ORIGINAL ARTICLE

Hemangioblastoma Stromal Cells Show Committed Stem Cell Phenotype

Cassandra M. Welten, Emily C. Keats, Lee-Cyn Ang, Zia A. Khan

ABSTRACT: ***Background:*** Hemangioblastomas are benign vascular tumors of the central nervous system that occur sporadically or in association with von Hippel-Lindau disease. These tumors are characteristically composed of a dense capillary network with intervening stromal/interstitial cells. To date, the histogenesis of hemangioblastoma remains unclear. We hypothesize that hemangioblastomas arise from a defective mesodermal stem cell, which gives rise to the atypical vasculature. ***Methods:*** To test our hypothesis, we have characterized the cellular composition of hemangioblastomas by immunophenotyping pluripotent and committed stem cells and vascular endothelial cells. ***Results:*** Our findings show that hemangioblastoma endothelial cells are positive for CD133, a stem and progenitor cell marker. Vascular endothelial cells also expressed nuclear Oct4. In addition to the endothelium, both CD133 and Oct4 were present in stromal and perivascular cells. Interestingly, neither the endothelium nor the stromal cells expressed Sox2 or Nanog suggesting a committed stem cell phenotype. ***Conclusions:*** From these findings, we believe that hemangioblastoma stromal cells are committed stem cells producing both vascular cell types. The findings also show an unusual CD133-positive endothelial phenotype in hemangioblastoma.

RÉSUMÉ: Les cellules stromales de l'hémangioblastome présentent un phénotype de cellules souches commises. ***Contexte :*** Les hémangioblastomes sont des tumeurs vasculaires bénignes du système nerveux central qui surviennent sporadiquement ou en association à la maladie de von Hippel-Lindau. Ces tumeurs sont composées d'un réseau capillaire dense intercalé de cellules stromales/interstitielles. À ce jour, l'histogenèse de l'hémangioblastome demeure obscure. Nous émettons l'hypothèse que les hémangioblastomes proviennent de cellules souches mésodermiques défectueuses qui donnent naissance à un réseau vasculaire anormal. ***Méthode :*** Nous avons caractérisé la composition cellulaire d'hémangioblastomes par immunophénotypage de cellules souches pluripotentes et de cellules souches commises ainsi que de cellules vasculaires endothéliales afin de vérifier notre hypothèse de travail. ***Résultats :*** Nos observations démontrent que les cellules endothéliales de l'hémangioblastome sont positives pour CD133, un marqueur des cellules souches et des cellules progénitrices. Les cellules endothéliales vasculaires exprimaient également l'Oct4 au niveau du noyau. En plus d'être présent dans l'endothélium, CD133 et Oct4 étaient également présents dans les cellules stromales et périvasculaires. À noter que ni l'endothélium ni les cellules stromales n'exprimaient Sox2 ou Nanog, ce qui suggère un phénotype de cellules souches commises. ***Conclusions :*** Ces observations nous laissent croire que les cellules stromales de l'hémangioblastome sont des cellules souches commises qui produisent les deux types de cellules vasculaires. Nous avons également observé un phénotype endothélial CD133 positif inusité dans l'hémangioblastome.

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Hemangioblastomas are benign vascular tumors of the central nervous system arising predominantly in the cerebellum, but may also be found in the brainstem, spinal cord, cerebrum, or the retina[1,2]. These neoplasms occur as a sporadic entity or in association with von Hippel-Lindau (vHL) disease, a disorder with an autosomal dominant pattern of inheritance[1]. The sporadic tumors usually occur in adults with an average age of presentation in the 5th and 6th decade, while the von Hippel-Lindau associated tumors may present earlier (3rd and 4th decades of life)[1,3,4]. Sporadic tumors occur largely in the hemispheres of the cerebellum, whereas von Hippel-Lindau predisposed hemangioblastomas (affecting 60–80 % of patients with the disease) may be multiple, arising in the cerebellum plus other locations along the craniospinal axis[2,5]. The presentation of hemangioblastomas is a well-demarcated large cyst adjoining a densely vascularized tumor nodule[6,7]. Microscopically, these tumors are composed of two principal components: a dense capillary network with intervening stromal/interstitial cells, considered to be the actual neoplastic cells of the tumor[1,5,8].

