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Neoplastic Stromal Cells of Intracranial Hemangioblastomas Disclose Pericyte-derived Mesenchymal Stromal Cells-like Phenotype

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Background: Stromal cells (SCs) of hemangioblastomas (HBs) have been regarded as true neoplastic components, but their ontogeny remains unclear. Convincing evidence suggests that embryonic mesenchymal cells may be the cells of origin of HBs. The aim of the present study was to investigate the immunophenotypic characteristics of neoplastic SCs using a set of markers against endothelial cells (ECs), vascular smooth muscle cells (vSMCs), mesenchymal stromal cells (MSCs), and pericytes. **Methods:** Intracranial HBs (n=46), angiolipoma (n=9), and pyogenic granuloma (n=11) were retrieved and the immunophenotypic profile of SCs was determined by immune stainings. **Results:** The MIB-1 labeling index was significantly higher in SCs compared to that of ECs and vSMCs, regardless of the type of lesion. The neoplastic SCs of HBs consistently expressed both MSC and pericyte markers, but did not express markers of ECs and vSMCs. Double immunofluorescent staining demonstrated that the neoplastic SCs of HBs expressing MSC or pericyte markers directly abutted onto the ECs of capillaries/venules. **Conclusions:** The results suggest that the neoplastic SCs of HBs share the immunophenotypic profile and distribution with those of pericyte-derived MSCs. Thus, HBs might originate from a distinctive population of pericyte-derived MSCs in the central nervous system.

Key Words: Hemangioblastoma; Stromal cells; Immunophenotype; Mesenchymal stromal cells; Pericytes

A hemangioblastoma (HB) is a highly vascularized neoplasm and generally occurs in a restricted area of the central nervous system (CNS). Considerable evidence demonstrates that HBs occur as a consequence of a somatic or germ-line mutation of the *VHL* gene.[1,2] Since the *VHL* gene mutation is exclusively detected in stromal cells (SCs), these intervening cells have been regarded as the true neoplastic components of HBs.[2] Loss of *VHL* function results in constitutive overexpression of hypoxia-inducible factor-1*a* and vascular endothelial growth factor in neoplastic SCs, which consequently leads to the proliferation of endothelial cells (ECs).[3] Even though many attempts have been made to elucidate the issue of the ontogeny of neoplastic SCs, their identity is still uncertain.[2,4-8] Recent studies demonstrated that the neoplastic SCs of HBs expressed markers of embryonic mesenchymal cells and hematopoietic stem cells.[8] Furthermore, isolated neoplastic SCs of HBs give rise to multiple hematopoietic and mesodermal lineage cells *in vitro*.[9] Thus, it has been suggested that the ontogeny of SCs may be embryonically arrested mesenchymal cells.

Pericytes are defined by distinctive features such as perivascular localization and expression of a set of pericyte markers.[10] Their function is not limited to regulating vascular stabilization and hemostasis but is extended to involve angiogenesis and tissue regeneration.[10] Recent convincing evidence suggests that certain populations of pericytes are multipotent mesenchymal stromal cells (MSCs), which give rise to a variety of mesodermal

and vasculogenic cells *in vitro* and *in vivo* and replace damaged cells.[11-15] Accumulating evidence demonstrates that the neoplastic SCs of HBs share morphologic and biologic features with pericyte-derived MSCs. First, the neoplastic SCs of HBs intimately contact with capillary ECs and distribute around the microvessels.[16] Second, neoplastic SCs share distinct expression profiles of chemokine receptors and their ligands with pericytes-derived MSCs.[3] Third, neoplastic SCs disclose variegated cytological features *in situ* and multipotent differential ability *in vitro*.[9] Considering all these characteristics, we speculated that the neoplastic SCs of HBs might originate from pericyte-derived MSCs.

In the present study, we analyzed the immunoreactivity of neoplastic SCs against markers for EC (CD31 and CD34), vascular smooth muscle cells (vSMC; desmin), MSC (vimentin, CD44, and CD105), and pericyte (α-smooth muscle actin [α-SMA], CD140b, and CD146). Furthermore, we evaluated the MIB-1 labeling index to determine whether SCs are a main neoplastic component in HBs. To validate the immunoreactivity of SCs against a set of markers, the present study used angiolipoma (AL) and pyogenic granuloma (PG), which typically exhibit proliferation of both ECs and intervening SCs.[17-20]

