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Original Article

Expression of CD44 and CD29 by PEComa cells suggests their possible origin of mesenchymal stem cells

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Abstract: Background: Perivascular epithelioid cell tumor (PEComa) is a rare mesenchymal tumor composed of histologically and immunohistochemically distinctive perivascular epithelioid cells. The perivascular epithelioid cell (PEC) co-expresses melanocytic and muscle markers. Since no normal counterpart to the PEC has ever been identified in any normal tissue, the cell origin of these tumors is still uncertain. Although, several hypotheses have recently been advanced to explain the histogenesis of PEComa, it remains unclear. Methods: The aim of this study was to discuss whether differential expression of stem cell-associated proteins could be used to aid in determining the histogenesis of PEComa. For this purpose, we detected the immunoexpression of 5 kinds of stem cell markers on PEComas, including CD29, CD44, CD133, ALDH1, and nestin. In addition to observed histopathologic morphology, we also performed PEComa relevant clinical diagnostic markers (HMB-45, SMA, melan-A, Desmin, Ki-67, S-100 and TFE3) to identify whether they belonged to PEComas. Results: Our study included 13 PEComa samples, and we obtained positive immunoexpression results as follows: CD29 (13/13), CD44 (8/13), ALDH1 (10/13), nestin (1/13), and CD133 (0/13). Conclusions: Since CD44 and CD29 are surface proteins associated with MSCs, these results suggest that PEComa might arise from MSCs. However, whether MSCs are the origin of PEComa needs to be further explored in the future.

Keywords: Perivascular epithelioid cell tumor, stem cell markers, mesenchymal stem cells

Background

The World Health Organization defines perivascular epithelioid cell neoplasms (PEComas) as "mesenchymal tumors composed of histologically and immunohistochemically distinctive perivascular epithelioid cells". PEComa was first named by Zamboni et al in 1996 [1]. Tumors belonging to the PEComa family include angiomyolipoma, lymphangioleiomyomatosis, renal capsuloma, clear cell "sugar" tumor (CCST) and clear cell myomelanocytic tumor. Cases of PEComa arising at virtually any anatomic site have been increasingly reported over the past decade, and it has now become a well-accepted entity. However, the histogenesis of PEComas is still unknown.

Several hypotheses have been advanced to explain the histogenesis of PEComa; Lim et al proposed that the perivascular epithelioid cell (PEC) originates from a pluripotent cell. Pluripotent cells are derived from the neural crest, which may give rise to smooth muscle cells and melanocytes [2]. Barnard and Lajoie, however, proposed that the cell of origin is a smooth muscle cell resembling a pericyte that exhibits unusual features, including melanocytic differentiation [3]. The concept that PECs might arise from a precursor cell, has also been mentioned elsewhere in the literature [4].

A novel concept considers PEComa to be a neoplasm of stem cells, which may have acquired a defect during differentiation [2]. In this study,

Table 1. Summary of patients' clinical information

Patient ID	Age	Sex	Site (s)	Tumor size (cm)	Follow-up time (months)	Outcome
1	43	F	Lung	2.7×2.0×2.0	22	Alive
2	64	F	Liver	3.6×3.0×1.7	8	Alive
3	54	F	Renal	4.5×2.5×2.3	5	Alive
4	59	F	Renal	1.6×1.0×0.8	26	Alive
5	48	F	Uterus	2.5×2.2×1.7	11	Alive
6	45	F	Liver	2.0×1.9×1.6	47	Alive
7	48	M	Liver	7.0×5.8×5.6	30	Alive
8	44	F	Uterus	0.8×0.6×0.5	24	Alive
9	45	M	Prostate	2.0×1.0×0.8	41	Alive
10	62	F	Renal	2.0×1.8×1.7	23	Not available
11	42	F	Uterus	3.3×2.8×2.0	63	Not available
12	47	F	Renal	2.7×2.5×1.6	41	Not available
13	42	F	Renal	6.0×4.0×4.0	41	Not available

we aimed to discuss whether differential expression of stem cell-associated proteins could be used to aid in determining the histogenesis of PEComa, and thus, antibody-based detection of a number of stem cell surface markers (CD29, CD44, CD133, ALDH1 and nestin) was performed on human paraffin-embedded tissue of PEComa. Antibodies against clinically relevant markers (HMB45, SMA, MelanA, Desmin, Ki-67, and S100) were used to confirm the diagnosis of PEComa. In addition, aberrant immunoreactivity for transcription factor E3 (TFE3) has been observed in some PEComas, suggestings that these lesions may represent a distinctive entity [5]; therefore, immunoexpression of TFE3 was also performed.