Recent studies have suggested that hemangioblastomas arise from stem cells that have the capacity to develop into blood vessels or blood cells. Vortmeyer and colleagues suggested that von Hippel-Lindau-associated hemangioblastomas are comprised of developmentally arrested hemangioblasts, cells with the potential to differentiate into hematopoietic or vascular cell types[9]. This was shown through the expression of erythropoietin (Epo) receptor in hemangioblastoma specimens. The research group postulated that stromal cells are the neoplastic cells of the tumor, and that these cells correlate morphologically to neoplastic angiomesenchyme. By demonstrating co-expression of Epo and its receptor EpoR (involved in embryonic blood island formation) in vHL-deficient stromal cells and by detecting fetal-type hemoglobin in

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these areas, the researchers suggested that the neoplastic cells are pluripotent. In agreement with this arrested hemangioblast hypothesis, Gläsker et al. reported that the hemangioblastoma protein profile is consistent with markers, such as stem cell leukemia (Scl), brachyury (Bry), colony stimulating factor-1 (Csf-1) and Gata-1, identified in the cellular differentiation of the hemangioblast[2]. Furthermore, Park et al arrived at similar conclusions by promoting expansion of mature hematopoietic and endothelial progeny from tumor specimen-derived cells[8]. However, it is difficult to conclude whether these isolated cells are the neoplastic cells or recruited cells (from circulation/bone marrow). No studies to date have investigated the expression of established stem and progenitor markers in hemangioblastoma. Hence, the identity of the tumor cells remains unknown.

In this study, we have attempted to profile the cellular components of hemangioblastomas. Specifically, we wanted to define the phenotype of the stem cells (if present) and of the vascular endothelial cells. To achieve this, we performed a series of immunohistochemical stains to evaluate expression, localization and relationship between these cell types in hemangioblastoma specimens. In developing a map of differentiation and stem cell involvement in this tumor, we hope to elucidate a model for hemangioblastoma pathogenesis.

Materials and Methods

Hemangioblastoma specimens

Tissue samples of resected cerebellar hemangioblastomas from 13 patients were obtained from the Brain Tumor Tissue Bank at the London Health Sciences Centre. The diagnosis was confirmed at the Department of Pathology and Laboratory Medicine, Division of Neuropathology, London Health Sciences Centre. The mean age of the patients was 41.84 ± 12.75 years (eight males and five females). Three tumor samples were associated with von Hippel-Lindau. Formalin-fixed/paraffin-embedded samples were serially sectioned at 5 μm thickness on positively charged slides. Studies were conducted following approval by the Research Ethics Board at the University of Western Ontario.

Immunostaining

Slides were deparaffinized in xylene, hydrated through a sequential ethanol gradient, and washed in phosphate-buffered saline (PBS). Slides were then subjected to antigen retrieval in Tris-EDTA buffer (10 mM Trizma-base, 1 mM EDTA, 0.05 % Tween-20, pH 9.0) and 120°C for 20 minutes using the Antigen Retriever™ (2100 Retriever, PickCell Laboratories). For immunofluorescence staining, slides were blocked using 5% blocking serum (serum species selected based on host of secondary antibody). Primary antibody (1:100) was then applied for one hour at room temperature. Antibodies used for immunostaining are listed in the Table. Slides were washed in PBS and incubated with AlexaFluor 488-labelled secondary antibody (Invitrogen) at 1:200 dilution for one hour. DAPI (4', 6-diamidino-2-phenylindole; VECTASHIELD® Mounting Medium with DAPI, Vector Laboratories) was used for nuclear counterstaining. Slides were mounted using Fluoromount™ Aqueous Mounting Medium (Sigma-Aldrich). Fluorescent images were captured using Olympus BX-51 microscope equipped with SPOT™ Pursuit Camera and SPOT™ imaging software.

Table: List of primary antibodies used in immunohistochemistry

Antigen	Description	Source	Reference no.
Stem cell markers**			
Brachyury (bry)	Rabbit anti-human	Abcam	ab20680
c-kit	Rabbit anti-human	Abcam	ab5506
CD133	Rabbit anti-human	Abcam	ab19898
Nanog	Rabbit anti-human	Abcam	ab21624
Nestin	Rabbit anti-human	Abcam	ab93666
Oct4	Rabbit anti-human	Abcam	ab19857
Sox2	Rabbit anti-human	Abcam	ab93689
Endothelial cell markers			
CD31	Mouse anti-human	Dako	M0823
Others			
α-SMA	Mouse anti-human	Sigma-Aldrich	A-2547
Glut1	Rabbit anti-human	Abcam	ab15309
HIF1α	Mouse anti-human	R & D Systems	MAB1935
HIF2α	Goat anti-human	R & D Systems	AF2997

**These antibodies were validated by using placenta specimens and infantile (skin) hemangioma specimens in our laboratory.

