Original Article

Ang-1 and Ang-2 expression in angiomyolipoma and PEComa family tumors

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ABSTRACT

Objective: Perivascular epithelioid cell tumors (PEComa) are an uncommon family of soft tissue tumors. Previously, we described that the presence of pericyte antigens among PEComa family tumors differs extensively by histologic appearance.

Methods: Here, we extend our findings using the pericyte antigens Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), using immunohistochemical detection in human tumor samples.

Results: While Ang-1 showed no expression across any PEComa family tumor, Ang-2 showed expression that like other pericyte markers was largely determined by cytologic appearance.

Conclusion/implications: Pericytic marker expression in PEComa may represent a true pericytic cell of origin, or alternatively aberrant pericyte marker adoption.

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1. Introduction

The perivascular epithelioid cell tumor (PEComa) family of tumors include a group of anatomically and histologically diverse neoplasms with dual myoid-melanocytic differentiation.¹ The most common PEComa family tumor is angiomyolipoma (or AML), which occurs predominantly in the kidney or liver, and has a characteristic triphasic histologic appearance including thick-walled blood vessels, myoid appearing perivascular cells, and lipid-distended cells resembling adipocytes.¹ On occasion, a predominant myoid component overshadows the vascular or lipid-filled components, which can have either a spindle or epithelioid cell morphology. Of these, epithelioid AML has been shown to have an aggressive clinical behavior.² Lymphangiomyoma, lymphangiomyomatosis, and clear cell sugar tumor (CCST) are rare, distinct entities.³ PEComas of other sites, including soft tissue and gynecologic origin have been well documented,⁴ while diverse sites have been described. Immunohistochemical features of

PEComa family tumors are distinct, and typically show co-expression of smooth muscle markers and melanocytic markers.⁵

Pericytes are mesenchymal cells that closely enwrap small blood vessels, regulating and supporting the microvasculature through direct contact with the endothelium. Pericytes demonstrate a distinct antigen expression, including co-localization of α SMA, CD146, PDGFR β (platelet-derived growth factor receptor β), and RGS5.^{6,7} Previously, we described the presence of pericyte antigens across a diverse group of PEComa family tumors.⁸ Results showed that pericyte antigens differed extensively by histologic appearance. Typical angiomyolipoma (AML) specimens showed variable expression of pericyte antigens among both perivascular and myoid-appearing cells. In contrast, AML specimens with a predominant spindle morphology showed diffuse expression of pericyte markers, including α SMA, CD146, and PDGFR β . AML samples with predominant epithelioid morphology showed a marked reduction or absence of immunoreactivity for pericyte markers.

Angiopoietin proteins are known to play an important role in angiogenesis and vascularization through the Tie2 pathway.⁹ Angiopoietin-1 (Ang-1) is produced by pericytes,¹⁰ and is hypothesized to be involved in the reciprocal communication between pericytes and endothelium. Angiopoietin-2 (Ang-2) is mainly expressed in the endothelium but also in pericytes,¹⁰ and appears

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to increase tumor vascularization and regulate angiogenesis.¹¹ Ang-2 has well described antagonistic functions on Ang-1/Tie2 signaling in the endothelium.¹² These antagonistic functions of Ang-2 may be cell specific, and in mesenchymal cells Ang-2 antagonism of Ang-1/Tie2 signaling may not occur.¹³

Angiopoietin/Tie signaling has primarily been interrogated in human vascular tumors. Buehler et al. found Ang-1 expression in the majority of angiosarcoma specimens, while Ang-2 expression was found in 42% of tumors.¹⁴ Further, increased Ang-1 expression by immunohistochemical detection correlated with improved overall survival in angiosarcoma.¹⁴ To the best of our knowledge, the expression of angiopoietin family members in PEComa family tumors has not yet been reported. In the present study, we examined the expression of Ang-1 and Ang-2 by immunohistochemical detection across a large set of PEComa family tumors.