Methods

Patients and tissue specimens

Thirteen PEComa samples were obtained from the Department of Pathology, the First Affiliated Hospital, Shihezi University School of Medicine, People's Hospital of Shihezi City and People's Hospital of Xinjiang Autonomous Region. Tumor tissues were fixed in 10% buffered formalin and embedded in paraffin. The diagnoses of all patients who received surgery were confirmed by histological and immunohistochemical analyses. Follow-up surveys were conducted. Clinical data including age, sex, sites, tumor size and the follow-up surveys were available in all cases (**Table 1**). This study was approved by the institutional ethics committee at the First Affiliated Hospital of Shihezi University School of Medicine.

Immunohistochemistry

The paraffin-embedded sections with 4 um were prepared for IHC staining. IHC staining was performed using EnVision two-step immunohistochemical kit (Dako, Glostrup, Denmark) and a 3.3'-diaminobenzidine peroxidase substrate kit (Dako, Glostrup, Denmark) were used to detect specific target proteins. Briefly, tissue sections were deparaffinized and rehydrated with ethanol, and then quenched with 3% hydrogen peroxide for 10 min. Slides were soaked in 0.01 M sodium citrate buffer and heat-induced antigen retrieval was performed for all primary antibodies (**Table 2**). **Table 2** shows the primary antibodies used in this study, including their respective dilutions, immunostaining methods and sources. Slides were incubated with primary antibodies overnight at 4°C. The samples were then washed with phosphate-buffered saline (PBS) and subsequently incubated with secondary antibodies for 30 min. The sections were visualized by incubating with diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. PBS was used in place of the primary antibody as negative control.

Results

Clinical and pathological features

Thirteen patients were included in our study group. The sex ratio was 11:2 (11 females, 2 males), and the median age at surgery was 49.5 years, with a range from 42 to 64 years. Anatomic locations of the tumors included the kidney, liver, lung, uterus and prostate (**Table 1**). All samples were primary tumors. Upon follow-up survey, nine of thirteen patients had a healthy prognosis without distant metastases while four patients were lost to follow-up.

Renal and liver PEComas were composed of an admixture of thick-walled hyalinized blood ves-

Table 2. Informations for primary antibodies

Antibody	Dilution	Pretreatment	Immunostaining	Clone number	Source	Positive control
HMB-45	1:400	PCA-CB	Envision	Polyclonal	DAKO, Glostrup, Denmark	Melanoma
Melan-A	1:100	PCA-CB	Envision	A103	DAKO, Glostrup, Denmark	Melanoma
S-100	1:3200	PCA-CB	Envision	Polyclonal	DAKO, Glostrup, Denmark	Melanoma
SMA	1:50	PCA-CB	Envision	1A4	DAKO, Glostrup, Denmark	Breast carcinoma
Desmin	1:100	PCA-CB	Envision	D33	DAKO, Glostrup, Denmark	Leiomyosacoma
Ki-67	1:600	PCA-CB	Envision	SP6	DAKO, Glostrup, Denmark	Breast carcinoma
CD29	1:1500	PCA-CB	Envision	EP1041Y	Abcam, Cambridge, UK	Breast carcinoma
CD44	1:200	PCA-CB	Envision	DF1485	DAKO, Glostrup, Denmark	Breast carcinoma
CD133	1:200	PCA-CB	Envision	Polyclonal	ARP, Waltham, America	Kidney
ALDH1	1:200	PCA-CB	Envision	EP1933Y	Abcam, Cambridge, UK	Kidney
Nestin	1:200	PCA-CB	Envision	SP103	Abcam, Cambridge, UK	Kidney
TFE3	1:500	PCA-EDTA	Envision	MRQ-37	ZSGB, Beijing, China	Renal cell carcinoma