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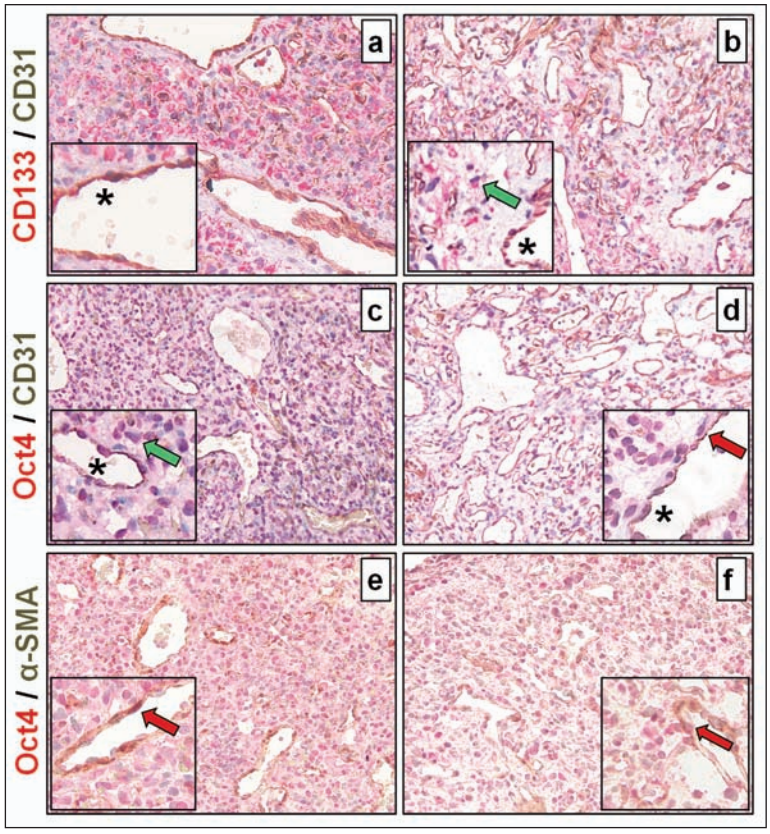

Figure 1: *Immunostaining of hemangioblastoma specimens for stem cell antigen CD133 (a and b) and pluripotency marker Oct4 (c-f). CD133 (red) and CD31 (brown) co-localized to endothelial lining of capillaries. CD133 was also found in the stromal cells that were negative for CD31. Oct4 (red) appeared in nuclei of endothelial cells, some perivascular cells, and stromal cells. Perivascular cell Oct4 positivity was confirmed by co-labeling with Oct4 (red) and perivascular cell marker, α-SMA (brown; e and f). No discernible differences were seen in hemangioblastomas associated with vHL (right side of the panels: b, d, and f) [images taken at 20X; inserts show high magnification; asterisk = endothelial positivity, green arrow = stromal positivity, and red arrow = perivascular positivity].*

A series of double stains were performed using Picture™ Plus Double Staining Kit (Invitrogen). As above, slides were deparaffinized, hydrated through a sequential ethanol gradient and washed in PBS. Following antigen retrieval, endogenous peroxidase activity was quenched with 3% H_2O_2 diluted in methanol for ten minutes. Slides were blocked and primary antibodies (Table; one mouse anti-human and one rabbit anti-human antibody combination) were applied simultaneously for one hour. Slides were rinsed in PBS containing 0.05% Tween-20. Goat anti-mouse IgG-horseradish peroxidase (HRP) polymer conjugate and goat anti-rabbit IgG-alkaline phosphatase (AP) polymer conjugate were then applied for 30 minutes. DAB chromogen and Fast Red were used for detection. Slides were counterstained with Mayer's hematoxylin (Sigma-Aldrich) for 30 seconds and mounted using ClearMount™ Mounting solution (Invitrogen).