MATERIALS AND METHODS

Cases selection

We retrieved 46 cases of HB, 9 cases of AL, and 11 cases of PG from the surgical pathology files of Pusan Paik Hospital, Inje University College of Medicine and Asan Medical Center, University of Ulsan College of Medicine in Korea, from 2004 to 2010. All enrolled cases were reviewed, and selected appropriated paraffin blocks were based on hematoxylin and eosin (H&E) staining. HBs disclosed an anastomosing network of delicate microvessels and contained varying numbers of granular or vacuolated SCs. HBs were obtained from the cerebellum (n = 45) and cerebrum (n = 1), and patient ages ranged from 14 to 68 years (mean, 41 years). ALs having both lipomatous and angiomatous components were selected. ALs were obtained from soft tissues in the arm (n = 4), abdomen (n = 1), breast (n = 1), and thigh (n = 3), and patient ages ranged from 22 to 63 years (mean, 40 years). PGs having enriched microvessels and intervening SCs were selected. PGs were obtained from the skin (n = 10) and nasal cavity (n = 1), and patient ages ranged from 6 to 73 years (mean, 23 years). All enrolled cases of ALs and PGs obviously disclosed active angiogenesis and prominent proliferation of intervening SCs, which were clearly differentiated from ECs and vSMCs.[19,20]

Immunohistochemical staining

To determine the immunophenotypic characteristics of neoplastic SCs, we conducted immunohistochemical staining using whole tissue paraffin blocks of HBs (n = 12) and ALs (n = 9) or microarray paraffin blocks of HBs (n = 34) and PGs (n = 11). Tissue microarray paraffin blocks were constructed using a Manual Tissue Arrayer (MTA-1, Beecher Instruments Inc., Sun Prairie, WI, USA). For antigen retrieval, paraffin sections were treated with 1 mM EDTA buffer at 98°C for 20 minutes in a PT module (LabVision, Fremont, CA, USA). Immunohistochemical stainings were performed in an Autostainer 480 (LabVision) using a Dako REAL EnVision detection system (Dako, Glostrup, Denmark). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide followed by incubation with primary antibodies at 37°C for 1 hour. The primary antibodies and their dilution folds used in the present study are listed in Table 1. The sections were incubated with horseradish-peroxidase (HRP)-conjugated anti-mouse/rabbit IgG (EnVision/HRP detection system, Dako) at 37°C for 30 minutes. The antigen-antibody complex was finally visualized using diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) and then counterstained with Meyer's hematoxylin. Rigorous washing three times with Tris-buffered saline (TBS) supplemented with 0.01% Tween 20 was accomplished between each step.

The evaluation of all immunostaining was carried out by two independent pathologists. To exclude the possibility of misinterpretation originating from the immunoreactivity of vSMCs or mural cells against pericyte or MSC markers, we interpreted and counted the immunoreactivity of SCs residing around mi-

Table 1. List of antibodies used in this study

Antibody	Clone	Dilution fold	Source
CD31	JC/70A	1 : 40	Dako
CD34	QBEnd/10	1 : 800	Neomarker
Desmin	D33	1 : 100	Dako
Vimentin	V9	1 : 200	Invitrogen
CD44	DF1485	1 : 40	Leica
CD105	35/CD105	1 : 500	BD
α-Smooth muscle actin	1A4	1 : 400	Dako
CD140b	Y92	1 : 300	Abcam
CD146	N1238	1 : 200	Abcam
MIB-1	7B11	1 : 50	Invitrogen
von Willbebrand factor	Polyclonal	1 : 150	Dako

crovessels without a mural layer, such as capillaries and venules. We used feeding microvessels having an obvious mural layer to validate the immunostaining by using them as the internal control. At least 1,000 cells from the most representative areas were analyzed and scored. The expression rate of neoplastic or reactive SCs in HBs, ALs, and PGs against each marker for EC, vSMC, MSC, and pericyte was semiquantitatively scored: "0," no positive SCs; "+," up to 10% positive SCs; "++," 11 to 50% positive SCs; and "+++," over 50% positive SCs. The MIB-1 index was evaluated as the % of positive cells according to each type of cell such as ECs, vSMCs, and SCs.

Double immunofluorescent staining

To determine the spatial approximation between neoplastic SCs and capillary ECs in HBs, double immunofluorescent staining was performed. Whole tissue paraffin sections of HBs were deparaffinized and rehydrated. After antigen retrieval using a 1 mM EDTA buffer, the sections were incubated with 10% rabbit serum suspended in TBS at 37°C for 30 minutes to minimize nonspecific signals. Subsequently, the sections were incubated with von Willebrand factor (vWF) at 37°C for 1 hour followed by incubation with fluorescein isothiocyanate-conjugated rabbit antigoat IgG (Sigma) at 37°C for 30 minutes. Following the first immunofluorescent staining, the sections were washed three times with TBS and then incubated with 10% goat serum suspended in TBS. Subsequently, the sections were incubated with another primary antibody against CD44, CD105, CD140b, CD146, or α-SMA at 37°C for 1 hour followed by incubation with Alexa Fluor 594-conjugated goat anti-mouse IgG (Invitrogen, Eugene, OR, USA) at 37°C for 30 minutes. The nuclei were counterstained with diamino-2-phenylindole (Invitrogen) and the sections were mounted with VECTASHIELD mounting media (Vector Labs, Burlingame, CA, USA). The signal was examined by microscopy (Eclipse 80i, Nikon, Tokyo, Japan) equipped with epifluorescence, and the signals were captured using a cooled CCD camera (ProgRes MFcool, Jenoptik, Jena, Germany).