2. Materials and methods

2.1. Histology and immunohistochemistry

Tumors were identified using a retrospective chart review of the pathology tissue archives of the Department of Pathology and Laboratory Medicine at the University of California, Los Angeles

(UCLA) using the search terms “angiomyolipoma, perivascular epithelioid cell tumor, and PEComa”. Slides were reviewed by two independent pathologists to ensure accuracy of diagnosis (S.M.D and A.W.J.). On re-review, tumors were assigned to one of five categories: (1) ‘typical’ AML which demonstrated a characteristic triphasic histologic appearance, (2) AML with predominant spindled cytomorphology (samples were combined under this designation which showed predominant spindled tumor cells, and with minimal lipid-laden cells), (3) AML with predominant epithelioid cytomorphology (epithelioid AML were defined as those tumors with predominant epithelioid cytomorphology of tumor cells and with minimal lipid-laden component), (4) malignant AML, and (5) lymphangiomyoma according to previously published diagnostic criteria.¹ Recognizing that agreement does currently not exist regarding criteria for malignancy in AML, we chose the criteria set forth by Brimo et al. to distinguish malignant potential in renal angiomyolipoma.¹⁵ Briefly, a designation of malignant AML was given when three of the following four criteria were present: (1) $\geq 70\%$ atypical epithelioid cells, (2) ≥ 2 mitoses per 10 HPF, (3) presence of atypical mitotic figures, and (4) presence of necrosis. Patient information was obtained, including age, sex, tumor location, tumor size, and previous immunohistochemical stains performed during the initial

Fig. 1. Typical histologic appearance of PEComa family tumors. Hematoxylin–eosin (H&E) staining of (A and B) typical angiomyolipoma (AML), (C and D) spindled AML, and (E and F) epithelioid AML. Black scale bar: 50 μ m.

diagnostic evaluation. Formalin fixed paraffin embedded (FFPE) tumor tissue from patients were acquired from the tissue archives, under UCLA IRB approval # 13-000918.

Immunohistochemistry for pericyte markers was performed using the ABC method (Vectastain Elite ABC; Vector Laboratories, Burlingame, CA) using diaminobenzidine (DAB) as the chromogen (ImmPACT DAB, Vector Laboratories). The following primary antibodies were used: goat anti-angiopoietin-1 IgG (1:50, R&D Systems, Minneapolis, MN, USA) and mouse anti-angiopoietin-2 IgG (1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The following secondary antibodies were used: polyclonal rabbit

biotinylated anti-goat IgG (1:500, Sigma, St Louis, MO) and polyclonal horse anti-mouse IgG (1:500, [H+L], Vector Laboratories).

Heat-mediated antigen retrieval was performed for all immunohistochemical stains in 1 mmol/L tris-EDTA, 0.01% Tween-20 (Sigma), pH 8. Nonspecific antibody binding was blocked (IHC-TEK Antibody Diluent, pH 7.4; IHC World, LLC, Woodstock, MD). Endogenous peroxidase and alkaline phosphatase blocking solution was used (BLOXALL endogenous peroxidase and alkaline phosphatase blocking solution, Vector Laboratories). Mayer's hematoxylin was used as a nuclear counterstain (1:5, ABCAM),

Fig. 2. Variation in histologic appearance of PEComa family tumors. Hematoxylin–eosin (H&E) staining of (A and B) spindled AML with hyalinization, (C and D) spindled AML with ossification, (E and F) lymphangiomyoma, and (G and H) atypical malignant PEComa. Black scale bar: 50 μ m.

and slides were mounted using aqueous media (VectaMount AQ, Vector Laboratories).

2.2. Immunohistochemical semi-quantitation

Semiquantitative grading of immunohistochemical stains was performed by 3 blinded independent observers, as previously performed.¹⁶ Intensity of staining was graded on a 3-point scale (0–3+), defined as follows: 0, absent stain; 1+, weak, focal cytoplasmic staining; 2+, moderate, focal to diffuse cytoplasmic staining; 3+, strong, diffuse cytoplasmic staining. In cases of disagreement between observers, tumor staining was reevaluated by the same observers and the majority opinion was selected. In addition, the percentage of tumor cells stained was also evaluated, using a 5% incremental scale. Means and standard deviations were calculated for all semiquantitative measurements.

