Original Article

Ang-2 but not Ang-1 expression in perivascular soft tissue tumors

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ARTICLE INFO

Article history:

Received 23 June 2016

Accepted 3 July 2016

Available online 28 November 2016

Keywords:

Glomus tumor
Myopericytoma
Angioleiomyoma
Pericyte
Angiopoietin

ABSTRACT

Perivascular soft tissue tumors are relatively uncommon neoplasms of unclear line of differentiation, although most are presumed to originate from pericytes. Previously, we reported a shared immunophenotype across these related tumor types. Here, we extend these findings to examine the expression of the pericyte markers angiopoietin-1 and -2 (Ang-1 and -2) among perivascular soft tissue tumors. Results showed consistent Ang-2 but not Ang-1 expression across tumor types. In summary, the absence of Ang-1 expression distinguishes perivascular from vascular soft tissue tumors. Ang-2 expression is present across perivascular soft tissue tumors, with some variation between histologic subtypes.

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1. Introduction

Perivascular soft tissue tumors are relatively uncommon neoplasms of unclear line of differentiation, although most have been hypothesized to originate from pericytes. Among these, glomus tumor, myopericytoma, and angioleiomyoma share a spectrum of histologic findings, including a perivascular growth pattern. Glomus tumor is a subcutaneous and soft tissue neoplasm, with recently discovered recurrent *MIR143-NOTCH* fusion gene.¹ Myopericytoma is composed of eosinophilic tumor cells with more distinct smooth muscle differentiation and a whorled perivascular pattern. Angioleiomyoma is commonly a painful subcutaneous nodule, with a histological appearance of more differentiated smooth muscle. Notably, there is well-recognized overlap between these tumors.²

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 immunohistochemical profile, including expression of CD146, PDGFR β , RGS5, and frequently α SMA, without endothelial differentiation.³ Ultrastructural examination has suggested either a modified pericyte or smooth muscle phenotype in glomus tumor, myopericytoma, and angioleiomyoma. Recently, we reported a shared pericyte immunophenotype among glomus tumor, myopericytoma, and angioleiomyoma, including diffuse immunoreactivity for α SMA, CD146, PDGFR β , and RGS5.^{4,5} However, these known pericyte antigens have relatively diverse expression profiles, both in normal and neoplastic tissues (see 6,7 for a review).

Angiopoietin proteins are known to play an important role in angiogenesis and vascularization through the Tie2 pathway.⁸ Angiopoietin-1 (Ang-1) is produced by pericytes and other perivascular cells as well as endothelium,⁹ and is hypothesized to be involved in the reciprocal communication between pericytes and endothelium. Ang-1 expression is associated with pericyte recruitment for vascularization and tumor growth and may promote the recruitment of Tie-2-expressing pericyte precursors to newly forming vessels.⁸ Angiopoietin-2 (Ang-2) is mainly expressed in the endothelium but also in pericytes,⁹ and appears to increase tumor vascularization and regulate angiogenesis.¹⁰ Ang-2

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has well described antagonistic functions on Ang-1/Tie2 signaling in the endothelium.¹¹ 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.¹²

Angiopoietin/Tie signaling has primarily been interrogated in human vascular tumors. Brown et al. identified angiopoietin family expression by *in situ* hybridization in Kaposi's sarcoma and angiosarcoma specimens.¹³ Recently, Ang/Tie signaling was reported to correlate with survival in human angiosarcoma.¹⁴ Specifically, 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 our knowledge, the expression of angiopoietin family members in perivascular 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 perivascular soft tissue 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 “glomus tumor,” “malignant glomus tumor,” “myopericytoma,” and “angioleiomyoma.” Slides were reviewed by 2 independent pathologists to ensure accuracy of diagnosis (SMD and AWJ). Diagnostic criteria for malignancy in glomus tumors were used as described by Folpe and colleagues,^{15,16} including deep-seated tumors greater than 2 cm, tumors with atypical mitotic figures, or tumors with moderate to high nuclear grade and >5 mitotic figures in 50 HPF. Patient information was obtained, including age, sex, tumor location, and tumor size. Formalin-fixed paraffin embedded (FFPE) tumor tissue from patients was acquired from the tissue archives, under 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), 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.⁴ 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 re-evaluated 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 semi-quantitative measurements.