PCA-CB, pressure cooker heating in citrate buffer (0.01 M, pH 6.0). PCA-EDTA, pressure cooker heating in ethylene diamine tetraacetic acid buffer (0.01 M, pH 9.0).

sels, smooth muscle cells and adipose tissue, with epithelioid cells having clear to pale eosinophilic granular cytoplasm arranged around blood vessels. However, PEComa in the liver showed more epithelioid cells than that in the kidney. PEComa of the lung was composed of a uniform population of clear-to-eosinophilic, round-to-polygonal epithelioid cells with thin-walled vasculature and a nested or somewhat alveolar appearance. Uterine PEComa showed multinodular growth of spindle to epithelioid cells with a clear cytoplasm oval hyperchromatic nuclei and thick-walled vasculature. Prostate PEComa was composed of epithelioid and spindle cells, with clear to granular cytoplasm arranged in nests and separated by a vascular stroma (**Figure 1**).

Clinical diagnosis relevant markers expression in PEComa

The immunohistochemical staining results are summarized in **Table 3**, and representative images are shown in **Figures 2** and **3**. PEComa expresses myogenic and melanocytic markers, HMB-45 (13/13), SMA (12/13) and melanA (10/13), and less desmin (3/13), with Ki-67< 6% (13/13) and almost no expression of S-100(1/13). TFE3 protein was positive in 7 of 13 cases (7/13) (**Table 3**; **Figure 2**).

Stem cell markers expression in PEComa

CD133 was completely negative in all cases (0/13), while CD29 was positive (13/13). CD44 was present in 8 of the 13 cases (8/13). ALDH1 was present in 9 of the 13 cases (9/13). Nestin was negative in 12 of the 13 cases; the scattered tumor cells were positive for this marker in only one case (1/13) (**Table 3**; **Figure 3**).

Discussion

PEComa is a heterogeneous and generally benign tumor, occurring throughout the body in a variety of tissues and organs. The most common sites are the kidneys, liver, lungs, and uterus, while a few reports also mention the bladder, prostate, ovary, pancreas, and soft tissues. Clinical data show that it predominantly affects women, and in the present study, we obtained samples from 13 patients (11 females and 2 males), and the tumor sites were the kidneys, liver, uterus, lungs, and prostate.

PEComas, arising in the kidney and liver, usually have similar morphology. However, as our results have shown, PEComa in the liver much more frequently shows a prominent component of large epithelioid cells than its renal counterpart does, and considerable numbers of liver cases are comprised nearly exclusively of such cells. PEComa of the lung is a very rare, benign, and usually incidentally detected neoplasm. It often presents with prominent thin-walled vasculature and a nested or somewhat alveolar appearance like the result described. The morphology of uterine PEComa not only overlaps with that of lung PEComa, but also with