3. Results

3.1. Demographics of patients with PEComa tumor samples

Nineteen PEComa specimens were identified in the pathology records. The majority of tumors were from women with a mean

age of 54.6 years. The majority of tumors were of renal/perirenal origin (68.4%), while other sites included the retroperitoneum/pelvis, liver, and mediastinum. Mean tumor size was 5.4 cm. Tumors were commonly positive for melanocytic markers, including HMB45 and MART1. When assessed smooth muscle markers were also primarily positive. Epithelial markers were uniformly negative when assessed. PEComa family tumors were next split into categories based on histologic appearance, including those tumors with the typical triphasic appearance (typical angiomyolipoma, $n = 5$), those with predominant spindle cell morphology ($n = 6$), those with predominant epithelioid morphology ($n = 4$), lymphangiomyoma ($n = 3$), and malignant angiomyolipoma ($n = 1$). See Supplemental Table 1 for a further breakdown of patient demographics and specimen characteristics.

3.2. Ang-1 expression in PEComa family tumors

Ang-1 expression was examined across PEComa family tumor samples, including typical AML ($n = 5$), spindled AML ($n = 6$), epithelioid AML ($n = 4$), lymphangiomyoma ($n = 3$), and malignant AML ($n = 1$). Each tumor type showed typical histologic features (Figs. 1 and 2). Results showed a complete absence of Ang-1

Fig. 3. Representative Ang-1 immunohistochemical staining among PEComa family tumors. (A and B) Ang-1 immunostaining in typical AML, (C and D) lymphangiomyoma, and (E and F) atypical malignant PEComa. Black arrows indicate Ang-1 expression in intralesional blood vessels. Black scale bar: 50 μ m.

Table 1

Summary of ANG1 and ANG2 immunohistochemical staining across PEComa family tumors.

Tumor type (n)	ANG1 intensity	ANG1 distribution (%)	ANG2 intensity	ANG2 distribution (%)
Typical AML (5)	0	0	3.00 (± 0)	88.00 (± 10.37)
Spindled AML (6)	0	0	2.17 (± 0.75)	81.67 (± 12.11)
Epithelioid AML (4)	0	0	1.00 (± 0)	36.25 (± 11.09)
Lymphangiomyoma (3)	0	0	2.33 (± 0.58)	53.33 (± 10.41)
Malignant AML (1)	0	0	1.00	50.00

Abbreviations: AML: angiomyolipoma; ANG1: angiopoietin-1; ANG-2: angiopoietin-2; PEComa: perivascular epithelioid cell tumor.

expression across all tumor types (Fig. 3, Table 1), with Ang-1 immunoreactivity limited to intralesional blood vessels only.

3.3. Ang-2 expression in PEComa family tumors

Next, Ang-2 immunohistochemical staining was performed across all PEComa family tumor samples. Results showed that some immunoreactivity for Ang-2 was present across all tumor types and in all tumor samples (Figs. 4–7, Table 1). Ang-2 expression was highest in typical AML, in which the majority of myoid-appearing cells showed strong staining (Fig. 4). Ang-2 immunoreactivity was present among those cells within and radiating outwards from blood vessels, but also those myoid cells interspersed between lipid-distended cells. All typical AML specimens showed strong intensity of staining with on average 88% of tumor cells stained. Spindled AML showed a similar pattern of immunoreactivity, which was also noted in examples with hyalinization and/or ossification (Fig. 5). Spindled AML specimens showed intermediate to strong intensity of Ang-2 immunostaining (2–3+) in the majority of tumor cells (average 81.67% of tumor cells).

In contrast, Ang-2 immunoreactivity was weak in epithelioid AML (1+) and was noted in the minority of tumor cells (average 36.25% of tumor cells) (Fig. 6). Like other spindled PEComa family tumors, lymphangiomyoma specimens showed consistent intermediate to strong expression (2–3+) in the majority of tumor cells (average 53.33% of tumor cells) (Fig. 7). In contrast, the malignant AML specimen examined showed weak Ang-2 immunostaining (Fig. 7C and D).