3. Results

3.1. Histologic appearance of perivascular soft tissue tumors

A collection of perivascular soft tissue tumors was interrogated for angiopoietin protein expression. Tumors included glomus tumor ($n = 9$), malignant glomus tumor ($n = 4$), myopericytoma ($n = 3$), and angioleiomyoma ($n = 9$). Each tumor type showed typical histologic features (Fig. 1). Clinical and demographic information for each patient sample are shown in Supplemental Table 1. Benign glomus tumors were all located on the digits, and showed either solid or glomuvenous architectural patterns (Fig. 1A, B, solid glomus tumor shown). Malignant glomus tumors were deep-seated (including deep soft tissue or visceral locations). Increased mitotic rate was seen in three of four tumors (9–25 mitoses per 10 HPF). Moderate to high nuclear grade was seen in a single case (Fig. 1C, D). Myopericytoma and angioleiomyoma specimens were found in the superficial soft tissue or dermis, most often of the lower extremity. Typical histologic features of myopericytoma included a perivascular proliferation of monomorphic ovoid to spindle cells with brightly eosinophilic cytoplasm (Fig. 1E, F). Angioleiomyoma tumors showed characteristics of both solid and venous growth patterns, being composed of eosinophilic tumor cells with indistinct cell borders, consistent with smooth muscle differentiation (Fig. 1G, H). Where appropriate, immunohistochemical stains were performed and supported each diagnosis (summarized in 4).

3.2. Ang-1 and -2 expression in non-lesional blood vessels

The normal distribution of Ang-1 and Ang-2 was first interrogated by immunohistochemical staining among small and large blood vessels not associated with tumors (Fig. 2). In small caliber vessels, Ang-1 was observed in both endothelial and perivascular cell types (Fig. 2A, B), while in larger vessels Ang-1 predominantly localized to the endothelium (Fig. 2C, D). Ang-2 expression was found in both endothelial and perivascular cells in both small and larger vessels (Fig. 2E–H). In aggregate, these data confirm previous observation that Ang-1 and -2 are present in both endothelial and perivascular cell types. Moreover, Ang-1 and -2 expression was not observed in other mesenchymal tissues, including no immunostaining in adipocytes, skeletal muscle, or stromal fibroblasts.

3.3. Ang-1 expression in perivascular soft tissue tumors

Next, Ang-1 expression was examined across perivascular soft tissue tumor samples. Results showed a near complete absence of Ang-1 expression across all tumor types (Fig. 3, Table 1). Ang-1 immunoreactivity was limited to intralesional blood vessels in most cases. The exception to this was malignant glomus tumor, which showed weak to moderate Ang-1 immunoreactivity (1–2+) in 50% of cases (2/4). Ang-1 expression in malignant glomus tumor was focal in all cases ($\leq 10\%$). See also Supplemental Tables 2–5 for a more detailed analysis of individual tumors.

Fig. 1. Typical histologic appearance of each perivascular soft tissue tumor. Hematoxylin–eosin (H&E) staining of (A, B) benign glomus tumor, (C, D) malignant glomus tumor, (E, F) myopericytoma, and (G, H) angioleiomyoma. Scale bar: 100 μ m.

3.4. Ang-2 expression is perivascular soft tissue tumors

Next, Ang-2 immunohistochemical staining was performed across all perivascular soft tissue tumor samples. Results showed

that Ang-2 expression was present throughout all tumor types and in all tumor samples (Fig. 4, Table 1). Ang-2 expression was higher in glomus tumor, malignant glomus tumor, and myopericytoma, with predominantly moderate staining intensity (2+) in the majority of tumor cells (66.1–82.5% of tumor cells showed positive

Table 1

Summary of Ang-1 and Ang-2 staining patterns within perivascular soft tissue tumors.

Tumor type	Ang-1 intensity (0–3+)	Ang-1 distribution (%)	Ang-2 intensity (0–3+)	Ang-2 distribution (%)
Glomus tumor	0	0	2 (± 0.5)	66.11 (± 23.95)
Malignant glomus tumor	0.75 (± 0.96)	5 (± 5.77)	2.25 (± 0.5)	82.5 (± 15.55)
Myopericytoma	0	0	2 (0)	70 (± 26.46)
Angioleiomyoma	0	0	1.44 (± 0.53)	30 (± 23.85)

Fig. 2. Ang-1 and -2 expression in non-tumor associated blood vessels. (A, B) Ang-1 in small caliber vessels. (C, D) Ang-1 in larger caliber vessels. (E, F) Ang-2 in small caliber vessels. (G, H) Ang-2 in larger caliber vessels. Black arrowheads indicate endothelial staining. Red arrowheads indicate perivascular staining. Scale bar: 100 μ m.

stain on average across all three diagnoses). In contrast, Ang-2 immunoreactivity was usually weak in angioleiomyoma. Similarly, the distribution of Ang-2 immunoreactivity was more limited in angioleiomyoma, and was most often noted in the minority of tumor cells within a given sample (30% of cells showed Ang-2 staining within angioleiomyoma). Among angioleiomyoma, no discernable difference in staining was observed with different architectural patterns (*i.e.* sheet-like *versus* venous growth).

