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Original contribution

Pericytic mimicry in well-differentiated liposarcoma/atypical lipomatous tumor☆,☆☆

Jia Shen PhD[a,1], Swati Shrestha BS[b,1], P. Nagesh Rao PhD[b], Greg Asatrian BS[a,b], Michelle A. Scott DDS, MBA[c], Vi Nguyen BS[b], Paulina Giacomelli BS[b], Chia Soo MD[d,e], Kang Ting DMD, DMedSci[a], Fritz C. Eilber MD[e], Bruno Peault PhD[d,f], Sarah M. Dry MD[b], Aaron W. James MD[a,b,d,*]

[a]*School of Dentistry, University of California, Los Angeles, CA 90095*
[b]*Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA 90095*
[c]*Nationwide Children's Hospital, Columbus, OH 43205*
[d]*Orthopedic Hospital Research Center, University of California, Los Angeles, CA 90095*
[e]*Department of Surgery, University of California, Los Angeles, Los Angeles, CA 90095*
[f]*Center for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom*

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Summary Pericytes are modified smooth muscle cells that closely enwrap small blood vessels, regulating and supporting the microvasculature through direct endothelial contact. Pericytes demonstrate a distinct immunohistochemical profile, including expression of smooth muscle actin, CD146, platelet-derived growth factor receptor *β*, and regulator of G-protein signaling 5. Previously, pericyte-related antigens have been observed to be present among a group of soft tissue tumors with a perivascular growth pattern, including glomus tumor, myopericytoma, and angioleiomyoma. Similarly, malignant tumor cells have been shown to have a pericyte-like immunoprofile when present in a perivascular location, seen in malignant melanoma, glioblastoma, and adenocarcinoma. Here, we examine well-differentiated liposarcoma specimens, which showed some element of perivascular areas with the appearance of smooth muscle (n = 7 tumors). Immunohistochemical staining was performed for pericyte antigens, including smooth muscle actin, CD146, platelet-derived growth factor receptor *β*, and regulator of G-protein signaling 5. Results showed consistent pericytic marker expression among liposarcoma tumor cells within a perivascular distribution. MDM2 immunohistochemistry and fluorescence in situ hybridization for *MDM2* revealed that these perivascular cells were of tumor origin (7/7 tumors), whereas double immunohistochemical detection for CD31/CD146 ruled out an endothelial cell contribution. These findings further support the concept of pericytic mimicry, already established in diverse malignancies, and its presence in well-differentiated liposarcoma. The extent to which pericytic mimicry has prognostic significance in liposarcoma is as yet unknown.

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* Corresponding author. Department of Pathology & Laboratory Medicine, University of California, Los Angeles, David Geffen School of Medicine, 675 Charles E. Young Dr. S., Rm 2641A Los Angeles, CA 90095.
E-mail addresses: Awjames@mednet.ucla.edu, aaronwjames1@gmail.com (A. W. James).

[1] The authors share equal responsibility for the work presented herein.

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1. Introduction

Pericytes are modified smooth muscle cells that closely enwrap small blood vessels, regulating and supporting the microvasculature through direct endothelial contact. Pericytes demonstrate a distinct immunohistochemical profile, including expression of smooth muscle actin (α-SMA), CD146, platelet-derived growth factor receptor β (PDGFRβ), and regulator of G-protein signaling 5 (RGS5), without evidence of endothelial differentiation [1,2]. Current interests in pericytes stem in large part from the growing understanding that this cell type represents a native mesenchymal stem cell (MSC) progenitor cell [1,3–9]. Indeed, purified pericytes give rise to multiple mesodermal tissues after in vitro differentiation or in vivo transplantation, including bone, adipose, cartilage, and muscle—features identical to traditionally derived bone marrow MSC [9,10].

Growing data suggest that when tumor cells adopt a perivascular location, they also adopt cell surface markers characteristic of pericytes. This phenomenon is best understood in malignant melanoma, where tumor cells adopt a perivascular migration pattern, or "angiotropism" [11–13]. This perivascular invasion, also called extravascular migratory metastasis, is an underrecognized route of tumor spread and associated with a poor prognosis in melanoma [13]. Similarly, in the malignant brain tumor glioblastoma, tumor cells adopt a pericyte-like location associated with perivascular invasion [14,15]. In fact, using cell-tracking techniques, investigators have found that most vessel-lining pericyte-like cells in glioblastoma are actually of tumor cell origin. Likewise, recent research suggests that pancreatic and prostatic adenocarcinoma exhibit perivascular invasion for regional spread, although this has been less well studied [16,17]. In summary, pericyte marker expression, also termed "pericyte mimicry," is a characteristic finding across all studied tumors with a perivascular tumor growth.