Statistical analysis

One-way ANOVA was performed using an SPSS ver. 10.0.7 (SPSS Inc, Chicago, IL, USA). All data were expressed as mean ± standard error. Statistical significance was set as p-value < 0.05.

RESULTS

SCs residing in HBs, ALs, and PGs disclosed the highest proliferative ability

The present study analyzed and compared the proliferative potential according to each cell type residing in HBs, ALs, and PGs. The MIB-1 (Ki-67) protein is absent from cells during resting stages (G0), whereas it appears on cells entering the cell cycle. In HBs, the MIB-1 labeling index ranged from 0 to 8% (mean, 1.70%) for ECs, 0 to 3% (mean, 0.54%) for vSMCs, and 1 to 21% (mean, 7.76%) for neoplastic SCs (Fig. 1). In ALs, the MIB-1 labeling index ranged from 1 to 18% (mean, 7.67%) for ECs, 0 to 1% (mean, 0.33%) for vSMCs, and 7 to 33% (mean, 16.67%) for SCs. In PGs, the MIB-1 labeling index ranged from 5 to 20% (mean, 11.55%) for ECs, 0 to 5% (mean, 1.55%) for vSMCs, and 10 to 40% (mean, 22.36%) for SCs. The HBs disclosed the lowest MIB-1 labeling index compared to that of ALs and PGs, which supports the premise that HBs are indolent, slow-growing neoplasms. Interestingly, SCs retained the highest proliferative potential compared to ECs or vSMCs regardless of the type of neoplasm or lesion ($p < 0.05$) (Fig. 1).

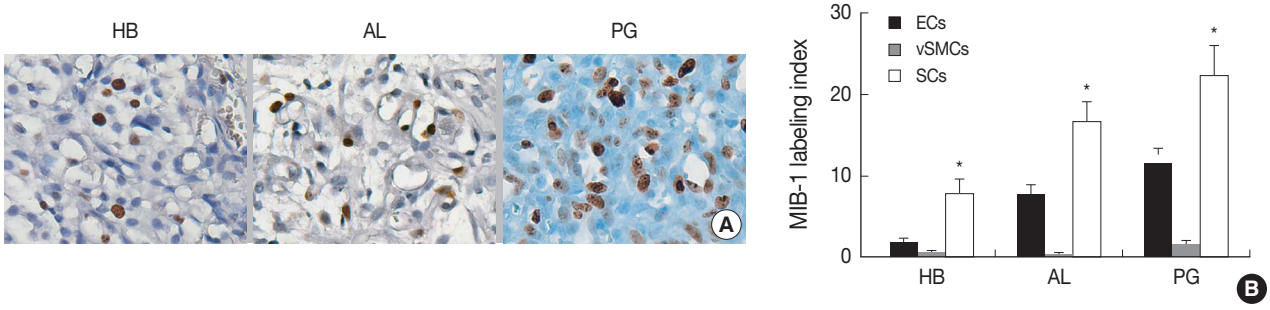

Fig. 1. Immunohistochemical stainings of MIB-1 in hemangioblastoma (HB), angiolipoma (AL), and pyogenic granuloma (PG). (A) Representative microphotographs of immunohistochemical stainings against MIB-1. (B) MIB-1 labeling index is semi-quantitatively analyzed according to endothelial cells (ECs), vascular smooth muscle cells (vSMCs), and stromal cells (SCs). *$p < 0.05$ compared to ECs and vSMCs.

Neoplastic SCs of HBs did not express any markers of EC and vSMC

Both CD31 and CD34 were generally used to identify the ECs lined in microvessels including arterioles, capillaries, and venules. The present study also demonstrated that both markers were exclusively expressed on the ECs of microvessels with or without a mural layer but were not expressed on vSMCs and SCs in all enrolled cases (Table 2, Fig. 2). Desmin was uniformly and consistently expressed on the vSMCs in microvessels with a mural layer such as arterioles or veins. Since capillaries or venules do not have a mural layer, desmin was not detected on these types of microvessels. As might be expected, neoplastic SCs residing around the pericapillaries or perivenules in HBs did not express any markers of EC and vSMC.