4. Discussion

The present study extends our previous findings, in which we identified variable expression of pericyte markers among PEComa family tumors that was highly dependent on tumor morphology.⁸ In our previous study, we found pericyte markers to be reduced or absent in AML with predominant epithelioid cytomorphology. Interestingly, in the present study we likewise found that while Ang-2 expression typifies the majority of PEComa family tumors, Ang-2 is weak to absent in epithelioid AML. Although incompletely understood, epithelioid AML tend to behave in a more aggressive fashion than spindled AML.² Perhaps the reduction or absence of pericyte markers including Ang-2 in epithelioid AML reflects a relative loss of pericyte differentiation that accompanies a more aggressive tumor behavior.

Recent evidence suggests that common tumors of the brain, pancreas, prostate and skin adopt a pericyte phenotype in order to spread along the abluminal surfaces of vessels. This tumor pathway has been termed pericytic mimicry and/or extravascular migratory metastasis (EVMM). During extravascular migration, angiotropic tumor cells migrate along the abluminal vascular surfaces of vessels in a pericyte location for microvessels, in a smooth muscle cell or adventitial cell location for larger vessels. This under recognized route of tumor spread is most well documented in melanoma. Similarly, in the malignant brain tumor glioblastoma multiforme, tumor cells adopt a pericyte-like location associated with perivascular invasion. Likewise, recent research suggests that pancreatic and prostatic adenocarcinoma

Fig. 4. Ang-2 expression in typical AML. Immunohistochemical staining of Ang-2 in representative features of typical AML including (A and B) thick-walled blood vessels, (C and D) myoid appearing perivascular cells, and (E and F) adipose rich areas. Black scale bar: 50 μ m.

Fig. 5. Ang-2 expression in spindled AML. Immunohistochemical staining of Ang-2 in (A and B) spindled AML, (C and D) spindled AML with hyalinization, and (E and F) spindled AML with ossification. Black scale bar: 50 μ m.

exhibit pericytic mimicry leading to regional invasion and/or EVMM.¹⁷ Recently from our research group, well-differentiated liposarcoma appears to show evidence of a similar phenomenon.¹⁸ However further studies are needed to define the precise immunophenotype of tumor cells involved in pericytic mimicry, and the links between this phenomenon and the phenotype we observed within PEComa family tumors

Although preliminary, our observations suggest that loss of Ang-2 may be a prognostic indicator in PEComa family tumors. Weak and focal Ang-2 immunoreactivity was predominantly

limited to epithelioid AML and malignant AML. Directed anti-angiopoietin therapies are possible (including Trebananib, AMG 386), and have been investigated as anti-angiogenic therapies in epithelial,¹⁹ hematopoietic,²⁰ and mesenchymal malignancies.²¹ For PEComa family tumors, however, it appears that those tumor types in most need to treatment would likely have low Ang-2 expression.

In summary, in the present study Ang-2 but not Ang-1 expression was observed in a spectrum of PEComa family tumors – which was largely predictable based on cytomorphology. Typical

Fig. 6. Ang-2 expression in epithelioid AML. Immunohistochemical staining of Ang-2 in (A–D) epithelioid AML. Black scale bar: 50 μ m.

Fig. 7. Ang-2 expression in lymphangiomyoma and malignant PEComa. (A and B) Lymphangiomyoma and (C and D) atypical malignant PEComa. Black scale bar: 50 μ m.

angiomyolipomas (AML) with triphasic differentiation showed strong and diffuse Ang-2 expression. AML with spindled morphology showed stronger and more diffuse Ang-2 expression, while AML with predominant epithelioid morphology showed reduced or absent Ang-2 expression. These findings are suggestive of pericytic differentiation within select members of the PEComa family tumors, which can be predicted based on cytomorphic appearance. Whether this represents true pericytic cell origins or rather aberrant adoption of pericytic marker expression is uncertain.