A subset of liposarcomas display heterologous differentiation, including smooth muscle, bone, and cartilage. Nonneoplastic adipose tissue is a rich source of pericytes [18–22], and it is currently assumed that tissue resident MSCs are the precursors for these heterologous components within liposarcoma. Of particular interest are areas with the appearance of smooth muscle within well-differentiated liposarcoma (WDLPS) [23–26]. Areas resembling smooth muscle often appear to arise from and radiate outward from intratumoral blood vessels, usually in a multifocal fashion [23]. These perivascular proliferations house atypical stromal cells characteristic of liposarcoma, often within the vessel wall. Of note, these perivascular cells demonstrate immunoreactivity to SMA [23], but a more thorough examination of pericyte markers has not yet been performed.

Here, we examine WDLPS specimens. We hypothesized that these perivascular areas with the appearance of smooth muscle would be associated with a pericyte immunophenotype, or pericytic mimicry, within liposarcoma tumor cells.

2. Materials and methods

2.1. Tissue identification and histology

Tumors were identified using a retrospective review of the pathology slide archives of the Department of Pathology and Laboratory Medicine at the University of California, Los Angeles (UCLA), examining 55 cases with a diagnosis of "WDLPS" or "atypical lipomatous tumor" (ALT). Slides were reviewed by 2 independent pathologists to ensure accuracy of diagnosis and to identify tumors with some element of perivascular areas with the appearance of smooth muscle (S. M. D. and A. W. J). Cases with dedifferentiation or greater than 5 mitotic figures per 10 high-power fields were not included. Patient information was obtained, including age, sex, tumor location, tumor size, and previous cytogenetic studies performed during the initial diagnostic evaluation. Formalin-fixed, paraffin-embedded tumor tissues from patients were acquired from the tissue archives, under UCLA institutional review board approval no. 13-000918.

2.2. Immunohistochemistry

Immunohistochemistry for pericyte markers was performed using the ABC method (Vectastain Elite ABC; Vector Laboratories, Burlingame, CA) using 3,3′-diaminobenzidine (DAB) as the chromogen (ImmPACT DAB; Vector Laboratories). Multiple antigens were detected by multiplexing the ABC method and DAB chromogen with an alkaline phosphatase polymer detection method (ImmPress-AP Polymer Detection, Anti-mouse IG; Vector Laboratories) and Vector Red chromogen (Vector Red Alkaline Phosphatase Substrate; Vector Laboratories).

The following primary antibodies were used: monoclonal mouse anti-MDM2 (1:100; EMD Millipore, Billerica, MA), monoclonal mouse anti–α-SMA (1:75 [1 A4]; Abcam, Cambridge, MA), monoclonal rabbit anti-CD146 (1:500, EPR3208: Abcam), monoclonal rabbit anti-PDGFRβ (1:100 [2E8E1], monoclonal mouse anti-RGS5 (1:100 [89C2]; Cell Signaling Technologies, Danvers, MA), and monoclonal mouse anti-CD31 (1:100 [89C2]; Cell Signaling Technologies). The following secondary antibodies were used: polyclonal goat biotinylated antirabbit IgG (1:500; Sigma, St Louis, MO), polyclonal horse antimouse IgG (1: 500 [H + L]; Vector Laboratories), and polyclonal goat antirat Ig (1:500; Becton Dickinson and Company, Franklin Lakes, NJ).

Heat-mediated antigen retrieval was performed for all immunohistochemical stains in 1 mM Tris-EDTA and 0.01% Tween-20 (Sigma), pH 8. Nonspecific antibody binding was blocked (IHC-TEK Antibody Diluent, pH 7.4; IHC World, Woodstock, MD). Endogenous peroxidase and alkaline phosphatase blocking solution was used (BLOXALL Endogenous Peroxidase and Alkaline Phosphatase Blocking Solution; Vector Laboratories). Mayer hematoxylin was used as a nuclear counterstain (1:5; Abcam), and slides were mounted using