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Case Report

CD31 is a surrogate marker for blastic plasmacytoid dendritic cell neoplasm: a case of a hematologic skin tumor immunohistochemically false negative for CD45

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Abstract: Round cell tumors of the skin in the adult constitute one of the diagnostic challenges for histopathologists, because their differential diagnosis covers a broad spectrum of tumors of epithelial as well as non-epithelial origins. As the first step towards the correct diagnosis, immunohistochemical screening of the tumor sample by CD45 (leukocyte common antigen, LCA) antibody is thought to be useful to examine whether the tumor is derived from hematopoietic lineages. Here, we report a diagnostically challenging case of CD31-positive malignant skin tumor with false immunonegativity for CD45, which turned out to be blastic plasmacytoid dendritic cell neoplasm (BPDCN). The tumor was composed of middle to large-sized round cells, which was supposed to be hematologic malignancy, probably of lymphoid or myeloid origins. However, immunohistochemistry of the tumor cells showed that they seemed to be CD45-negative. We considered the result to be false negative because immunohistochemistry of another CD45 antibody from a different supplier revealed that the tumor cells were positive. It turned out that CD45 antibodies from different suppliers were derived from different clones with presumably distinct specificity. On the other hand, CD31 immunopositivity of the tumor cells suggested the possibility of vascular origin of the tumor. However, ERG immunohistochemistry of the tumor cells showed weaker staining than that of vascular endothelium, enabling us to reach the correct diagnosis of BPDCN, a CD31-positive hematologic malignancy. We also discuss how to avoid these pitfalls and potential implications of CD31 expression as a surrogate marker for hematologic malignancy.

Keywords: Blastic plasmacytoid dendritic cell neoplasm, CD31, CD45, immunohistochemistry, false negative

Introduction

Round cell tumors of the skin in the adult constitute one of the diagnostic challenges for histopathologists, because their differential diagnosis covers a broad spectrum of tumors of epithelial as well as non-epithelial origins. As the first step towards the correct diagnosis, immunohistochemical screening of the tumor sample by pankeratin AE1/AE3 and CD45 (leukocyte common antigen, LCA) antibodies is thought to be useful to examine whether the tumor is derived from epithelial or hematopoietic lineages, respectively. In many cases, this initial characterization works well, although some exceptions exist that may be potential sources of diagnostic pitfalls.

First, hematologic tumor may not always be CD45-positive in immunohistochemistry. For example, typical cases of classical Hodgkin lymphoma show inherent immunohistochemical negativity of CD45 [1]. It is also not unexpected that B-acute lymphoblastic leukemia/lymphoblastic lymphoma (B-ALL/LBL) and plasmacytic tumor including plasmacytoma are CD45-negative in immunohistochemistry or flow cytometry [2-4]. Other subtypes of lymphomas are also reported to be CD45-immunonegative, including diffuse large B-cell lympho-

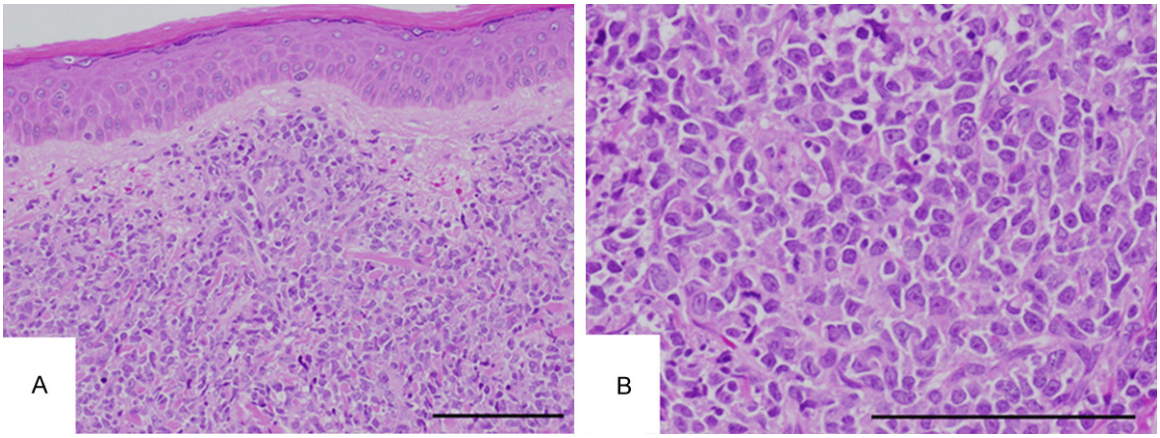

Figure 1. Hematoxylin-eosin (HE) image of the skin tumor. A. Low power image. Original magnification: × 200. Bar: 100 μm. B. High power image. Note prominent intercellular slits. Original magnification: × 400. Bar: 100 μm.

ma and NK/T-cell lymphoma [5-7]. Therefore, CD45-immunonegativity does not rule out the possibility of hematopoietic tumors.