4. Discussion

In summary, Ang-2 but not Ang-1 expression is a consistent feature of perivascular soft tissue tumors. Only malignant glomus

tumor showed Ang-1 expression, although this was not a consistent feature. Ang-2 expression was higher in glomus tumor, malignant glomus tumor, and myopericytoma, in comparison to angioleiomyoma. Thus, perivascular soft tissue tumors seem to somewhat depart from non-neoplastic pericytes in their expression profile of angiopoietin proteins. It is interesting to note that Ang-1 was not found in the perivascular component of the majority of larger blood vessels examined. This lack of Ang-1 in larger vessels mirrors the apparent lack of Ang-1 in perivascular soft tissue tumors.

Previously, we observed that perivascular soft tissue tumors (including glomus tumor, myopericytoma, and angioleiomyoma) share a unique immunohistochemical profile including expression

Fig. 3. Ang-1 immunohistochemical staining among perivascular soft tissue tumors. (A, B) Benign glomus tumor, (C, D) malignant glomus tumor, (E, F) myopericytoma, and (G, H) angioleiomyoma. Across the majority of tumor samples, Ang-1 was essentially limited to intraluminal blood vessels. Malignant glomus tumor samples showed focal staining in 2/4 of cases. Scale bar: 100 μ m.

of α SMA, CD146, PDGFR β , and RGS5.^{4,5} These markers are quite similar to antigens normally expressed in non-neoplastic pericytes. Perivascular epithelioid cell tumors (PEComa) to some extent share this staining profile.¹⁷ Our results for the first time a pericyte marker (Ang-1) that is not shared by perivascular soft tissue tumors. Whether this related family of soft tissue tumors is derived from an Ang-1 negative perivascular cell, or rather if Ang-1 expression is lost with neoplastic transformation remains a matter of speculation.

Directed anti-angiopoietin therapies are possible (including Trebananib, AMG 386), and have been investigated as anti-angiogenic therapies in epithelial,^{18–22} hematopoietic,²³ and

mesenchymal malignancies.²⁴ The most promising results for Trebananib therapy appear to be in ovarian cancer (reviewed in 18). In terms of mesenchymal tumors, D'Angelo et al. reported on the use of Trebananib in angiosarcoma.²⁴ In this case, lack of response led to early closure of the study.²⁴ From a theoretical perspective, our expression profile of Ang-2 suggests that anti-angiopoietin therapy can be considered in malignant glomus tumor.

In summary, Ang-2 but not Ang-1 expression is a consistent feature of perivascular soft tissue tumors. Moderate to strong Ang-2 expression was observed in glomus tumor, malignant glomus tumor, and myopericytoma.

Fig. 4. Ang-2 expression among perivascular soft tissue tumors. (A, B) Benign glomus tumor, (C, D) malignant glomus tumor, (E, F) myopericytoma, and (G, H) angioleiomyoma. Scale bar: 100 μ m.

Conflicts of interest

The authors have none to declare.

Acknowledgments

The present work was supported by the Orthopaedic Research and Education Foundation with funding provided by the Musculoskeletal Transplant Foundation, and NIH/NIAMS K08 AR068316-01. The authors thank the staff of UCLA Translational Pathology Core Laboratory, and A.S. James for their excellent technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jor.2016.07.003](https://doi.org/10.1016/j.jor.2016.07.003).