Table 2. Summary of immunohistochemical staining against markers of neoplastic or reactive stromal cells in hemangioblastoma, angiolipoma, and pyogenic granuloma

Marker	Hemangioblastoma (n=46)				Angiolipoma (n=9)				Pyogenic granuloma (n=11)			
	0	+	++	+++	0	+	++	+++	0	+	++	+++
CD31	46	0	0	0	9	0	0	0	11	0	0	0
CD34	46	0	0	0	9	0	0	0	11	0	0	0
Desmin	46	0	0	0	9	0	0	0	11	0	0	0
VMT	0	0	0	46	0	0	0	9	0	0	0	11
CD44	0	17	18	11	0	5	4	0	0	10	1	0
CD105	0	25	17	4	0	2	6	1	0	7	4	0
SMA	0	21	24	1	0	1	6	2	0	1	6	4
CD140b	0	17	20	9	0	0	2	7	0	3	7	1
CD146	0	17	26	3	0	6	2	1	0	9	2	0

Expression rate of stromal cells is semiquantitatively scored; "0," no positivity cell; "+," up to 10 % positive cells; "++," 11 to 50 % positive cells; "+++," over 50% positive cells.
SMA, smooth muscle actin.

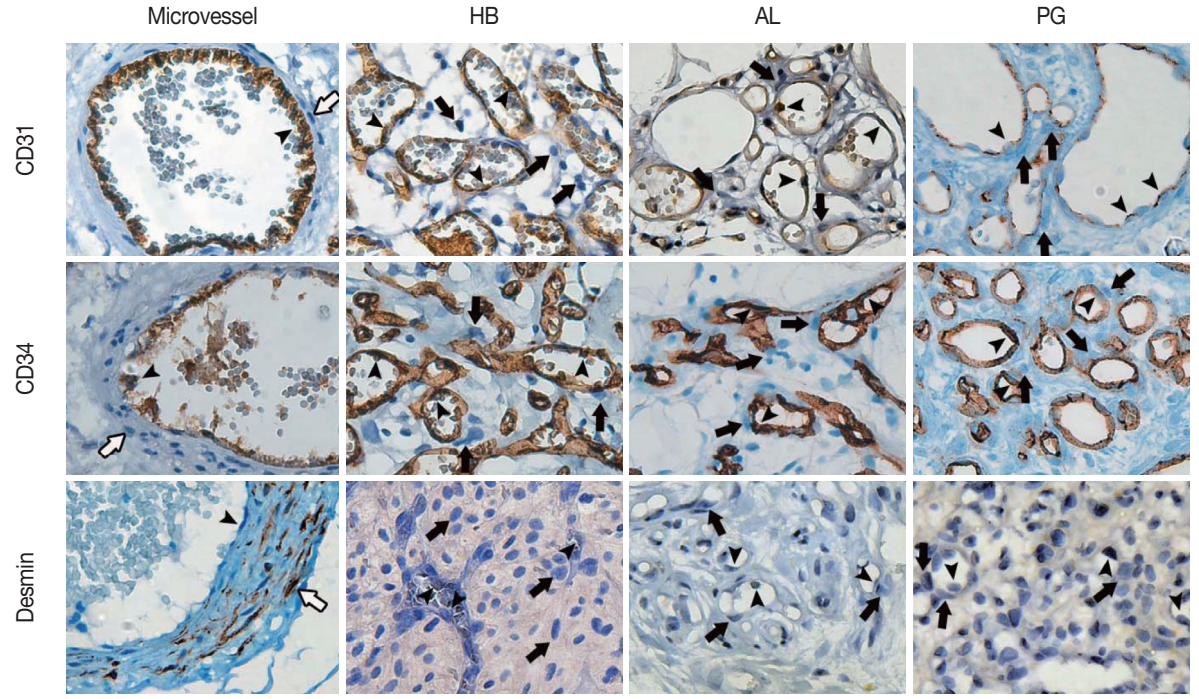

Fig. 2. Immunohistochemical stainings against markers of endothelial cells (ECs; CD31 and CD34) and vascular smooth muscle cells (vSMCs; desmin) in hemangioblastoma (HB), angiolipoma (AL), and pyogenic granuloma (PG). Feeding microvessels (microvessel) having a distinct mural layer are used as an internal control to validate immunostaining. Both CD31 and CD34 are exclusively expressed on the ECs (arrowhead) of microvessels, whereas desmin is exclusively expressed on vSMCs (white arrow). No stromal cells (black arrow) in HBs, ALs, and PGs expressed any markers of CD31, CD34, and desmin.

