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BRIEF REPORT

Parvalbumin immunohistochemical expression in the spectrum of perivascular epithelioid cell (PEC) lesions of the kidney

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Abstract

Parvalbumin is a cytosolic calcium-binding protein expressed in the distal convoluted tubule of the renal nephron. Among epithelial renal tumors, the reactivity for parvalbumin is observed in chromophobe renal cell carcinomas and frequently in oncocytomas. On the other hand, there are no data available on parvalbumin expression in the mesenchymal tumors of the kidney. Therefore, the purpose of this study was to evaluate the expression of parvalbumin in the spectrum of PEC (perivascular epithelioid cells) lesions of the kidney. Sixty-six PEC lesions (37 classic angiomyolipomas, 10 microscopic angiomyolipomas, 7 epithelioid angiomyolipomas/pure epithelioid PEComas, 5 leiomyoma-like angiomyolipomas, 3 lipoma-like angiomyolipomas, 2 intraglomerular lesions, 1 angiomyolipoma with epithelial cysts (AMLEC), and 1 sclerosing angiomyolipoma) were immunohistochemically stained with parvalbumin. Overall, parvalbumin immunostain was found in fifty-six PEC lesions (85%) and absent in the remaining ten cases (15%). Classic angiomyolipomas were positive in almost all cases (97%). Intraglomerular lesions and AMLEC showed parvalbumin immunolabeling as well. None of the 7 epithelioid angiomyolipomas/pure epithelioid PEComas or the only sclerosing angiomyolipoma expressed parvalbumin. In conclusion, we demonstrated the immunolabeling of parvalbumin in almost all PEC lesions of the kidney, but not in the epithelioid angiomyolipoma/pure epithelioid PEComa. This finding could shed light on some biological characteristics observed in the PEC lesions such as the plasticity of their cellular component. Moreover, parvalbumin may be another useful tool in the differential diagnosis among epithelioid angiomyolipoma/pure epithelioid PEComa with other renal eosinophilic tumors, such as oncocytoma and chromophobe renal cell carcinoma.

Keywords Angiomyolipoma · Parvalbumin · Epithelioid angiomyolipoma · PEC · PEComa · MITF · Tuberous sclerosis · Immunohistochemistry

Introduction

Parvalbumin is a cytosolic calcium-binding protein of the EF-hand family protein acting as a calcium buffer and calcium transporter/shuttle protein [1]. It has been found in the brain, skeletal and heart muscles, parathyroid glands, and kidney, mainly in the distal convoluted tubule of the renal nephron in which the role of calcium is functionally important [2]. Parvalbumin immunohistochemical expression has been studied in the most frequent renal cell neoplasms, especially eosinophilic renal cell tumors. Reactivity for parvalbumin is constantly observed in almost all chromophobe renal cell carcinomas and roughly 70% of oncocytomas [3–5], two neoplasms rich in mitochondria with differentiation toward the renal distal tubules. Concerning the other most common histotypes, clear cell renal cell carcinomas, as well as the majority papillary renal cell carcinomas, are negative for parvalbumin [6, 7]. Clear cell papillary renal cell carcinoma [7, 8] and MiT family translocation renal cell carcinoma [9] are also reported to be usually negative for this staining. On the other hand, there are no data available on parvalbumin expression in the mesenchymal tumors of the kidney. Among those, angiomyolipoma, a neoplasm belonging to

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Table 1 Clinicopathological and immunohistochemical features of PEC lesions