References

1. Mosquera JM, Sboner A, Zhang L, et al. Novel MIR143-NOTCH fusions in benign and malignant glomus tumors. *Genes Chromosomes Cancer*. 2013;52:1075–1087.
2. Granter SR, Badizadegan K, Fletcher CD. Myofibromatosis in adults, glomangiopericytoma, and myopericytoma: a spectrum of tumors showing perivascular myoid differentiation. *Am J Surg Pathol*. 1998;22:513–525.
3. Corselli M, Chen CW, Sun B, Yap S, Rubin JP, Peault B. The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. *Stem Cells Dev*. 2012;21:1299–1308.
4. Shen J, Shrestha S, Yen YH, et al. Pericyte antigens in perivascular soft tissue tumors. *Int J Surg Pathol*. 2015;23:638–648.
5. Shen J, Shrestha S, Yen YH, et al. The pericyte antigen RGS5 in perivascular soft tissue tumors. *Hum Pathol*. 2016;47:121–131.
6. Shih IM, Nesbit M, Herlyn M, Kurman RJ. A new Mel-CAM (CD146)-specific monoclonal antibody, MN-4, on paraffin-embedded tissue. *Mod Pathol*. 1998;11:1098–1106.
7. Palman C, Bowen-Pope DF, Brooks JJ. Platelet-derived growth factor receptor (beta-subunit) immunoreactivity in soft tissue tumors. *Lab Invest*. 1992;66:108–115.

8. 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.
9. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res*. 2005;97:512–523.
10. Falcón BL, Hashizume H, Koumoutsakos P, et al. Contrasting actions of selective inhibitors of angiopoietin-1 and angiopoietin-2 on the normalization of tumor blood vessels. *Am J Pathol*. 2009;175:2159–2170.
11. Maisonpierre PC, Suri C, Jones PF, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science*. 1997;277:55–60.
12. Witzanbichler B, Maisonpierre PC, Jones P, Yancopoulos GD, Isner JM. Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J Biol Chem*. 1998;273:18514–18521.
13. Brown LF, Dezube BJ, Tognazzi K, Dvorak HF, Yancopoulos GD. Expression of Tie1, Tie2, and angiopoietins 1, 2, and 4 in Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol*. 2000;156:2179–2183.
14. Buehler D, Rush P, Hasenstein JR, et al. Expression of angiopoietin-TIE system components in angiosarcoma. *Mod Pathol*. 2013;26:1032–1040.
15. Folpe AL, Fanburg-Smith JC, Miettinen M, Weiss SW. Atypical and malignant glomus tumors: analysis of 52 cases, with a proposal for the reclassification of glomus tumors. *Am J Surg Pathol*. 2001;25:1–12.
16. Goldblum JR, Folpe AL, Weiss SW, Enzinger FM. *Enzinger and Weiss's Soft Tissue Tumors*. 6th ed. Philadelphia, PA: Saunders/Elsevier; 2014.
17. Shen J, Shrestha S, Yen YH, et al. Pericyte antigens in angiomyolipoma and PEComa family tumors. *Med Oncol*. 2015;32:210.
18. Al Wadi K, Ghatage P. Efficacy of trebananib (AMG 386) in treating epithelial ovarian cancer. *Expert Opin Pharmacother*. 2016.
19. Chiu JW, Hotte SJ, Kollmannsberger CK, et al. A phase I trial of ANG1/2-Tie2 inhibitor trebaninib (AMG386) and temsirolimus in advanced solid tumors (PJC008/NCI#9041). *Investig New Drugs*. 2016;34:104–111.
20. Li X, Zhu S, Hong C, Cai H. Angiogenesis inhibitors for patients with ovarian cancer: a meta-analysis of 12 randomized controlled trials. *Curr Med Res Opin*. 2016;32:555–562.
21. Atkins MB, Gravis G, Drosik K, et al. Trebananib (AMG 386) in combination with sunitinib in patients with metastatic renal cell cancer: an open-label, multicenter, phase II study. *J Clin Oncol*. 2015;33:3431–3438.
22. Diamond JR, Wu B, Agarwal N, et al. Pharmacokinetic drug–drug interaction study of the angiopoietin-1/angiopoietin-2-inhibiting peptibody trebananib (AMG 386) and paclitaxel in patients with advanced solid tumors. *Investig New Drugs*. 2015;33:691–699.
23. Aguirre Palma LM, Flamme H, Gerke I, Kreuzer KA. Angiopoietins modulate survival, migration, and the components of the Ang-Tie2 pathway of chronic lymphocytic leukaemia (CLL) cells in vitro. *Cancer Microenviron*. 2016.
24. D'Angelo SP, Mahoney MR, Van Tine BA, et al. Alliance A091103 a phase II study of the angiopoietin 1 and 2 peptibody trebananib for the treatment of angiosarcoma. *Cancer Chemother Pharmacol*. 2015;75:629–638.