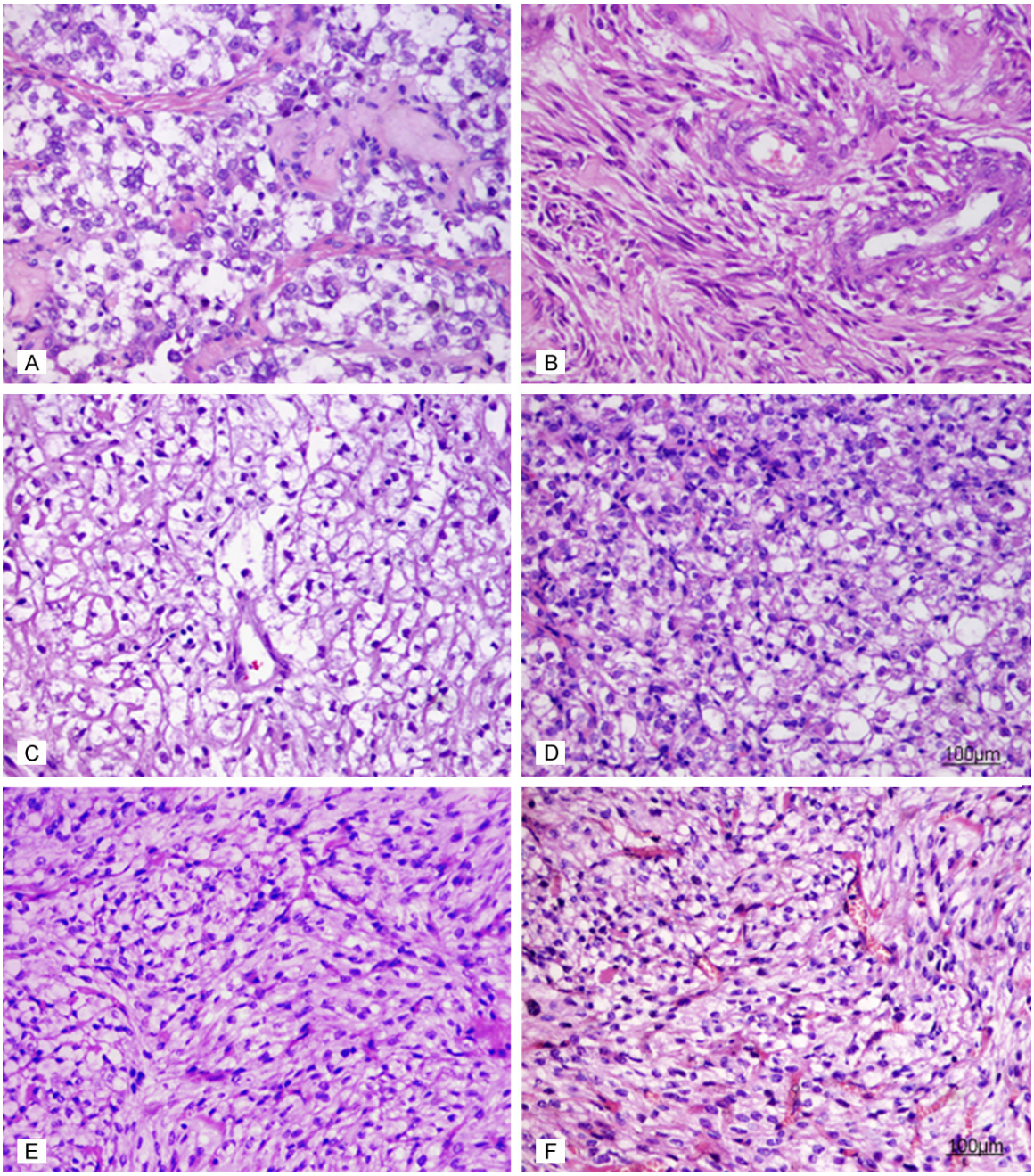

Figure 1. A. Uterine PEComa composed of epithelioid cells with a clear cytoplasm and oval hyperchromatic nuclei (HE.×200); B. Renal PEComa: perivascular epithelioid cells arranged around a blood vessel (HE.×200); C. Lung PEComa composed of epithelioid cells with a clear cytoplasm and well-defined cell borders (HE.×200); D. Liver PEComa composed of epithelioid cells with a clear cytoplasm (HE.×200); E, F: Prostate PEComa was composed of epithelioid and spindle cells, with clear to granular cytoplasm arranged in nests and separated by a vascular stoma (HE.×200).

lymphangioleiomyomatosis. However, we found that uterine PEComas had thick-walled vasculature, while the vasculature of lung PEComa was thin. To the best of our knowledge, only two prostate PEComas have been reported until now. The first tumor was described as a malignant tumor first reported in 2003. The tumor cells had mild-to-moderate nuclear pleomorphism and a low mitotic activity [6]. The other tumor was benign [7]. The prostate PEComa in

Table 3. Immunohistochemical staining for all antibodies

Patient ID	Site(s)	Clinical diagnosis relevant markers						Stem cell markers					
		HMB45	Melan-A	SMA	Desmin	Ki-67	S-100	TFE3	CD29	CD44	CD133	ALDH1	Nestin
1	Lung	+	+	+	-	1%	-	+	+	+	-	+	Scattered+
2	Liver	+	+	+	-	5%	Focally+	+	+	+	-	+	-
3	Renal	+	-	+	+	3%	-	+	+	+	-	-	-
4	Renal	Focally+	-	+	+	<1%	-	+	+	+	-	-	-
5	Uterus	+	+	+	-	3%	-	+	+	-	-	-	-
6	Liver	+	-	+	-	2%	-	-	+	+	-	+	-
7	Lung	+	+	-	-	1%	-	+	+	Scattered+	-	+	-
8	Uterus	+	+	+	-	3%	-	–	+	+	-	Weakly+	-
9	Prostate	+	+	+	-	2%	-	-	+	-	-	+	-
10	Renal	+	+	+	-	-	-	-	+	-	-	+	-
11	Uterus	+	-	+	-	2%	-	+	+	+	-	+	-
12	Renal	+	+	+	-	1%	-	-	+	-	-	+	-
13	Renal	+	+	+	Focally+	4%	-	-	+	-	-	+	-