On the other hand, AE1/AE3-immunopositivity of the tumor does not always guarantee that the tumor is of epithelial origin. For example, angiosarcoma is a non-epithelial tumor that sometimes mimics epithelial tumor in histology as well as AE1/AE3-positivity in immunohistochemistry. Typical angiosarcomas exhibit tubular vascular channels and epithelioid adhesiveness. The potential diagnosis of angiosarcoma may easily be reached by histopathologists if they notice that the tumor is reddish and localized at the forehead of the old or if they observe red blood cells in the vascular channels. However, it may be a diagnostic challenge to distinguish poorly differentiated or AE1/AE3-positive angiosarcomas from epithelial tumors. In these cases, it is often useful to immunohistochemically examine the expression of such markers as CD31 or ERG, which are thought to be specific for vascular endothelium.

Here, we report a diagnostically challenging case of CD31-positive malignant skin tumor with false immunonegativity for CD45, which turned out to be blastic plasmacytoid dendritic cell neoplasm [8].

Case report

An 83-year-old Japanese man was admitted to Takarazuka Municipal Hospital for the examination of multiple erythemas. It had first appeared 5 months before in his right forearm with the size of around 1 cm in diameter. It seemed to regress upon steroid ointment. However, the erythema reappeared 2 months before not only in the right forearm but also in the trunk and the shoulder. The largest erythema on the shoulder was around 3 cm in diameter.

On admission, there were erythematous lesions on the left shoulder as well as on the trunk and the bilateral forearms. The largest one was on the left shoulder, which was a purple-red plaque-like tumor. It was palpable but appeared to be fixed on the subcutaneous tissue. In addition to this tumor, there were multiple palpable erythemas of up to 2 cm in diameter on the trunk and the bilateral forearms.

Laboratory test for peripheral blood showed pancytopenia with low hemoglobin (12.7 g/dL; normal range: 13.5-17.5), white blood cell (20.2 × 10^2; normal range: 50-80 × 10^2) and platelet (117 × 10^9/L; normal range: 140-379 × 10^9) counts, and high serum lactate dehydrogenase (264 U/L; normal range 100-210). In order to explore the cause of pancytopenia, needle aspiration biopsy of the bone marrow was performed to find myelodysplasia with trisomy 8 (data not shown). Other laboratory tests showed unremarkable findings. His past history included colon cancer at the early stage 5 years before, which was successfully resected laparoscopically, and transient liver dysfunction 18 months before. Chest and abdominal computed tomography (CT) studies showed no apparent mass or space-occupying lesions in the internal organs and lymph nodes (data not shown). ^{18F}-fluorodeoxyglucose-positron emis-