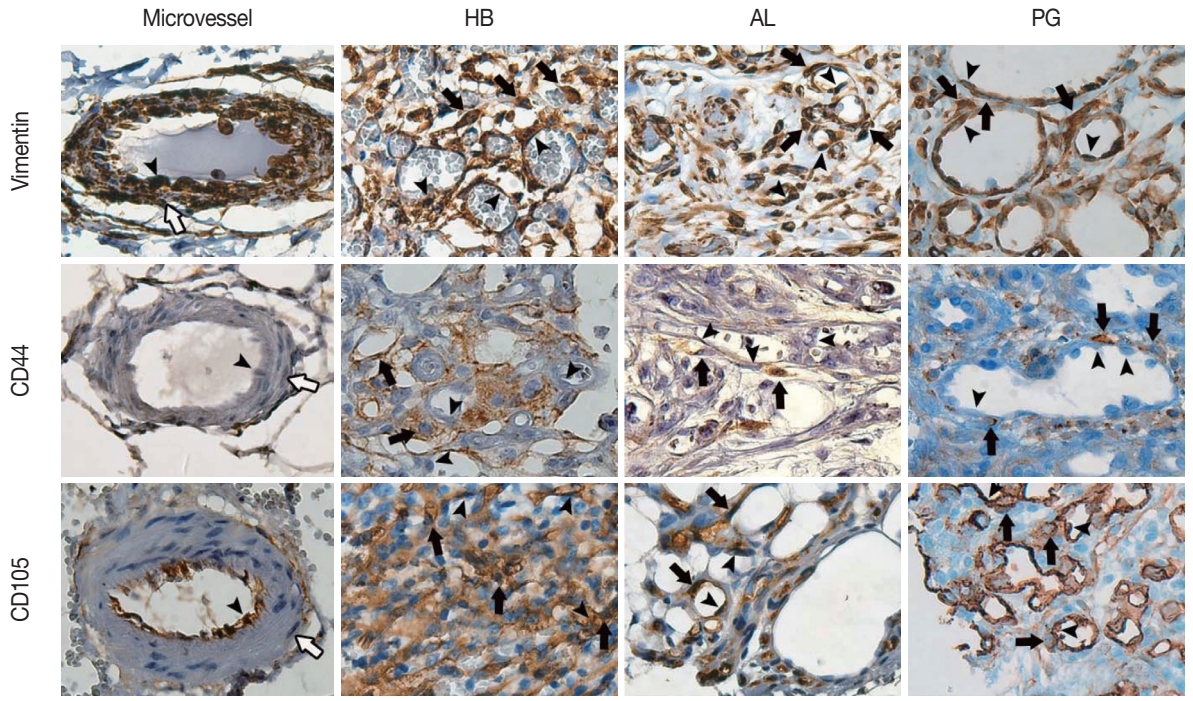

Fig. 3. Immunohistochemical stainings against mesenchymal stromal cell markers in hemangioblastoma (HB), angiolipoma (AL), and pyogenic granuloma (PG). Feeding microvessels (microvessel) having a distinct mural layer are used as an internal control to validate immunostaining. Vimentin is expressed on all types of cells including endothelial cells (ECs, arrowhead), vascular smooth muscle cells (vSMCs, white arrow), and stromal cells (SCs, black arrow) in HBs, ALs, and PGs. CD44 is exclusively expressed on the SCs in HBs, ALs, and PGs but not on ECs and vSMCs. CD105 is expressed on both the ECs and SCs in HBs, ALs, and PGs but not on vSMCs.

Neoplastic SCs of HBs consistently expressed MSC markers

The present study used a set of markers including vimentin, CD44, and CD105 to identify MSCs. Since vimentin has been generally used to identify generic mesenchymal cells, this marker was homogeneously and consistently expressed on all types of cells in feeding microvessels including ECs, vSMCs, and adventitial cells (Fig. 3). CD44 and CD105 were expressed differently according to the type of cells residing in the feeding microvessels. CD44 was exclusively expressed on adventitial cells around the feeding microvessels but was not expressed on ECs and vSMCs. However, CD105 was expressed on both the ECs and adventitial cells of the feeding microvessels but was not expressed on vSMCs. In all enrolled cases of HBs, ALs, and PGs, neoplastic or reactive SCs were consistently, but not homogeneously, expressed on all MSC markers including vimentin, CD44, and CD105. Vimentin was diffusely expressed on neoplastic or reactive SCs and the surrounding ECs of capillaries and venules (Fig. 3). CD44 was not expressed on the ECs of capillaries and venules, but it was consistently expressed on the neoplastic or reactive SCs around the microvessels in all enrolled cases (Table 2, Fig. 3). CD105 was consistently expressed on the ECs of capillaries and venules and on the neoplastic or reactive SCs around microvessels in all enrolled cases of HBs, ALs, and PGs (Table 2, Fig. 3). Interestingly, the $CD44^+$ and $CD105^+$ SCs of HBs and ALs were distributed in peri- and para-capillaries/venules, whereas the $CD44^+$ and $CD105^+$ SCs of PGs were limitedly distributed on the microvascular wall (Fig. 3). The expression rate of CD44 and CD105 was higher in the neoplastic SCs of HBs compared to that of ALs and PGs, but no significant difference was observed (Table 2).