our study displayed benign behavior without mitotic activity or vascular invasion. The tumor was composed of epithelioid and spindle cells, with clear to granular cytoplasm arranged in nests separated by a vascular stroma.

As with the morphology described above, PEComa presents epithelioid morphology and expresses melanocytic markers. Both epithelial tumors and mesenchymal tumors can be confused with PEComa. For undefined cases, immunohistochemistry is useful for differential diagnosis.

The co-expression of melanoma cell markers and muscle cell markers at the same time is characteristic of PEComa, but the expression of strength and sensitivity of the two markers show differences in different cases. In the melanoma cell markers, the most sensitive one is HMB-45, followed by melan-A, for which mainly the epithelioid cells are positive. In muscle cell markers, SMA and vimentin are commonly positive, mainly for the spindle-shaped cells. In addition, vimentin is positive, with less desmin. Generally, S-100 is negative in PEComa, nevertheless, some cases of immunoexpression of S-100 by PEComas have been reported [8, 9]. The PEComa relevant clinical diagnosis markers we screened in our study showed that: HMB-45 (13/13), SMA (12/13), melan-A (10/13), Desmin (3/13), and S-100 (1/13). The results of immunohistochemical staining were accordance with the characteristics of PEComa and the reported studies.

PEComa usually has shown benign behavior, but given its rarity, it is unclear whether any of these tumors can be declared to be benign or whether all PEComas should be considered to have uncertain malignant potential. Ki-67 is a proliferation marker, which is a useful marker in distinguishing malignant from benign PEComa [10]. Perivascular epithelioid cell tumors with a Ki-67 labeling, index of less than 1%, have neither recurred nor metastasized [11]. However, Ki-67 labeling of 5% of neoplastic cells has been observed in uterine PEComas that have behaved aggressively [12]. In our study, all thirteen patients were Ki-67 ≤5%, who were still alive without recurring or metastasizing.

Transcription factor E3 (TFE3) gene is a member of the MiTF family of transcription factors, locaed on Xp11.2. Recently, immunoreactivity for TFE3 has been reported in PEComas [13-16], those TFE3-positive PEComas showed predominant alveolar architecture and epithelioid cell morphology, minimal immunoreactivity for muscle markers SMA, and a young age tendency [5, 16, 17]. According to our results, nuclear TFE3 immunopositivity was identified in 7 of 13 cases and all cases present alveolar or nested architecture. However the SMA was strong positive and only 2 cases showed SMA negative. Furthermore, the seven patients (from 43 to 64 years) were not as young as previously described [5]. TFE3 protein is a sensitive and specific marker of neoplasms harboring TFE3 gene fusions. To the best of our knowledge, at least 6 kinds of TFE3-related gene fusions (ASPS-TFE3, PRCC-TFE3, PSF-TFE3, CLTC-TFE3, NonO-TFE3 and SFPQ/PSF-TFE3) [5, 18, 19] had been performed in PEComa, but only the SFPQ/PSF-TFE3 gene fusion was confirmed in