Conflicts of interest

The authors have none to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jor.2016.12.001](https://doi.org/10.1016/j.jor.2016.12.001).

References

- SW W, GJ J. *Enzinger and Weiss's Soft Tissue Tumors*. 6th ed. Mosby Elsevier; 2008.
- Hornick JL, Fletcher CD. PEComa: what do we know so far? *Histopathology*. 2006;48:75–82.
- Liebow AA, Castleman B. Benign clear cell ("sugar") tumors of the lung. *Yale J Biol Med*. 1971;43:213–222.
- Folpe AL, Mentzel T, Lehr HA, et al. Perivascular epithelioid cell neoplasms of soft tissue and gynecologic origin: a clinicopathologic study of 26 cases and review of the literature. *Am J Surg Pathol*. 2005;29:1558–1575.
- Pea M, Bonetti F, Zamboni G, et al. Melanocyte-marker-HMB-45 is regularly expressed in angiomyolipoma of the kidney. *Pathology*. 1991;23:185–188.
- Corselli M, Chen CW, Sun B, et al. The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. *Stem Cells Dev*. 2012;21:1299–1308.
- Murray JR, West CC, Hardy WR, et al. Natural history of mesenchymal stem cells, from vessel walls to culture vessels. *Cell Mol Life Sci: CMLS*. 2014;71:1353–1374.
- Shen J, Shrestha S, Yen YH, et al. Pericyte antigens in angiomyolipoma and PEComa family tumors. *Med Oncol*. 2015;32:659.
- De Palma M, Venneri MA, Galli R, et al. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell*. 2005;8:211–226.
- Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res*. 2005;97:512–523.
- Falcon BL, Hashizume H, Koumoutsakos P, et al. Contrasting actions of selective inhibitors of angiotensin-1 and angiotensin-2 on the normalization of tumor blood vessels. *Am J Pathol*. 2009;175:2159–2170.
- Maisonpierre PC, Suri C, Jones PF, et al. Angiotensin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science*. 1997;277:55–60.
- Witzenbichler B, Maisonpierre PC, Jones P, et al. Chemotactic properties of angiotensin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J Biol Chem*. 1998;273:18514–18521.
- Buehler D, Rush P, Hasenstein JR, et al. Expression of angiotensin-TIE system components in angiosarcoma. *Mod Pathol*. 2013;26:1032–1040.
- Brimo F, Robinson B, Guo C, et al. Renal epithelioid angiomyolipoma with atypia: a series of 40 cases with emphasis on clinicopathologic prognostic indicators of malignancy. *Am J Surg Pathol*. 2010;34:715–722.
- Shen J, Shrestha S, Yen YH, et al. Pericyte antigens in perivascular soft tissue tumors. *Int J Surg Pathol*. 2015;23:638–648.
- Levy MJ, Gleeson FC, Zhang L. Endoscopic ultrasound fine-needle aspiration detection of extravascular migratory metastasis from a remotely located pancreatic cancer. *Clin Gastroenterol Hepatol*. 2009;7:246–248.
- Shen J, Shrestha S, Rao PN, et al. Pericytic mimicry in well-differentiated liposarcoma/atypical lipomatous tumor. *Hum Pathol*. 2016.
- Al Wadi K, Ghatage P. Efficacy of trebananib (AMG 386) in treating epithelial ovarian cancer. *Expert Opin Pharmacother*. 2016.
- Aguiarre Palma LM, Flamme H, Gerke I, et al. Angiotensins modulate survival, migration, and the components of the Ang-Tie2 pathway of chronic lymphocytic leukaemia (CLL) cells in vitro. *Cancer Microenviron*. 2016.
- SP DA, Mahoney MR, Van Tine BA, et al. Alliance A091103 a phase II study of the angiotensin 1 and 2 peptidomimetic trebananib for the treatment of angiosarcoma. *Cancer Chemother Pharmacol*. 2015;75:629–638.