Neoplastic SCs of HBs expressed pericyte markers

The present study used a set of pericytes markers including α-SMA, CD140b, and CD146 to determine whether neoplastic SCs disclose pericyte-like phenotype. All markers to identify pericytes were homogenously and consistently expressed on the vSMCs of feeding microvessels but were not expressed on ECs, except for CD146 (Fig. 4). CD146 was expressed on both the vSMCs and ECs of feeding microvessels. Neoplastic or reactive SCs consistently expressed all pericyte markers regardless of the type of neoplasm or lesion, but the distribution of cells express-

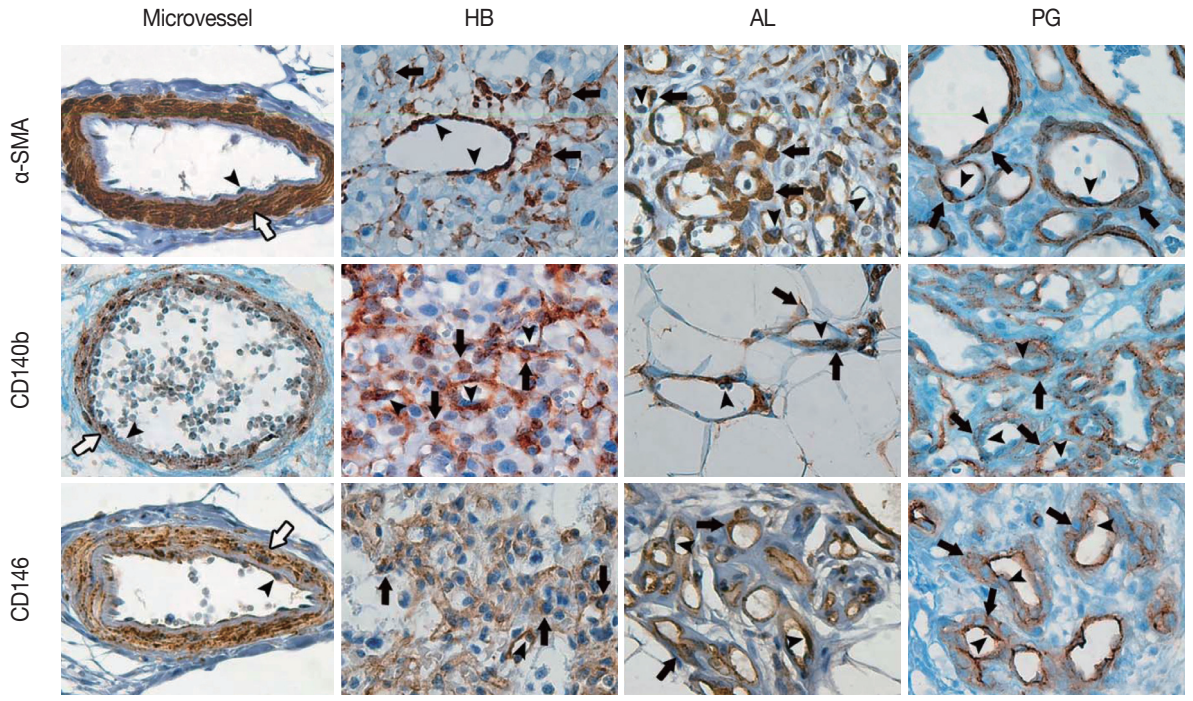

Fig. 4. Immunohistochemical stainings against pericyte markers in hemangioblastoma (HB), angiolipoma (AL), and pyogenic granuloma (PG). Feeding microvessels (microvessel) having a distinct mural layer are used as an internal control to validate immunostaining. Both alpha-smooth muscle actin (α-SMA) and CD140b are expressed on both the vascular smooth muscle cells (vSMCs, white arrow) and stromal cells (SCs, black arrow) in HBs, ALs, and PGs but not on endothelial cells (ECs, arrowhead). CD146 expressed on ECs, vSMCs, and SCs in HBs, ALs, and PGs. SCs are not homogeneously expressed pericyte markers, but are consistently expressed. SCs expressing α-SMA, CD140b, and CD146 are distributed around the capillaries and venules in HBs and ALs, whereas these cells are restricted within the walls of capillaries and venules in PGs.

ing these markers was distinct (Table 2). Neoplastic SCs expressing pericyte markers more radially and individually scattered around capillaries and venules in HBs and ALs (Fig. 4). However, SCs expressing pericyte markers were closely approximated with the walls of capillaries and venules in PGs. Among the pericyte markers, CD140b was more diffusely expressed on the neoplastic SCs in HBs, whereas α-SMA and CD146 were restrictively expressed on the neoplastic SCs around capillaries and venules.

Neoplastic SCs closely approximated with ECs and expressed both pericyte and MSC markers

Double immunofluorescent staining against vWF and markers of MSCs or pericytes could highlight spatial approximation between ECs and neoplastic SCs in HBs. The neoplastic SCs in HBs were intimately contacted with the ECs of capillaries or venules and were arranged in a sheet-like structure (Fig. 5, H&E). All markers of MSCs and pericytes, except for CD105 and CD146, were exclusively expressed on the neoplastic SCs around the capillaries and venules on immunofluorescent staining (Fig. 5). CD105 and CD146 were expressed on the ECs of capillaries and venules and neoplastic SCs. Neoplastic SCs expressing pericyte or MSC markers directly abutted on the vWF^+ ECs of capillaries and venules and were radially scattered. Based on abluminal localization of capillaries or venules and the expression of both pericyte and MSC markers, certain populations of neoplastic SCs in HBs disclose a pericyte-derived MSCs phenotype.