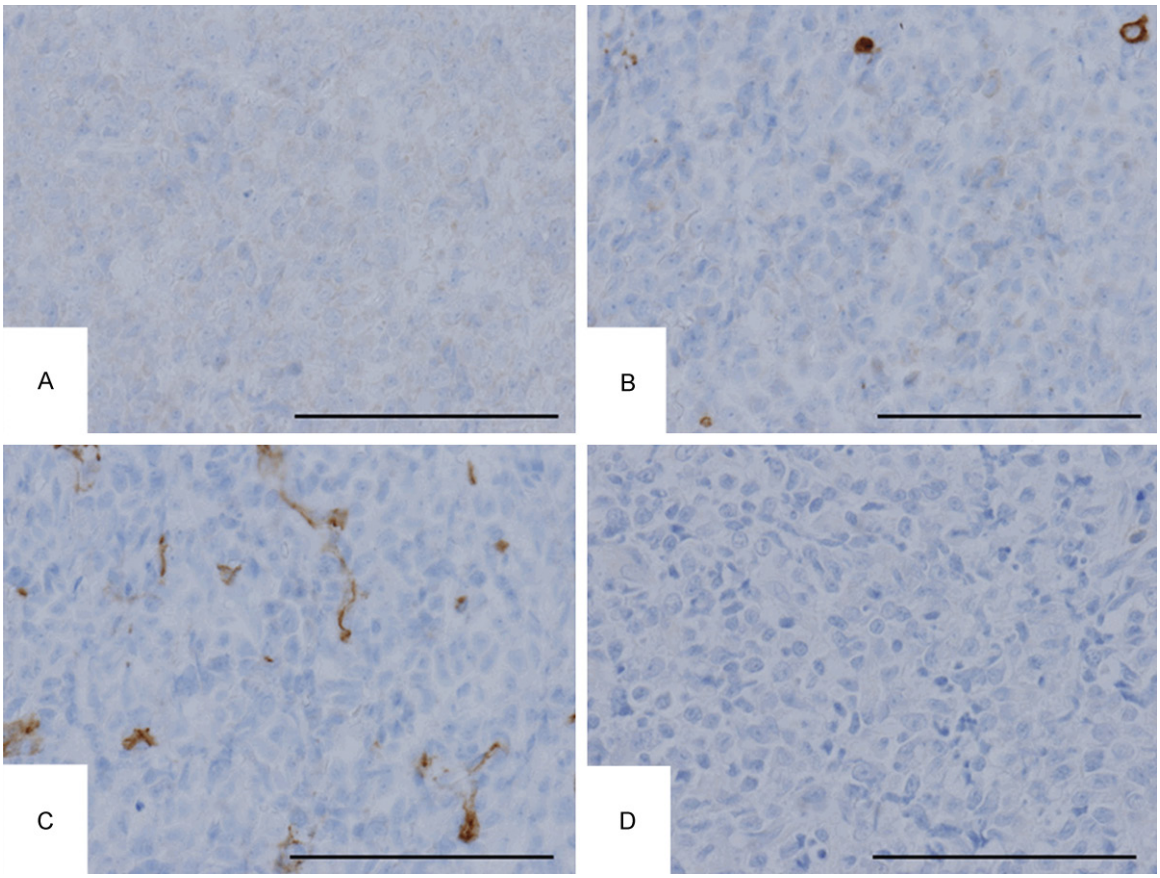

Figure 2. The tumor cells were immunohistochemically negative for CD20, CD3, CD34, and myeloperoxidase. A. CD20. B. CD3. C. CD34. D. Myeloperoxidase. Original magnification, × 400. Bar, 100 μm.

sion tomography (FDG-PET)/CT study showed strong uptake of FDG corresponding to the tumor of the left shoulder (data not shown).

The excisional biopsy of the skin tumor was performed for histological examination. The HE image at low power showed that the tumor was localized mainly in the dermis as nodular and diffuse proliferation of atypical lymphoid cells without apparent epidermotropism (**Figure 1A**). At high power, the tumor cells were middle to large-sized lymphoid cells, suggesting a hematologic malignancy, particularly of B-cell lineage (**Figure 1B**). In addition, we noticed prominent intercellular slits, some of which were filled with red blood cells, leading us to suspect the presence of vascular channels (**Figure 1B**). Together with partially epithelioid morphology of the tumor cells, this reminded us of another possibility of epithelioid vascular tumor as a rare differential diagnosis. Based on these HE findings, we first examined the possibility of lymphoid malignancy and tried to exclude a rare possibility of poorly differentiated angiosarcoma.

Immunohistochemical analysis showed that the tumor cells were stained negative for CD20 (**Figure 2A**), CD79a (data not shown), CD3 (**Figure 2B**), CD34 (**Figure 2C**), and myeloperoxidase (**Figure 2D**). Further immunohistochemical examination showed that the tumor cells were negative for Pax5, CD68, CD30, ALK, and AE1/AE3 (data not shown). Taken together, it seemed that these data almost excluded the possibility of lymphoid and epithelial malignancy.

On the other hand, the tumor cells were immunohistochemically positive for CD31 and ERG, both of which are considered to be specific markers for a majority of vascular tumors (**Figure 3A** and **3B**). However, a closer look at the ERG staining image revealed that the staining intensity of ERG in the tumor cells were weaker than that in the capillary endothelium (**Figure 3B**).

