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Pericyte Antigens in Mesenchymal Tumors

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ABSTRACT

Pericytes are modified smooth muscle cells that lie in intimate association with the vasculature. Once thought to simply regulate blood flow and angiogenesis, current understandings suggest that pericytes have wide ranging roles in regulating inflammation, fibrosis and neoplasia. In order to detect pericytes, a combination of cell surface markers appears to be specific, including αSMA (Smooth Muscle Actin), CD146, PDGFRβ (Platelet Derived Growth Factor Receptorβ) and RGS5 (Regulator of G protein signaling 5). In the following concise review, we will describe what is known regarding pericyte antigen expression among mesenchymaltumors. Pericyte antigen expression is common across a group of related perivascular soft tissue tumors, including glomus tumor, my opericytoma, and angioleiomyoma. Pericyte antigen expression is also observed within the PEComa family tumor group, although pericyte marker expression is more variable and depends on the tumor cell morphology. In contrast, pericyte markers are absent in solitary

fibrous tumor, a sarcoma once postulated to arise from pericytes. Bone marrow-derived pericytes have also been shown to support the tumor vasculature of sarcomas, such as in Ewing's sarcoma. In summary, pericyte antigen expression is increasingly studied in soft tissue and skeletal tumors. In theory, this may represent a true pericytic cell of origin, a specific line of differentiation, or aberrant marker adoption reflecting a perivascular residence.

Keywords: Pericyte; Perivascular Stem Cell; CD146; Platelet Derived Growth Factor Receptor; RGS5; Glomus Tumor; Myopericytoma; Angiomyolipoma; PEComa.

INTRODUCTION

Pericytes are mesenchymal cells that closely enwrap capillaries and microvessels, regulating and supporting the microvasculature through direct contact with the endothelium. Pericytes have distinct antigen expression, including a combination of αSMA, CD146, PDGFRβ, and RGS5 [1,2]. Conversely, they lack expression of the endothelial markers CD34, CD31, and von Willebrand factor [3]. Current research interests in pericytes span a variety of scientific disciplines, including atherosclerosis and peripheral vascular disease [4], cancer and regulation of tumor blood flow and metastasis [5] and even mesenchymal stem cell biology [6]. For some time, it has been recognized that pericytes are multipotent cells with the ability to undergo osteogenesis or chondrogenesis, among other lines of differentiation [3]. More recently, it has been established that pericytes represent native *in vivo* progenitors of Mesenchymal Stem Cells **(MSC)** [1,6-12]. For example, pericytes have been shown to express typical markers that identify MSC, including CD44, CD73, CD90 (Thy-1), and CD105 (Endoglin). In addition, when isolated and cultured *in vitro*, pericytes demonstrate multilineage differentiation potential on a clonal level, including differentiation down osteogenic, adipogenic, myogenic,and chondrogenic lineages [6,13] (see also[14] for a review). While research into the MSC properties of non-neoplastic pericytes continues [15-18], the expression of pericyte markers within mesenchymal tumors has only recently been investigated.

PERICYTES IN PERIVASCULAR SOFT TISSUE TUMORS

Perivascular soft tissue tumors represent a group of related and relatively uncommon entities presumed to originate from pericytes or a modified perivascular cell. Among these soft tissue tumors, glomus tumor, myopericytoma and angioleiomyoma share a spectrum of histologic findings, including a prominent perivascular growth pattern. Glomus tumor is a subcutaneous and soft tissue neoplasm, most commonly found on the digits, often dark in color owing to its rich blood supply [19,20]. Glomus tumors have a characteristic cytomorphology including enlarged and monomorphic round to ovoid nuclei, and moderate amounts of amphophilic cytoplasm, and are accompanied by a variable myxoid matrix. Recently, recurrent *MIR143-NOTCH* fusion genes have been identified in a large subset of glomus tumors [21]. My opericytoma bears some histologic resemblance to glomus tumor, but is most common on the lower extremities and is composed of moreeosinophilic tumor cells with a prominent whorled perivascular growth pattern [22]. Other

histologic features of myopericytoma include a perivascular proliferation of tumor cells around adjacent vessels. Angioleiomyoma generally presents as a painful subcutaneous nodule, again common on the lower extremities, with a histological appearance of more differentiated smooth muscle cells, arranged in perivascular or fascicular growth pattern. Importantly, there is well-recognized overlap between these perivascular tumors, leading to the use of hybrid terms in the past, such as 'glomangiomyoma' or 'glomangiopericytoma'[23, 24].