DISCUSSION

Even though several independent studies have been conducted over the last two decades, these attempts have provided a contradictory conclusion for identifying the ontogeny of neoplastic SCs in HBs from entirely different tissues: glial cells,[4] embryonic choroid plexus,[5] arachnoid cells,[6] fibrohistiocytes,[7] neuroectodermal cells,[21] reticuloendothelial cells,[22] or as yet unidentified cell types.[23] A series of immunohistochemical studies have revealed contradictory and inconsistent immunoreactivity of neoplastic SCs for various markers against epithelial cells (cytokeratin),[1] vSMCs (muscle-specific actin, desmin, and cal-

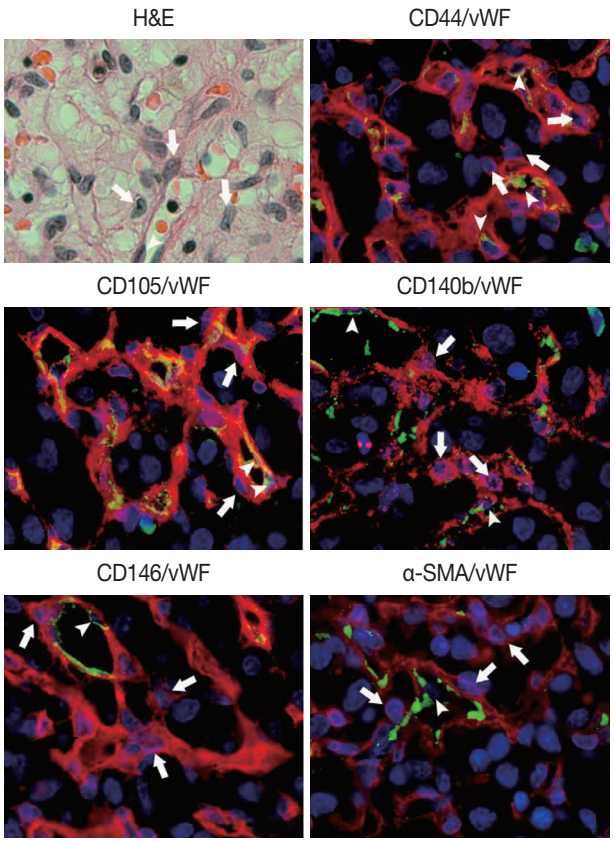

Fig. 5. Double immunofluorescent staining against von Willebrand factor (vWF, green) and markers (red) of pericytes and mesenchymal stromal cells to determine the spatial approximation between the stromal cells (SCs) and endothelial cells (ECs) in hemangioblastoma (HB). Neoplastic SCs (arrow) expressing CD44, CD105, CD140b, CD146, and alpha-smooth muscle actin (α-SMA) intimately abutted onto vWF+ECs (arrowhead) and are radially distributed.
H&E, hematoxylin and eosin.

ponin),[23] and neuroectodermal cells (CD56, S-100, neuron-specific enolase, and glial fibrillary acidic protein).[1,21] Based on previous immunohistochemical studies, markers for identifying vasculogenic cells such as ECs and vSMCs were consistently absent from the neoplastic SCs of HBs. The present study also demonstrated that the neoplastic SCs of HBs did not show any immunoreactivity against ECs markers (CD31, CD34, and vWF) and the vSMC marker (desmin). Among the various markers used in previous and present studies, the neoplastic SCs of HBs consistently and homogeneously expressed vimentin. Vimentin is a generic marker for mesenchymal cells, not a lineage-specific marker for committed mesenchymal cells. Even though previous ultrastructure studies have failed to provide conclusive evidence for elucidating the ontogeny of HBs, these trials have demonstrated that the neoplastic SCs of HBs disclose morphologic similarity with embryonic mesenchymal cells.[16] Overall, the present results, in conjunction with the literature, demonstrate that neoplastic SCs exhibit morphologic and phenotypical features of uncommitted or primitive mesenchymal cells.