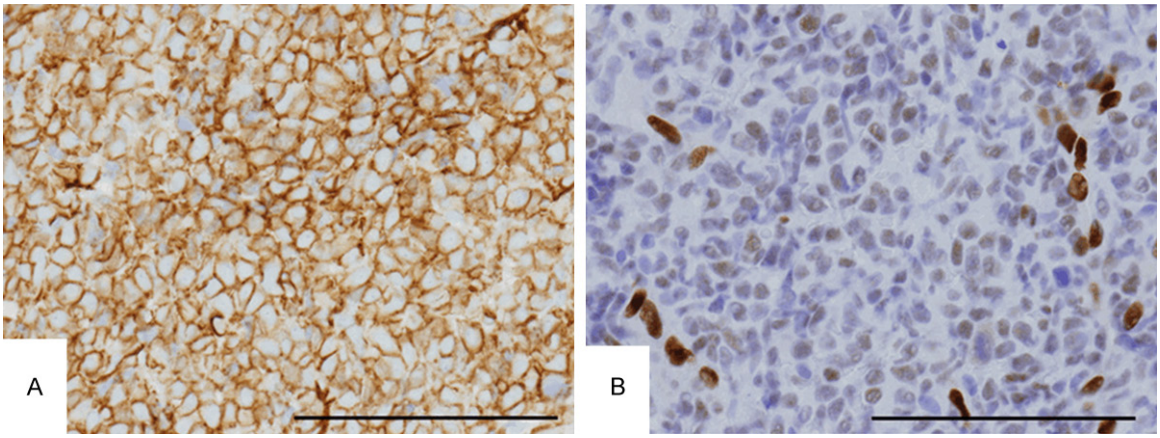

Figure 3. The tumor cells were immunohistochemically positive for vascular endothelial markers CD31 and ERG. A. CD31. B. ERG. Original magnification, × 400. Bar, 100 μm.

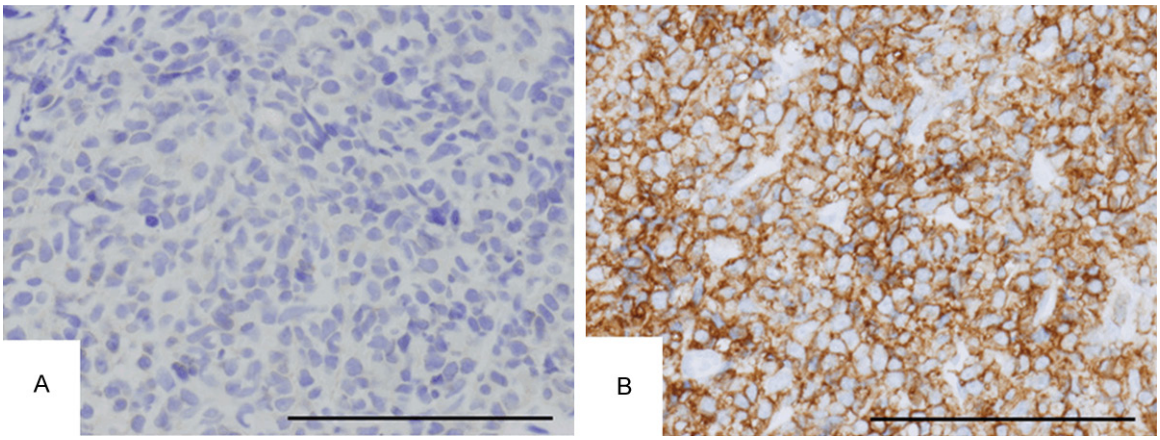

Figure 4. CD45 immunohistochemistry of the tumor cells by different antibodies. A. Antibody from Leica. B. Antibody from DAKO. Original magnification, × 400. Bar, 100 μm.

The apparent discrepancy between CD31 and ERG immunostaining led us to reconsider the possibility of non-vascular malignancy. Before concluding that the tumor was of vascular origin, we tried to confirm the expression of CD45, which is considered to be expressed in a broad spectrum of hematologic malignancies. When we used a CD45 antibody purchased from Leica, the tumor cells were negatively stained (**Figure 4A**). However, surprisingly, when we used a CD45 antibody purchased from DAKO, the tumor cells were shown to be CD45-positive (**Figure 4B**). We confirmed that both CD45 antibodies specifically labeled lymphoid cells in tonsils (data not shown). It turned out that these CD45 monoclonal antibodies are derived from different clones: the Leica one is a mixture of clones RP2/18 and RP2/22, while the DAKO one is a mixture of clones 2B11 and PD7/26. Taken together, we reasoned that the immunonegativity of the tumor cells by the Leica CD45 antibody was false negative, suggesting that the tumor was in fact a hematologic malignancy.