Case	Age	Gender	Size (cm)	Diagnosis	Parvalbumin (%)
1	27	F	3.5	Classic AML	20
2	80	F	2.5	Classic AML	20
3	56	M	15	Classic AML	1
4	68	F	n.a.	Classic AML	1
5	47	F	5	Classic AML	10
6	70	F	3	Classic AML	0
7	33	F	2.5	Classic AML	20
8	52	F	3	Classic AML	15
9	57	M	2	Classic AML	5
10	58	M	3	Classic AML	15
11	68	F	8.5	Classic AML	30
12	76	F	n.a.	Classic AML	20
13	52	M	2	Classic AML	40
14	49	F	0.7	Classic AML	10
15	31	F	10	Classic AML	5
16	59	F	15	Classic AML	10
17	48	F	4.2	Classic AML	10
18	62	M	1.9	Classic AML	1
19	55	F	7	Classic AML	1
20	69	F	5.8	Classic AML	5
21	76	F	1.3	Classic AML	5
22	53	M	14	Classic AML	1
23	53	F	2	Classic AML	10
24	52	F	2.5	Classic AML	5
25	60	F	5	Classic AML	1
26	72	F	1.5	Classic AML	1
27	70	F	3	Classic AML	100
28	71	F	1.5	Classic AML	20
29	48	F	2.6	Classic AML	10
30	65	F	3	Classic AML	1
31	46	F	15	Classic AML	5
32	56	F	0.5	Classic AML	30
33	58	F	3	Classic AML	10
34	47	M	n.a.	Classic AML	5
35	25	M	2.5	Classic AML	100
36	52	F	n.a.	Classic AML	1
37	58	M	n.a.	Classic AML	40
38	48	M	1.5	Leiomyoma-like AML	0
39	67	M	2	Leiomyoma-like AML	1
40	27	F	3.5	Leiomyoma-like AML	1
41	49	M	n.a.	Leiomyoma-like AML	80
42	61	F	6.5	Leiomyoma-like AML	60
43	42	F	0.9	Sclerosing AML	0
44	73	M	4	Lipoma-like AML	10
45	62	F	6	Lipoma-like AML	10
46	60	M	7	Lipoma-like AML	10
47	39	F	0.3	Microscopic AML	100
48	55	F	0.9	Microscopic AML	60
49	65	F	0.5	Microscopic AML	90

Table 1 (continued)

Case	Age	Gender	Size (cm)	Diagnosis	Parvalbumin (%)
50	48	M	0.2	Microscopic AML	20
51	56	F	0.5	Microscopic AML	10
52	44	F	0.5	Microscopic AML	90
53	53	F	0.3	Microscopic AML	50
54	55	F	0.3	Microscopic AML	80
55	37	F	0.7	Microscopic AML	40
56	51	F	0.2	Microscopic AML	40
57	42	F		Intraglomerular lesion	100
58	38	F		Intraglomerular lesion	100
59	64	F	3	AMLEC	1
60	48	M	12	Epithelioid AML	0
61	36	F	8	Epithelioid AML	0
62	53	F	17	Epithelioid AML	0
63	60	M	5	Epithelioid AML	0
64	30	F	2	Epithelioid AML	0
65	14	M	11	Epithelioid AML	0
66	65	F	2	Epithelioid AML	0

n.a. not available, *F* female, *M* male, *AML* angiomyolipoma, *AMLEC* angiomyolipoma with epithelial cysts

the PEComas "family" [10–13], is the most common. Therefore, the purpose of this study was to evaluate the expression of parvalbumin in the spectrum of the PEC (perivascular epithelioid cells) lesions of the kidney.

Materials and methods

Sixty-six renal PEComas have been retrieved from the files of the Department of Pathology of the University of Verona and Pederzoli Hospital (in-house cases). All the tumor slides were reviewed from two authors (GM, AC). These included 46 renal angiomyolipomas of variable size (37 composed of a mixture of fat, spindle and epithelioid smooth muscle cells, and abnormal thick-walled blood vessels; 3 composed predominantly of fat (lipoma-like angiomyolipoma); 5 almost exclusively composed of spindle-shaped smooth muscle cells (leiomyoma-like angiomyolipoma); and 1 with extensive fibrosis (sclerosing angiomyolipoma/PEComa)): 10 microscopic angiomyolipomas, 2 intraglomerular lesions, 7 epithelioid angiomyolipomas/pure epithelioid PEComas, and 1 angiomyolipoma with epithelial cysts (AMLEC).

Control cases consisted of 50 clear cell renal cell carcinomas, 20 papillary renal cell carcinomas, 20 chromophobe renal cell carcinomas, 20 renal oncocytomas, 10 clear cell papillary renal cell carcinomas, and the normal renal parenchyma of kidneys harboring the neoplasms.

Whole slide sections from tissue blocks of the 66 renal PEC lesions and control cases were immunohistochemically stained with parvalbumin (clone PARV-19, dilution 1:500, Sigma-Aldrich St. Louis, MO, USA). All samples were processed using a sensitive "bond polymer refine" detection system in an automated Bond immunohistochemistry instrument (Leica Biosystems). Labeling for parvalbumin was recorded as the percentage of tumor cells stained of each tumor.