Interestingly, diffuse immune reactivity for pericyte antigens was seen in this group of related tumors [25]. This included expression of αSMA, CD146, and PDGFRβ across benign glomus tumor, myopericytoma and angioleiomyoma (Figure 1). Moreover, the strongest immunoreactivity for all 3 markers was located in areas exhibiting the most prominent perivascular whorling of tumor cells. These findings are line with related antigens that may be seen in these perivascular tumors, including Caldesmon and Calponin. Of note, pericyte markers were also seen in examples of malignant glomus tumors, although this was seen to a lesser degree. On the other hand, pericytic marker expression was not seen within Solitary Fibrous Tumors **(SFT)**, a tumor type once hypothesized to have pericytic differentiation.

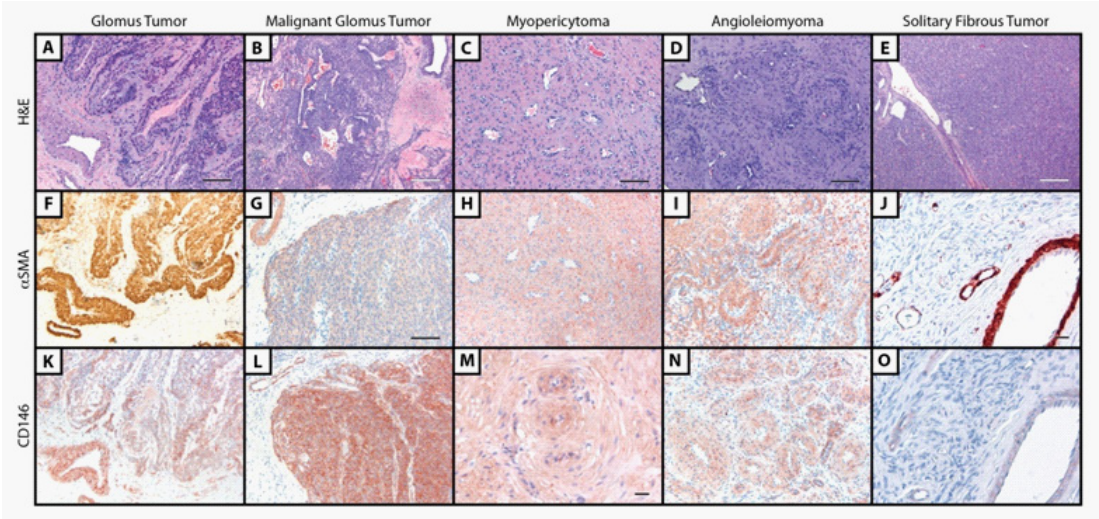

Figure 1: Pericyte marker expression among soft tissue tumors. (A-E) Characteristic histologic appearance of Glomus tumor, malignant glomus tumor, myopericytoma, angioleiomyoma, and solitary fibrous tumor. (F-J) α Smooth Muscle Actin (αSMA) expression within each tumor. With the exception of solitary fibrous tumor, each shows αSMA immunoreactivity. (K-O) CD146 expression within each tumor. Likewise, all show expression of CD146 with the exception of solitary fibrous tumor. White scale bar: 500 μm; Black scale bar: 200 μm.

In theory, this diffuse pattern of pericyte marker expression may represent a true pericytic cell of origin among this group of tumors, or alternatively simply bereflective of a pericytic line of differentiation. Importantly, the three pericyte markers examined (αSMA, CD146, and PDGFRβ)

did not show discordance throughout the tumors examined. Given the distinctive histologic appearance of these perivascular tumors and the widespread use of αSMA in the clinical practice of pathology, it seems unlikely that the addition of CD146 and PDGFRβ would of substantive clinical benefit. Nevertheless, co-expression of these markers solidifies the WHO classification of these related entities as perivascular tumor types.