At this stage, we determined to examine the possibility of CD31-positive hematologic malignancy, particularly focusing on blastic plasmacytoid dendritic cell neoplasm (BPDCN), which was reported to be immunohistochemically CD31-positive [9]. The tumor cells were immunohistochemically positive for CD4 (**Figure 5A**) and CD56 (**Figure 5B**), which is consistent with their aberrant expression in myeloid malignancy. In addition, they were positive for CD123 (**Figure 5C**) as well as CD2AP (**Figure 5D**), both

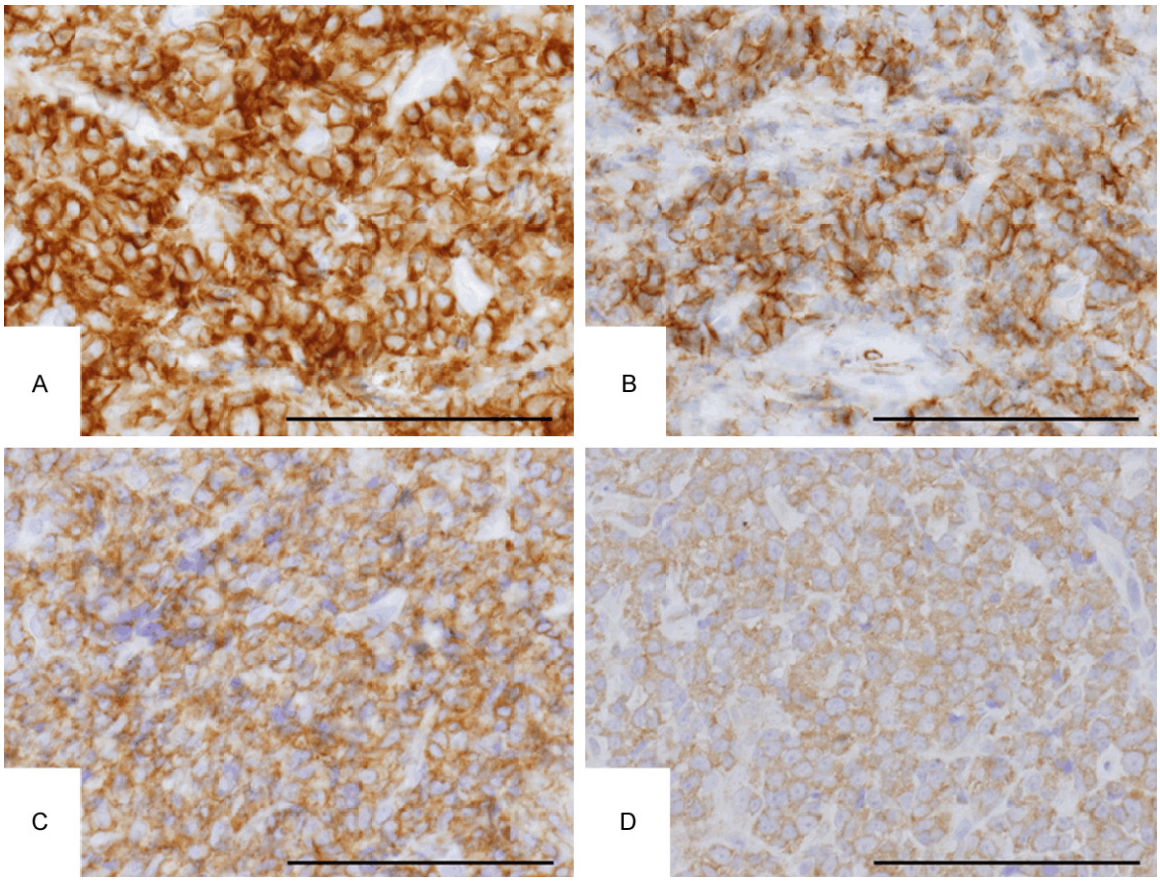

Figure 5. The tumor cells were immunohistochemically positive for markers of blastic plasmacytoid dendritic cell neoplasm. A. CD4. B. CD56. C. CD123. D. CD2AP. Original magnification, × 400. Bar, 100 μm.

of which are considered to be specific markers for BPDCN. From these results, a final diagnosis of BPDCN was established.

Discussion

Here, we report a diagnostically challenging case of CD31-positive malignant skin tumor with false immunonegativity for CD45, which turned out to be BPDCN. BPDCN is a malignant tumor of plasmacytoid dendritic cells regulating mainly innate immunity [8]. It is a rare tumor mainly observed in elder males. The typical examples of BPDCN manifest themselves as so-called myeloid sarcoma in the skin. Although 10-20% of BPDCN is associated with myeloid leukemias during the disease process, the diagnosis is often complicated by its presentation as myeloid sarcoma in the skin, together with its rarity.