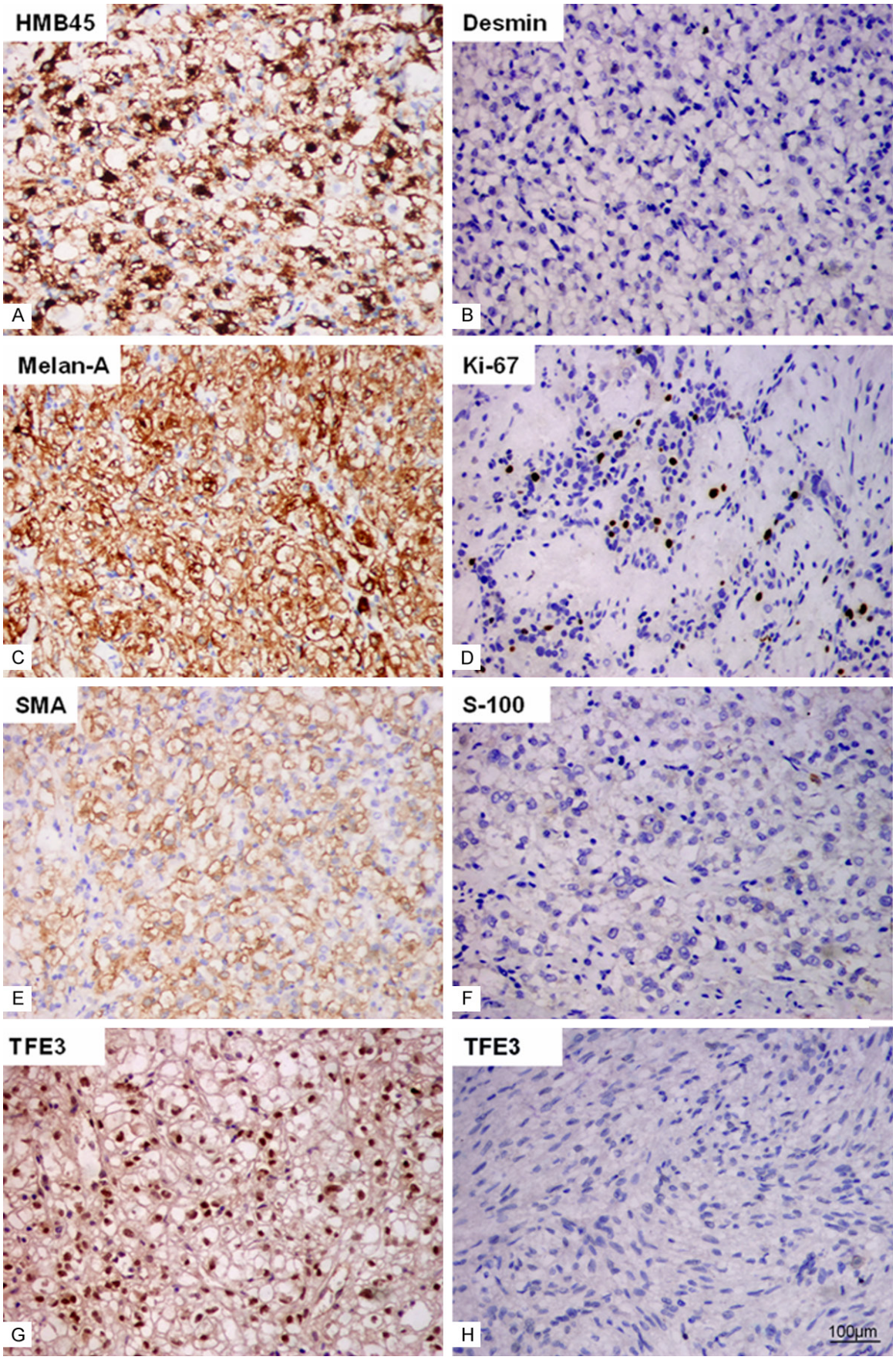
HMB45
A
Desmin
B
Melan-A
C
Ki-67
D
SMA
E
S-100
F
TFE3
G
TFE3
H
100μm

Figure 2. Immunohistochemical features of relevant clinical diagnosis markers of PEComa. The tumor cells were positive for HMB-45 (A). Melan-A (C) and SMA (E), negative for Desmin (B) and S-100 (F), with Ki-67<6% positive (D). TFE3 was nuclear immunopositivity (G, H) Immunostaining, ×200.