Results

The clinicopathological and immunohistochemical characteristics are detailed in Table 1. Forty-eight patients (73%) were female and eighteen (27%) male, the patients' age at diagnosis ranged from 14 to 80 years old (mean: 53 and median: 54), and the maximum diameter of the tumors varied from 0.2 to 17 (mean: 4 cm, median: 2.8 cm), excluding the intraglomerular lesion present in the cohort.

Nuclear and cytoplasmic staining of parvalbumin was found in fifty-six PEC lesions (85%) with a percentage of tumor cells stained varying from 1 to 100% and was absent in the remaining ten cases (15%). In detail, staining of parvalbumin was observed in 36 of 37 (97%) classic angiomyolipomas, 4 of 5 (80%) leiomyoma-like angiomyolipomas, and in all lipoma-like angiomyolipomas, microscopic angiomyolipomas, intraglomerular lesions, and the only angiomyolipoma with epithelial cysts (AMLEC) in the solid extracystic component with the morphologic features of a leiomyoma-like angiomyolipoma and not in the cystic epithelium (Fig. 1). Among 56 positive cases, the expression of parvalbumin was observed in the scattered spindle and epithelioid smooth muscle cells of 19 tumors (< 5% of neoplastic cells). None of the 7 epithelioid angiomyolipomas/pure

epithelioid PEComas or the only sclerosing angiomyolipoma in the cohort expressed parvalbumin (Fig. 1).

Among the control tumors, all chromophobe renal cell carcinomas and 17 of 20 (85%) oncocytomas were diffusely positive for parvalbumin whereas none of the clear cell renal cell carcinomas, papillary renal cell carcinomas, clear cell papillary renal cell carcinomas (Table 2), and the glomeruli of the normal kidney showed any immunohistochemical expression.

Discussion

Parvalbumin, a cytosolic calcium-binding protein described in the skeletal muscle, neurons, and distal convoluted tubule cells of the nephron [2], is known to be expressed in chromophobe renal cell carcinomas and frequently oncocytomas [3–5]. Parvalbumin acts as a slow-onset calcium buffer, determining spatial and temporal modification in calcium transient, and several studies correlate parvalbumin cellular content in cells with