PERICYTES IN PERIVASCULAR EPITHELIOID CELL TUMORS (PECOMA)

The Perivascular Epithelioid Cell Tumor **(PEComa)** families of tumors include a group of diverse neoplasms with dual myoid-melanocytic differentiation [26]. The most common PEComa family tumor is angiomyolipoma, which occurs predominantly adjacent to the kidney or within the liver [27]. As the name implies, angiomyolipoma has a triphasic appearance including thick-walled blood vessels, myoid appearing perivascular cells, and lipid-distended adipocyte-like cells [26]. As briefly mentioned, the immunohistochemical features of PEComa family tumors are distinct, and show co-expression of smooth muscle (αSMA) and melanocytic markers (including HMB45, MART1, S100, Tyrosinase)[28]. Occasionally, the myoid appearing perivascular cells overshadow the other cell populations. In these cases, tumor cells may adopt a predominant epithelioid to spindled morphology, often designated 'epithelioid angiomyolipoma' and 'spindled angiomyolipoma.' Although incompletely understood, epithelioid angiomyolipoma tend to behave in a more aggressive fashion [29,30].

Siroky et al. investigated the possible origin of renal angiomyolipoma among patients with tuberous sclerosis and found that tumor cells stained positively for the pericytic markers PDGFRβ, Desmin and αSMA [31]. Additionally, the angiomyolipoma cells displayed intense staining for VEGFR2 and ANGII. Thus, these results suggest a pericytic origin or line of differentiation for renal angiomyolipoma.These findings have also been confirmed in a larger and more heterogenous group of PEComa family tumors [32], although the degree of staining depended on the tumor morphology. One of the main and somewhat unexpected findings was the marked difference in pericyte markers between angiomyolipoma with spindled vs. epithelioid cytomorphology. Spindled angiomyolipomas showed significant expression of pericyte markers, including αSMA, CD146, and PDGFRβ. In contrast, the intensity of all pericyte markers was reduced to absent in epithelioid angiomyolipoma.Pericytic marker expression was also examined across lymphangiomyoma samples, which exhibited a variable range of αSMA, CD146, and PDGFRβ distribution (0 to 51.67%).

These findings bring up interesting concepts of the meaning of pericyte marker expression in PEComa family tumors. Perhaps the marked difference in pericyte marker expression between spindled and epithelioid angiomyolipoma indicates a different cell of origin for these related tumors. Alternatively, the reduction or absence of pericyte markers in epithelioid angiomyolipomamay reflects a relative loss of pericyte differentiation that accompanies a more aggressive tumor behavior.

PERICYTES IN EWING'S SARCOMA

In addition toperivascular soft tissue tumors, pericyte distribution has also been studies in Ewing's sarcoma [5]. The second most common pediatric primary bone tumor, Ewing's sarcoma is defined by characteristic chromosomal translocations most commonly resulting in fusion of the *EWSR1* gene with members of the ETS gene family, including *FLI1* or *ERG* [33]. From a histological perspective, Ewing's sarcoma is commonly characterized by the surrounding of blood vessels by small round blue cells. *In vivo* murine studies have shown how pericyte biology contributes to Ewing's sarcoma tumor vascularization [34,35]. For example, human CD34+ and murine Sca1+/Gr1+ and VEGFR2+ bone marrow stem cells act as pericyte precursors in Ewing's sarcoma and migrate towards TC71 tumor sites, where they adopt a perivascular distribution [34]. Moreover, these cells express markers of pericytic differentiation including αSMA and Desmin [34]. In addition to confirming the previous findings, another study revealed that bone marrow cells also migrate to sites of lung metastasis, where they display pericytic location and expression of the antigens αSMA, Desmin, and PDGFRβ [35]. Thus, these studies suggest that pericytes derived from bone marrow stem cell precursors support the vasculature of both Ewing's sarcoma primary tumors and lung metastases.

PERICYTIC MIMICRY

Recent evidence suggests that common malignancies spread along the abluminal (outer) surface of vessels and without intravascular spread in a process termed Extravascular Migratory Metastasis **(EVMM).** This is promoted via angiotropism, in which tumor cells migrate towards a pericytic or adventitial cell location without intravasation. This adoption of pericyte markers when residing and infiltrating in a perivascular manner has been coined 'pericytic mimicry.'Together, EVMM, angiotropism, and pericyte mimicry represent an alternative mechanism of cancer dissemination, which classically occurs intravascularly via the blood or lymph.