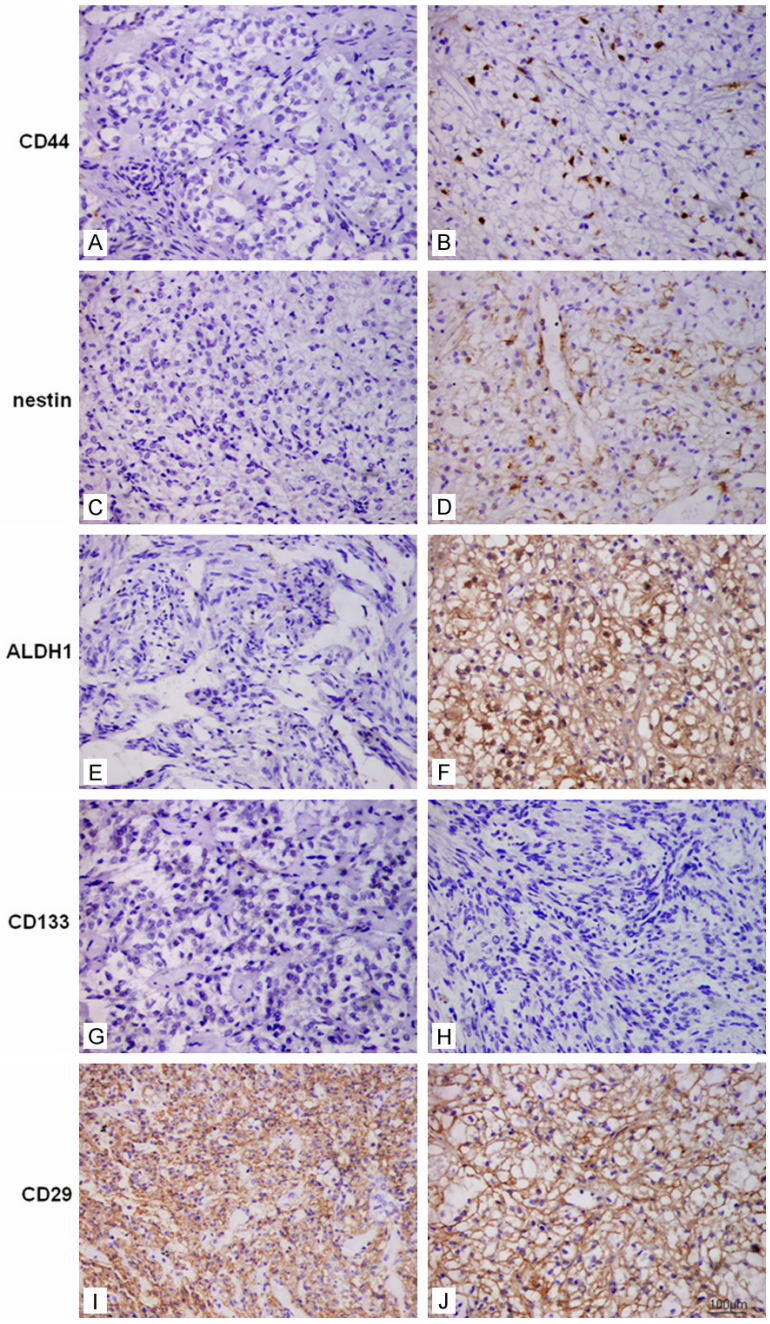

Figure 3. A, B. CD44 was present in 8 of 13 cases. C, D. Nestin was focally expressed in most small blood vessel endothelia, scattered positive in tumor cells in one case, and the rest of the samples were negative. E, F. ALDH1 was present in 10 of 13 cases. G, H. CD133 was completely negative in all cases. I, J. CD29 was positive. Immunostaining, ×200.

tive PEC. It is a tumor of complicated pathogenesis and uncertain histology. Although, at present, PEC does not have a known normal counterpart, several hypotheses have been advanced to explain the histogenesis of PEComa.

Some researchers support the hypothesis that PEComas may originate from the neural crest, which gives origin to pericytes and smooth muscle cells of blood vessels of the face and the forebrain [23]. Studies on neural crest precursors have demonstrated that these precursors have the ability to differentiate into smooth muscle cells [24] and adipocytes [25] depending on the culture condition in vitro. These precursors express the neural stem cell markers Neuroglia-2 (NG2) and L1 (a neural cell adhesion molecule), which have both been found in angiomyolipomas [2]. However, due to the lack of expression of S-100, some researchers disagree with this hypothesis. Besides, neural crest cells could express the stem cell marker nestin [26], while our study showed that, nestin was positive in only one sample and was negative in the other twelve.

