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Original contribution

Phenotypic characterization of perivascular myoid cell neoplasms, using myosin 1B, a newly identified human pericyte marker☆,☆☆

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Summary Our aims were to identify pericyte-specific markers for the analysis of formalin-fixed, paraffin-embedded human tissue samples, and to characterize perivascular myoid cell neoplasms phenotypically. Previously identified pericyte markers failed to distinguish pericytes from other cellular types, such as vascular smooth muscle cells (vSMCs) and fibroblasts, in immunohistochemistry analysis. However, we compared gene expression profiles between pericytes, vSMCs, and fibroblasts, and performed human skin vasculature immunohistochemistry analysis, which led to the identification of myosin 1B (MYO1B) as a novel pericyte marker. Afterward, we investigated the expression levels of MYO1B and h-caldesmon (h-CD) in perivascular myoid cell neoplasms, angioleiomyomas (n = 28), glomus tumors (n = 23), and myopericytomas (n = 3). Angioleiomyomas were shown to contain MYO1B-negative and h-CD–positive ($MYO1B^{-}hCD^{+}$) tumor cells, with vSMC features. Glomus tumors were predominantly composed of the $MYO1B^{+}hCD^{+}$ tumor cells, with the intermediate features between pericytes and vSMCs, whereas $MYO1B^{+}hCD^{-}$ tumor cells with pericytic features and/or the $MYO1B^{-}hCD^{+}$ tumor cells with vSMC features were frequently found in these tumors. The perivascular concentric pattern of 2 myopericytoma cases was composed of $MYO1B^{+}hCD^{+}$ tumor cells, whereas that of one myopericytoma contained $MYO1B^{-}hCD^{+}$ tumor cells. These results indicate that the ability to distinguish between these cell types may allow us to understand the differentiation and origin of perivascular myoid cell neoplasms. This is

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the first study to identify cell properties of perivascular myoid cell neoplasms by using a pericyte-specific marker with considerably lower expression in vSMCs and fibroblasts.

1. Introduction

Two types of vascular mural cells have been identified as follows: pericytes and vascular smooth muscle cells (vSMCs). These cells localize to the abluminal side of endothelial cells and adluminal side of the adventitial fibroblasts, covering small and large blood vessels, respectively [1]. They belong to the same lineage and form a morphological and functional continuum along blood vessels. Previous ultrastructural studies demonstrated that pericytes and vSMCs share common features, but pericytes lack dense bodies and have fewer myofilaments [1-7]. Ultrastructural analysis represents an essential method for the elucidation of pericytic features, but they are not always accessible for diagnostic purposes. Therefore, pericyte-specific immunohistochemistry (IHC) markers for the analysis of formalin-fixed, paraffin-embedded (FFPE) tissues are necessary.

Previous studies demonstrated that adenosine triphosphate–binding cassette, subfamily C member 9 (ABCC9), α-smooth muscle actin (αSMA), CD13, CD73, CD90, CD146, CD248, δ-like 1 homolog (DLK1), desmin, potassium inwardly rectifying channel subfamily J member 8 (KCNJ8), low-affinity nerve growth factor receptor (LNGFR), myosin heavy chain 9 (MYH9), neuroglia 2 (NG2), platelet-derived growth factor receptor type B (PDGFRB), and tropomyosin may represent molecular markers specific for pericytes in humans and rodents [8-13]. However, little is known about the expression levels of most of these markers in pericytes and other cellular types in FFPE human tissues.

According to the classification of soft tissue tumors, provided by the World Health Organization, glomus tumors have been categorized as pericytic (perivascular) tumors [14]. Ultrastructural studies demonstrated that glomus tumor cells are more similar to vSMCs than to pericytes [15,16]. Therefore, whether tumor cells with pericytic features exist at all remains unknown, and to identify these cells, human pericyte molecular markers should be identified.

In this study, we aimed to identify pericyte-specific markers for the analysis of FFPE human tissues and to characterize phenotypically perivascular myoid cell neoplasms.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Animal Care and Use Committee of the Hamamatsu University School of Medicine (No. 2015008). NG2 discosoma red fluorescent protein (DsRed) transgenic mice and C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME) and SLC (Hamamatsu, Shizuoka, Japan), respectively. All animal experiments complied with Animal Research: Reporting of In Vivo Experiments guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals. The study design and all experiments, where human FFPE tissue samples were used, were approved by the Medical Ethics Committee of Hamamatsu University School of Medicine (No. 14-366), which waived the need for written or oral consent. This study was performed according to the Declaration of Helsinki.

2.2. Antibodies

Antibodies were purchased from several manufacturers (Supplementary Table 1).

2.3. Tissue samples

A total of 54 perivascular myoid cell neoplasm samples were retrieved from the archives of the Hamamatsu University School of Medicine Hospital, Japan. These samples included 28 angioleiomyoma cases, 23 glomus tumor cases, and 3 cases of myopericytomas. Hematoxylin and eosin (H&E)–stained FFPE tissues were examined by 2 independent pathologists, and the diagnosis was established based on the most recent criteria provided by the World Health Organization [14]. Patients' data, including age, sex, tumor location, and tumor size, were obtained from the relevant medical records.

2.4. IHC and immunofluorescence analysis

Conventional IHC analysis was performed after antigen retrieval (Supplementary Table 1). Chromogenic double staining was performed using anti–myosin 1B (MYO1B) antibody and peroxidase-conjugated Universal Immuno-enzyme Polymer antirabbit solution, stained with 3,3′-diaminobenzidine + substrate-chromogen (Nichirei Biosciences, Tokyo, Japan), followed by a treatment with anti-CD34 antibody and alkaline phosphatase–conjugated Universal Immuno-enzyme Polymer antimouse solution, stained bluish purple with fast blue BB salt (Sigma-Aldrich, St Louis, MO). Immunofluorescence staining was performed after antigen retrieval as well. Deparaffinized sections were incubated with anti-MYO1B and anti–h-caldesmon (h-CD) antibodies, and Hoechst 33342 (Sigma-Aldrich). Afterward, the sections were incubated with the Alexa Fluor 546–conjugated goat antirabbit and Alexa Fluor 488–