This under-recognized route of tumor spread is most well documented in melanoma, for which it has been found to be a poor prognostic marker [36-39]. EVMM in melanoma has also been compared to Neural Crest Cell **(NCC)** migration and angiogenesis. When transplanted into the chick embryonic neural crest microenvironment, human metastatic C8161 melanoma cells respond to cues from surrounding host tissues by migrating in a NCC-like pattern [40]. Additionally, aberrant regulation of neural crest developmental genes and receptor tyrosine kinases promotes plasticity and invasiveness in melanoma [40]. Likewise, dysregulation of angiogenesis can result in the recruitment of invasive melanoma cells rather than pericytes towards microvessels[41]. This pericytic mimicry is further evidenced by the up regulation of PDGFB, which is normally expressed by pericytes during angiogenesis, in angiotropic melanoma cells [37,41]. In addition, the interaction between melanoma cells and the abluminal surface of the endothelial cells triggers differential expression of genes correlated to cancer cell migration, progression, epithelial to mesenchymal transition, and embryonic/stem cell properties; for

example, Selectin E **(SELE)**, Intercellular Adhesion Molecule 1 **(ICAM1)**, and chemokine (C-C motif) ligand 2 are all over expressed more than 2-fold [37]. Interestingly, in addition to initiating DNA mutation in melanocytes, UV irradiation also enhances melanoma tumor cell expansion along abluminal blood vessels and increases incidence of lung metastases [39]. Specifically, UV-exposed keratinocytes release High Mobility Group Box 1**(HMGB1)**, which initiates Toll-Like Receptor 4 **(TLR4)**/MyD88-driven neutrophilic inflammation and promotes pericyte mimicry and metastatic dissemination.

A similar phenomenon has been described in glioblastoma, the most common malignant brain tumor, in which the majority of cells found in a pericytic location are in fact tumor derived[42-44]. Glioblastoma cells share the same embryonic origin (NCC) as melanocytes and also exhibit pericyte mimicry [45, 46]. Similarly, other research groups have found that pancreatic and prostatic adenocarcinoma as well as ovarian carcino sarcoma also may invade tissues in an angiotropic manner [47-49].

DISCUSSION

Importantly, no known pericytic markers are absolutely specific and additional pericyte markers have yet to be examined in detailed in soft tissue tumors. For example, CD146 is expressed in a variety of cell types including endothelium, smooth muscle, and Schwann cells, among others [50]. Similarly, PDGFRβ is also present in diverse cell types including fibroblasts, endothelium, and smooth muscle [51]. With this diverse expression profile, it is understandable a single 'pericyte' markeris seen across multiple non-pericyte tissue and tumors. Nevertheless, a combination of αSMA+ CD146+ PDGFRβ+ and RGS5 appears specific for pericytic and/or smooth muscle differentiation. Other potentially more specific pericyte markers have yet to be investigated in soft tissue tumors, including Ang-1 [52], Nestin [53,54], and NG2 [6,55,56].

New and promising areas of investigation include the investigation of pericyte marker expression in malignant soft tissue tumors, or sarcomas. Several sarcomas exhibit a characteristic perivascular growth pattern. For example Malignant Peripheral Nerve Sheath Tumor **(MPNST)** commonly exhibits a distinctive perivascular condensation of tumor cells. A similar phenomenon is also observed in most Fibromyxoid Sarcoma **(FMS)** specimens. An interesting investigation would include whether a phenomenon analogous to 'pericyte mimicry' exists among sarcomas with a prominent perivascular growth pattern. If this theory holds true, targeted therapy against pericyte specific markers, including tyrosine kinase inhibitors (e.g. anti-PDGFRβ), could be considered on a biologic basis.

In summary, although in it's relatively infancy, the study of pericytic markers in mesenchymal tumors has already yielded key insights. A group of related perivascular tumors demonstrates diffuse expression of key pericyte antigens, including glomus tumor, myopericytoma, and angioleiomyoma. Angiomyolipoma and related PEComa tumors show more variable pericyte marker expression For example, epithelioid angiomyolipoma is essentially lacking in pericyte

marker expression.The contribution of bone marrow derived pericytes to Ewing's sarcoma growth and metastasis has also been examined. In summary, pericytic differentiation is increasingly studied in neoplasia. In theory, this pattern of pericyte marker expression may represent a true pericytic cell of origin, a specific line of differentiation, or aberrant marker adoption reflecting a perivascular residence. Markers of pericytic differentiation may be of future diagnostic utility for the evaluation of mesenchymal tumors or identify actionable signaling pathways for future therapeutic intervention.

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