Fukunaga thought perhaps the original mesenchymal cells have the ability to differentiate into smooth muscle cells and HMB45 positive epithelioid cells, as different cases show various degrees of differentiation [27]. It is possible to speculate that it is derived from genetically modified mesenchymal stem cells of perivascular origin [28]. Furthermore, classic renal AML is histologically composed of smooth mus-

cle, adipose tissue and thick blood vessel walls. This tripartite-tissue composition also had made some researchers hypothesize that renal AML may arise from mesenchymal stem cells (MSCs).

Mesenchymal stem cells (MSCs), which are considered to be multipotent cells, have been demonstrated to localize to the connective tissues of numerous organs, including liver, kidney and lung [29]. Sources for MSCs are not restricted to the bone marrow. Recent studies have shown that MSCs can reside in the perivascular region in multiple organs, such as adipose tissue [30], brain [31] and umbilical cord [32]. Moreover, MSCs have some specific surface proteins, including CD29, CD44, CD73, CD90 CD105, CD146 and CD166 [33].

A recent study described the successful isolation and culture of MSC-like cells from six renal AML tumors which expressed CD29, CD44, CD73, CD90 and CD105. Cells from the tumors had the ability to differentiate into adipogenic and osteogenic cells, demonstrating for the first time that renal AML-derived adhesive cells possess the characteristics of MSCs [34]. According to the results of our study, CD29 was observed in all samples, and CD44 was positive in 8 of 13 cases. Given that, the co-expression of CD29 and CD44 is a surface biomarker for MSCs [35], we hypothesized that PEComa may arise from MSCs.

Besides its specific markers, MSC residing in the perivascular region also expressed CD133 [36]. CD133 was initially described as a specific marker to select human hematopoietic progenitor cells [37, 38]. Later, it was widely used as a cancer stem cell marker in numerous tumors, including sarcoma [39] and other cancers. $CD133^+$ hemangioma-derived MSCs have been obtained [40] and the $CD133^+$ stem cells selected from proliferating infantile hemangioma, expressed CD29 and CD44, but not CD34 and CD45 [41]. However, our results showed CD133 was completely negative in all 13 specimens, contrary to the reported. This indicated that the hemangioma-derived MSC and the MSC giving rise to PEComa may be not originate from the same source.

As a stem cell marker [42], high ALDH1 activity has been found in stem cell populations in many cancer types including human multiple myeloma, acute myeloid leukemia [9], pancreatic cancer [43], and breast cancer [44]. Therefore, ALDH1 activity might be usable as a common marker for both normal malignant stem cell populations [42]. A recent study demonstrated that ALDH1 can serve as a potential marker for cancer stem cells in sarcoma [45]. Several independent study groups have reported that ALDH1 expression can be used as a poor prognostic marker for epithelial cancers. Nevertheless, 4 of 13 samples presented negative for ALDH1 in our study: two renal PEComas and two uterus PEComas. In addition to the two uterus PEComas that presented negative, ALDH1 showed weakly positive in another uterus PEComa. These results indicated that PEComas locating in the uterus may be different from other PEComas. In addition, according to our follow-up study, most patients do not have a poor prognosis at this time. As this study was limited by the small number of clinical specimens, the function of the activity of ALDH1 in PEComa still requires further study.

According to the results of another study about solitary fibrous tumors (SFT) from our group (data unpublished), ALDH1 was highly expressed, while CD29 and CD44 were almost negative. The results meant that these two kinds of mesenchymal tumors may not share the same origin. Given the uncertain biological behavior of PEComa and the possibility of local recurrences/distant metastasis, close long-term follow-up is warranted to monitor the prognosis.

Conclusions

The aim of the present study was to explore a possible origin of PEComa. For this purpose, the immunoexpression of 5 stem cell markers (CD29, CD44, CD133, nestin, and ALDH1) was detected in paraffin-embedded human tissue samples of PEComa. Based on the results described, we proposed that MSCs may give rise to PEComa. However, due to the restriction of sample numbers, our study has some limitations; the process of tumor formation may also be affected by the tumor stem cell microenvironment. Additional studies with larger numbers of specimens and cell culture are necessary to explore whether PEComa originates from MSCs.

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Disclosure of conflict interest

None.

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