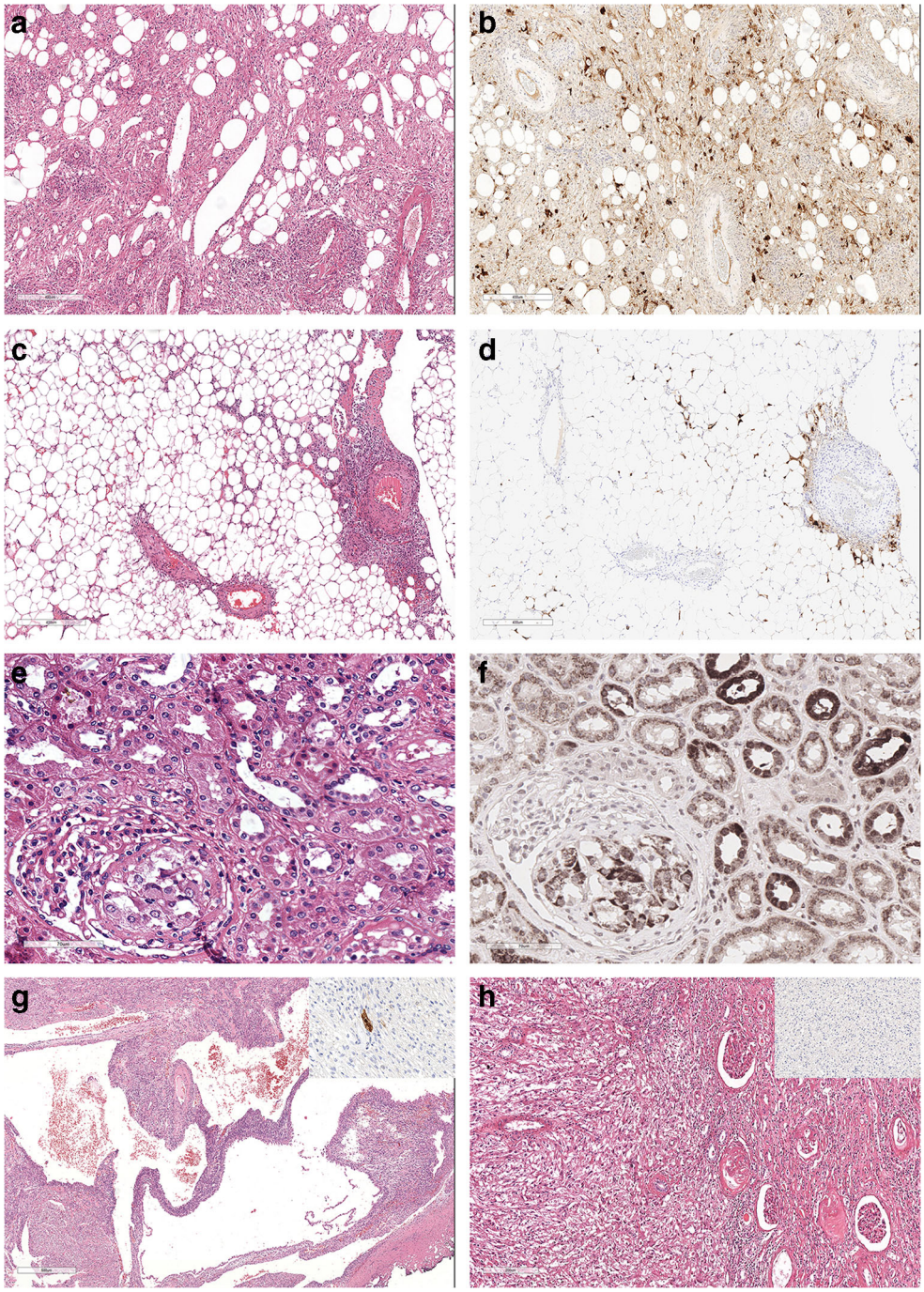

Fig. 1 Histological and immunohistochemical features of PEC lesions. A triphasic angiomyolipoma (**a**) and lipoma-like angiomyolipoma (**c**) with immunoreactivity for parvalbumin (**b**, **d**). An intraglomerular lesion (**e**) highlighted by parvalbumin (**f**). The only case of angiomyolipoma with epithelial cysts with scattered cells positive for parvalbumin (insert) (**g**). None of the epithelioid angiomyolipoma/pure epithelioid PEComa stained for parvalbumin (**h**)

dynamic changes in morphology and volume of the mitochondria and the whole cells [14]. For instance, in the skeletal muscle, mitochondrial inner and outer membranes present a wide spectrum of calcium channels with whom this organelle participates in the finest regulation of action potential [14]. In parvalbumin-devoid cell cultures, the volume of the mitochondria increases, and the surface of the whole cell is larger, probably due to a compensatory mechanism of cells in the attempt to replace the lack of this calcium buffer [14]. Interestingly, the prototype of morpho-immunophenotypical plasticity among the PEC lesions is the angiomyolipoma in which the PEC cells can show smooth muscle differentiation with spindle and epithelioid shape and acquiring the features of adipocytes [11, 12, 15].

In this study, we found that parvalbumin is expressed in the PEC lesions of the kidney including classic angiomyolipomas, microscopic angiomyolipomas, intraglomerular lesions, and angiomyolipoma with epithelial cysts, even though with a wide spectrum of staining, ranging from scattered positivity to diffuse expression, but not in epithelioid angiomyolipomas/pure epithelioid PEComas. The knowledge of this immunoreactivity may be valuable in the differential diagnosis between epithelioid angiomyolipomas/pure epithelioid PEComas which are negative and eosinophilic tumors (chromophobe renal cell carcinoma and oncocytoma) which are positive. Although PAX8 is a useful marker in this challenging diagnosis since epithelioid angiomyolipoma/pure epithelioid PEComa is negative whereas chromophobe renal cell carcinoma and oncocytoma are positive, it is known in clinical practice that nuclear immunoreactivity for PAX8 might be faint or patchy. Therefore, the lack of expression of parvalbumin might be another diagnostic tool in this setting.