Immunophenotypically, MSCs have been defined by the homogeneous expression of MSCs markers (CD44, CD73, CD90, and CD105) and multipotency, which was proposed by the International Society of Cellular Therapy.[24] Therefore, the present study used CD44 and CD105 to determine whether the neoplastic SCs of HBs reveal a similar phenotypic profile to that of MSCs. Although CD44 and CD105 were not evenly expressed on all the neoplastic SCs of HBs, MSC markers were consistently expressed on neoplastic SCs. Research has demonstrated that MSCs are widely deposited along the microvascular wall in various organs and tissues.[11-14] Convincing studies have clearly demonstrated that MSCs expressing CD44 or CD105 are directly abutted on capillary ECs and give rise to multiple committed mesenchymal cell lineages.[13-15] Interestingly, our results demonstrated that the perivascular distribution of $CD44^+$ and $CD105^+$ neoplastic SCs in HBs and ALs is similar to the *in situ* distribution of MSCs. $CD44^+$ and $CD105^+$ neoplastic SCs in HBs and ALs intimately resided along capillaries and venules and were radially scattered. Even though we did not determine the multipotent differentiation ability *in vitro*, our results demonstrated for the first time that the neoplastic SCs of HBs disclose typical *in situ* features of MSCs.

Subsequently, we determined whether neoplastic SCs exhibit pericyte-like phenotype using a set of pericyte markers. To date, a number of pericyte markers have been proposed, but no single marker is able to identify all pericytes.[10] Furthermore, pericytes are able to dynamically and variously express these markers depending upon the pathophysiologic condition, species, and anatomical location.[10,25] Similar to the distribution of pericytes in a place of active angiogenesis, neoplastic SCs expressing α-SMA, CD140b, and CD146 were centrifugally distributed around the microvessels in HBs and ALs, and were not restricted within a microvessel wall. All pericyte markers used in the present study consistently expressed on both vSMCs and SCs regardless of the type of neoplasm or lesion. Although vSMCs expressed pericyte markers, we could exclude the possibility that the immunoreactivity of neoplastic SCs against pericyte markers was not derived from entrapped vSMCs, because the neoplastic SCs exhibited a complete absence of immunoreactivity against desmin and did not form any organoid structure such as a mural layer. Collectively, our results suggested that the neoplastic SCs in

HBs disclosed a pericyte-like phenotype, and certain populations of neoplastic SCs might originate from the pericytes around microvessels without a mural layer.

Recent comprehensive studies have demonstrated that the neoplastic SCs of HBs expressed embryonic mesodermal and hematopoietic stem cell markers such as brachyury, CD133, Flk-1, and stem cell leukemia.[8,9] In addition, isolated neoplastic SCs retained the capacity to differentiate into fibroblasts, adipoblasts, erythroblasts, and immature myeloid cells *in vitro*.[9] Therefore, it has been suggested that the neoplastic SCs of HBs originate from embryonically arrested primitive mesenchymal cells.[9] Recent accumulating evidence demonstrates that primitive mesenchymal cells are not restricted in embryonic tissue but also occur in postnatal organs such as bone marrow, adipose tissue, skeletal muscle, and the brain.[12-15] Postnatal MSCs also retained a differentiation potential in both mesodermal and vasculogenic lineage cells.[12-14] Accumulating evidence demonstrates that these postnatal MSCs disclose a pericyte-like feature by way of unique perivascular localization and coexpression of both pericyte and MSC markers.[12-14] Therefore, a distinctive population of pericytes is regarded as tissue-resident MSCs, which have an important role in tissue-specific regeneration and angiogenesis. α-SMA$^+$/NG2$^+$ or CD44$^+$/CD90$^+$ multipotent MSCs have been discovered in the CNS.[26,27] These MSCs also disclosed a multipotent differentiation capacity into not only glial and neuronal cells but also ECs and other mesodermal cells.[26,27] Interestingly, MSC populations were distributed in normal tissues and were also discovered in neoplasm such as hemangioma.[28] Hemangioma-derived MSCs expressed markers of MSCs and pericytes and disclosed multipotent differentiation capacity. Collectively, the present results, in conjunction with the literature, suggest the possibility that neoplastic SCs in HBs might be derived from postnatal MSCs residing in the CNS.

Inhibin A has been proposed as a diagnostic marker in distinguishing HBs from metastatic clear cell renal cell carcinoma, since inhibin A is exclusively expressed on neoplastic SCs.[29] While inhibin A, a polypeptide of the transforming growth factor-β (TGF-β) superfamily, has substantial roles in reproduction- and endocrine-related neoplasms, their possible implication in the tumorigenesis of HBs is still unclear.[30] Inhibin, a polypeptide of the TGF-β superfamily, is exerted with activin which has significant roles in the cell proliferation and differentiation of embryonal stem cells.[30] It has been suggested that inhibin competes with activin for binding to activin receptors, blocks the TGF-β signaling pathway, and consequently enhances the mesodermal differentiation of embryonal stem cells.[30] The roles of inhibin on the TGF-β signaling pathway may be related to the tumorigenesis of HBs.

In summary, the present study demonstrated for the first time that neoplastic SCs consistently expressed markers of MSCs and pericytes and were distributed around capillaries and venules. The unique immunoreactivity and localization of neoplastic SCs in HBs are similar to those of the *in situ* pericyte-derived MSCs in extracranial tissues or organs. Thus, our results suggest a new concept whereby neoplastic SCs of HBs might originate from pericyte-derived MSCs.

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