endothelial cell neoplasms, such as hemangioendotheliomas and angiosarcomas. Furthermore, more than half of epithelioid sarcomas are positive for CD34 that is also variably expressed in angiosarcoma. Fortunately, the other endothelial markers: CD31, claudin 5, and prox 1 are discriminatory, as they are expressed in the vast majority of angiosarcomas and not in epithelioid sarcoma, with very rare exceptions. In one epithelioid sarcoma metastatic to lung CD31 was focally detected. In this case, antigen adsorption from a hemorrhagic background is a possible explanation for focal membrane staining.

The biologic significance and origin for ERG expression in 38% of epithelioid sarcomas remains unclear. Although ERG expression could be considered a trait related to endothelial differentiation, the general lack of expression of other endothelial markers (CD31, claudin 5, Prox1) argues against this. Lack of ERG gene rearrangements in epithelioid sarcoma does not support the possibility that ERG expression in this setting is due to an ERG-involving translocation, as is the case in a 40–50% of prostatic carcinomas^{9,10} and 10% of Ewing sarcomas.¹² Similarly, the lack of ERG gene rearrangement in angiosarcomas argues against the expression being related to ERG-involving translocations and supports the idea that ERG expression in angiosarcoma merely reflects the endothelial phenotype.

Podoplanin, usually detected with a D2-40 antibody, is often used as a marker for endothelial cells of lymphatic origin, but a majority of angiosarcomas are also positive.^{14,15} Based on our observations, podoplanin is also expressed in a majority of epithelioid sarcomas. Because it is also expressed in a variety of carcinomas, especially squamous cell carcinomas, mesothelioma, and some mesenchymal tumors^{15,16}, podoplanin expression in epithelioid sarcoma does not have major differential diagnostic value, and neither does it have any specific histogenetic significance.

Keratins and EMA are nearly consistently expressed in epithelioid sarcoma, and furthermore, epithelioid sarcoma has a complex pattern of keratins including common expression of keratin 19 and keratins detected by the AE1/AE3 keratin cocktail. Although angiosarcomas, especially the epithelioid ones and epithelioid hemangioendothelioma, can express keratins, specifically keratin 18 and the former also keratin 8, they are less commonly positive for keratin 19 and keratin cocktail AE1/AE3. Furthermore, angiosarcomas are very rarely positive for EMA, so that these epithelial markers are useful in the differential diagnosis of epithelioid sarcoma versus epithelioid angiosarcoma.¹⁷

INI1/SMARCB1 gene product is a chromatin remodeling protein known to be lost in virtually all epithelioid sarcomas through inactivating mutations and genomic losses involving its locus at 22q11.^{18,19} For the sake of clarity, we considered this feature definitional for epithelioid sarcoma. In fact, our observations (Miettinen et al, unpublished) indicate that the number of potential INI1-positive epithelioid sarcomas is so small that this diagnosis warrants close scrutiny in such instances. Our survey of large numbers of angiosarcomas showed INI1/SMARCB1 protein loss in only 1 case, a soft tissue epithelioid angiosarcoma of the buttock positive for CD31, ERG, and claudin 5. This case also indicates that INI1/SMARCB1 loss cannot be considered totally pathognomonic for epithelioid sarcoma necessitating the use of a panel of markers to separate epithelioid sarcoma from angiosarcoma. Loss of INI1/SMARCB1 is also well known in malignant rhabdoid tumor (renal and extrarenal), and atypical teratoid rhabdoid tumor in the central nervous system, and genomic losses involving SMARCB1 locus have also been reported in schwannomatosis.^{20–22}

Epithelioid sarcoma-like hemangioendothelioma, also known as pseudomyogenic hemangioendothelioma is a tumor that can both histologically and immunohistochemically mimic variants of both epithelioid sarcoma and other malignant vascular tumors in its dual positivity for endothelial markers and keratins.^{23,24} Immunohistochemically it is positive for keratins and variably positive for CD31^{18,19}, usually positive for ERG⁸, but negative for CD34.^{23,24} However, it retains INI1/SMARCB1, in strong contrast with epithelioid sarcoma.²⁴

In summary, we show that a significant subset (38%) of epithelioid sarcomas express ERG transcription factor, known to be expressed in endothelial neoplasms. This diagnostic pitfall necessitates the use of a panel of endothelial and epithelial markers to separate epithelioid sarcoma and angiosarcoma. The biologic significance of ERG expression in epithelioid sarcoma remains open, but this is not likely related to ERG-involving translocations, based on negative FISH-results for ERG gene split rearrangement.

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Fig. 1. Epithelioid sarcoma showing a zone of coagulative necrosis. The tumor cells show nuclear positivity for ERG, but lack nuclear INI1-immunoreactivity, in contrast to stromal cells, indicating INI1-loss. CD31 is restricted to vascular endothelial cells.

Fig. 2.
Another example of epithelioid sarcoma showing nuclear ERG-positivity, loss of INI1, and lack of CD31 expression.

Fig. 3. Dual-color interphase FISH of ERG-positive epithelioid sarcoma demonstrates presence of an intact ERG locus and lack of the ERG gene rearrangement (no split of red and green fluorescence signals).

Fig. 4.
A poorly differentiated epithelioid angiosarcoma shows nuclear positivity for ERG, but exceptionally shows loss of INI1. The tumor cells are strongly positive for CD31.

Table 1

Antibodies, their dilution, sources and epitope retrieval modalities. High pH buffer from Leica was used for epitope retrieval for all antibodies.

Antibody	Clone	Source	Catalog#	Dilution
CD31	JC70A	Dako Cytomation, Carpinteria, CA	MO823	1:100
CD34	QBEND/10	Dako Cytomation	M7165	1:100
Claudin 5	Polyclonal	Abcam, Cambridge, MA	Ab53765	1:500
EMA	E29	Dako Cytomation	MO613	1:100
ERG	9FY	Biocare Medical, Concord, CA	CM421	1:100
INI1/SMARCB1	MRQ-27	Cell Marque, Rocklin, CA	CXC311	1:100
Keratins	AE1/AE3	Chemicon, Temecula, CA	MAB3412	1:1500
Keratin 19	RCK108	Dako	MO888	1:200
Podoplanin	D240	Covance/Signet, Princeton, NJ	730-01	1:100
Prox1	Polyclonal	Angiobio, Delmar, CA	11-002P	1:250

Table 2

Comparison between ERG-negative and ERG-positive epithelioid sarcomas.

Category	Age in years Range (median)	Sex (M:F)	Sites U/L/G/T/M/Unk	CD34 positive	AE1/AE3 positive	Ker19 positive	EMA positive
ERG-negative ES (n = 68)	4–74 (30)	41:24	32/12/7/7/4/6	30/63 (48)	55/61 (90)	43/57 (75)	55/57 (96)
ERG-positive ES (n = 41)	15–65 (27)	30:9	25/9/4/0/1/2	21/35 (60)	36/37 (97)	25/37 (68)	29/29 (100)

Sites are abbreviated as: U = upper extremities, L = lower extremities, G = genital region, T = trunk, M = metastases, Unk = unknown. The numbers in parentheses are percentages, except for age, they represent median values.