Angiomyolipoma can occur sporadically showing a female predominance and a bimodal age distribution [16] or in patients with tuberous sclerosis harboring *TSC1*/*TSC2* germline mutation [17]. In those patients, angiomyolipomas are usually multiple and variable in size [11, 12] and associated with microscopic angiomyolipomas and especially intraglomerular lesions, which may be subtle to identify [18]. To date, cathepsin K but not HMB45 can be helpful to detect these microscopic lesions [19]. Parvalbumin, constantly negative in normal glomeruli, could be another important tool in this particular setting, highlighting the presence of intraglomerular lesions and suggesting the possibility of a diagnosis of tuberous sclerosis. This finding could have a biological explanation. In cellular models of tuberous sclerosis, it has been demonstrated a progressive accumulation of mitochondria and in general an impaired mitophagy [20]. Interestingly, this alteration of mitochondria homeostasis might be related to cytosolic calcium levels which are regulated by parvalbumin.

Another fascinating aspect is the different expressions of parvalbumin in classic angiomyolipoma and epithelioid angiomyolipoma/pure epithelioid PEComa, which are all negative in contrast to cathepsin K, HMB45, and Melan-A reported in both tumors [19, 21]. In PEC lesions, it has been demonstrated that the expression of genes codifying for melanogenesis proteins (HMB45 and Melan-A) and cathepsin K is regulated by the microphthalmia transcription factor (MITF) [19, 22]. The MITF subfamily transcription factor includes MITF itself, TFE3, TFEB, and TFEC which act together as homodimers and/or heterodimers. Calcineurin, a calcium-dependent phosphatase, plays a major role in TFEB-dephosphorylation promoting TFEB nuclear translocation

Table 2 Immunohistochemical expression of parvalbumin in renal tumors

	Parvalbumin expression *N* (%)
Tumor subtypes	
Mesenchymal tumors	
Classic angiomyolipoma	36/37 (97%)
Leiomyoma-like angiomyolipoma	4/5 (80%)
Lipoma-like angiomyolipoma	3/3 (100%)
Sclerosing angiomyolipoma	0/1 (0)
Microscopic angiomyolipoma	10/10 (100%)
Intraglomerular lesion	2/2 (100%)
AMLEC	1/1 (100%)
Epithelioid angiomyolipoma	0/7 (0)
Epithelial tumors	
Clear cell renal cell carcinoma	0/50 (0)
Papillary renal cell carcinoma	0/20 (0)
Clear cell papillary renal cell carcinoma	0/10 (0)
Chromophobe renal cell carcinoma	20/20 (100%)
Oncocytoma	17/20 (85%)

AMLEC angiomyolipoma with epithelial cysts

[23] and probably other translocation factors of MITF subfamily which act as a dimer with it. This suggests that TFEB nuclear localization might be influenced by changes in intracellular calcium levels which, in angiomyolipoma, might be modulated by parvalbumin, as calcium buffer protein. The wide spectrum of staining of parvalbumin observed in the entire spectrum of PEC lesions could be due to the plasticity of the PEC. Regarding the negativity of the staining observed in epithelioid angiomyolipoma/pure epithelioid PEComa, this finding could reasonably represent the extreme end of the variability of the expression of parvalbumin. Moreover, the lack of parvalbumin could explain the presence of larger epithelioid cells observed in epithelioid angiomyolipoma/pure epithelioid PEComa rather than in common angiomyolipoma.

In conclusion, we demonstrated the immunolabeling of parvalbumin in almost all perivascular epithelioid cell (PEC) lesions of the kidney but not in the epithelioid angiomyolipoma/pure epithelioid PEComa. The expression of this calcium-binding protein might be important for the plasticity of the perivascular epithelioid cells and some of their biological features (i.e., MITF nuclear expression) in angiomyolipoma and related lesions. Moreover, this finding may be another useful tool, particularly in the differential diagnosis between epithelioid angiomyolipoma/pure epithelioid PEComa and other renal eosinophilic tumors, such as oncocytoma and chromophobe renal cell carcinoma.

Authors' contributions A Caliò and G. Martignoni: designed and coordinated the study. A. Caliò, S. Ammendola, G. Martignoni, M. Brunelli, and S.Gobbo: collected the samples. A. Caliò, S. Ammendola, G. Martignoni, M. Brunelli, S. Gobbo and S. Pedron: performed the histopathological and immunohistochemical analyses. A. Caliò, S. Ammendola, and G. Martignoni: wrote the paper. All the authors approved the final version of the manuscript.

Compliance with ethical standards

The study was conducted in accordance with the Helsinki Declaration.

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

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