The diagnosis of this case was complicated by false immunonegativity of CD45 at the initial stage of examination. It turned out that CD45 monoclonal antibodies commercially available for immunohistochemistry are derived from several different clones with presumably distinct epitope specificity. Hematologic malignancies can be immunohistochemically CD45-negative in the cases of classical Hodgkin lymphoma, B-ALL/LBL, or else, as described in Introduction [1-7]. In addition, as illustrated in the present case, immunohistochemical false-negativity for CD45 could occur depending on which CD45 antibody is used, because there are CD45 monoclonal antibodies derived from different clones with presumably different specificity. We previously reported immunohistochemical false-positivity of CD4 antibody due to incompatible combination of autostainers and the antibodies from different suppliers [10]. Taken together, the diagnostic possibility of hematologic malignancy should not be excluded even in the case that the tumor shows immunonegativity for CD45.

How can we circumvent this pitfall to erroneously discard the possibility of myeloid malignancy based on false immunonegativity for CD45? If available, immunonegativity for CD45 by one antibody may be reconfirmed by the other CD45 antibody derived from a different clone. Alternatively, CD56 and/or CD4 immunohistochemistry can be examined before excluding the possibility of hematologic malignancy. Because myeloid malignancies including BPDCN frequently show aberrant expression of CD56 and/or CD4, inclusion of immunohistochemistry for CD56 and/or CD4 at the early stage of the analysis may reduce the risk of excluding the possibility of myeloid malignancy.

The BPDCN case in this report is immunohistochemically positive for CD31. Most textbooks of diagnostic histopathology strengthens that CD31 and ERG are immunohistochemical markers specific for vascular endothelial lineages. However, in reality, it is not surprising that hematologic malignancies express CD31 from the following perspectives. In addition, our case illustrates that CD31 is a useful surrogate marker for the diagnosis of BPDCN.

First, there are developmental and physiological basis for CD31 expression in hematopoietic cells. In the embryonic stage, it is reported that hematopoietic stem cells originate from hemangioblasts, a common precursor for hematopoietic and vascular lineages, although some controversy exists [11]. Physiologically, CD31 expression of hematopoietic progenitor cells may have roles in their regulation in the bone marrow vascular niche [12]. Also in the context of tissue inflammation, circulating leukocytes first adhere to vascular endothelium via homophilic binding of CD31 to transverse it to the damaged tissue [13, 14]. Therefore, it is reasonably assumed that CD31 may be transiently expressed in these hematopoietic cells for transient homophilic adhesion to vascular endothelium. In fact, CD31 is broadly expressed in almost all non-erythroid hematopoietic cells including neutrophils, macrophages, monocytes, mast cells, natural killer cells, naïve B cells, naïve T cells, and platelets [13].

Second, a rare subtype of leukemia is associated with chromosomal translocations involving ERG gene encoding transcription factors considered to be specific for vascular endothelial phenotypes [15]. Speculatively, this may be a reminiscence of the common developmental origin, the hemangioblast, between hematopoietic cells and vascular endothelial cells. Therefore, it is not unexpected that CD31 protein, generally considered to be a vascular endothelial marker, is expressed in some subtypes of leukemic cells [16].

Third, although not comprehensive, there are some reports on CD31 expression in hematologic malignancies including B-ALL, BPDCN, and plasmacytic lesions including plasmacytoma [3, 9, 17]. CD31 expression in BPDCN in our case is consistent with the previous report [3]. Interestingly, it is reported that some cases of CD45-negative B-ALL are positive for Fli-1, a transcription factor comparatively specific for vascular endothelium [3]. This may suggest reciprocal regulation between hematopoietic-specific and vascular-specific gene expression, which may also be related to the presence of common precursor to both lineages, a hemangioblast, and to the importance of vascular endothelium-specific transcription factors ERG in a rare subtype of leukemia [15].

In conclusion, there are two take-home messages for diagnostic histopathologists in this report. First, immunonegativity of CD45 of the tumor does not exclude the possibility of hematologic malignancy. False-negativity of CD45 immunohistochemistry can occur. The use of alternative CD45 antibody and/or the inclusion of CD56/CD4 immunohistochemistry may circumvent this pitfall. Second, CD31-immunopositivity of the tumor may not always suggest vascular endothelial origins but hematopoietic ones in some cases. This is not only important as a useful surrogate marker in diagnostic histopathology but also interesting in biological viewpoints on how the development, physiology, and pathology of vascular endothelial and hematopoietic lineages are interrelated.

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Disclosure of conflict of interest

None.

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