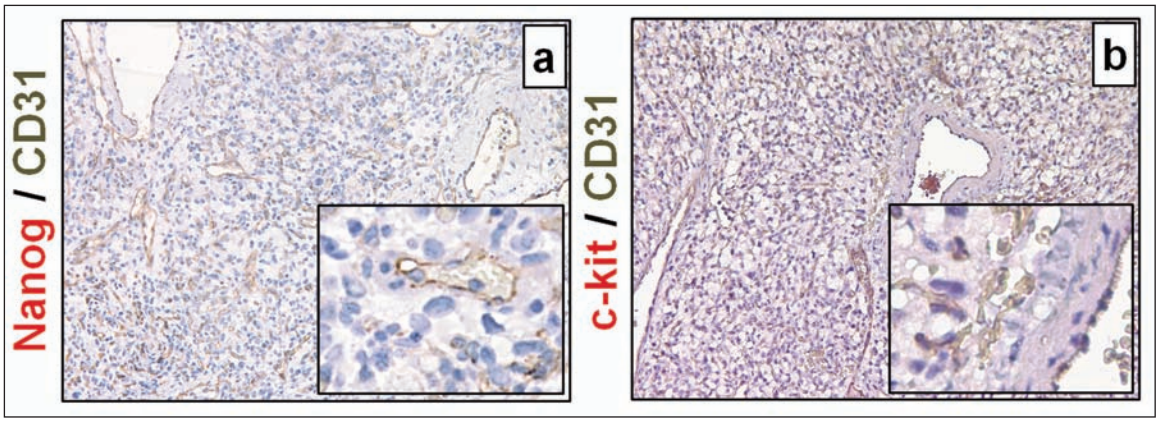

Figure 2: *Representative images of double-labeling Nanog/CD31 (a) and c-kit/CD31 (b) immunohistochemistry showing complete negativity of hemangioblastoma cells for Nanog (red) and c-kit (red). Only CD31 immunoreactivity is seen (brown) [images taken at 20X; inserts show high magnification].*

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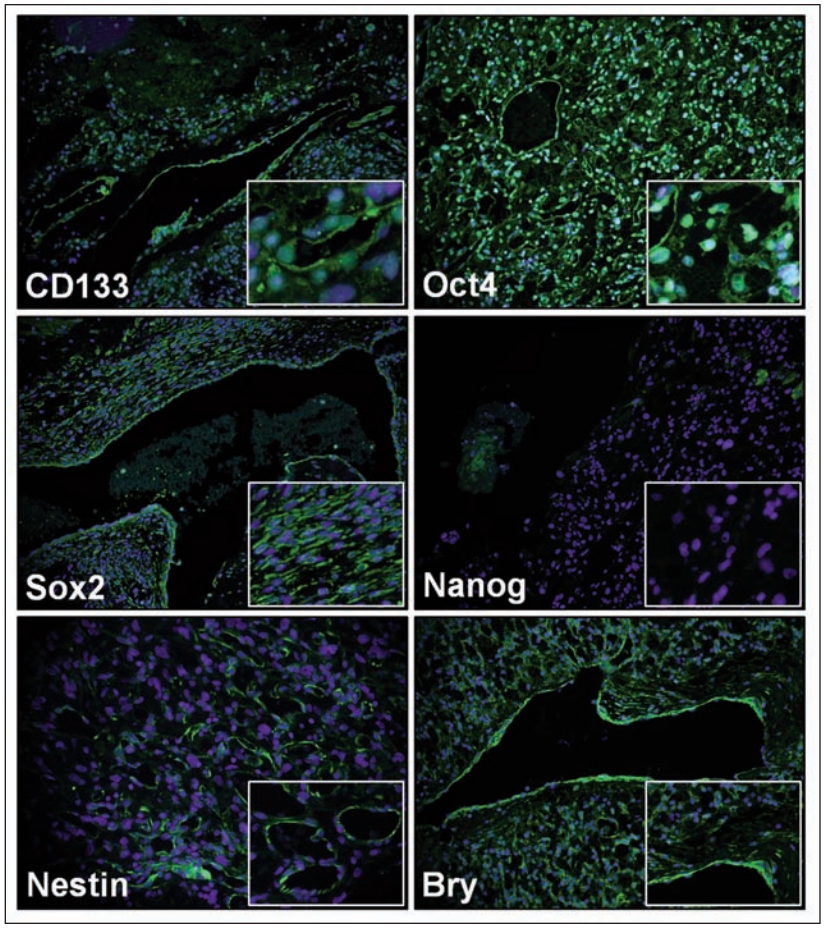

Figure 3: *Immunofluorescence staining was performed to confirm the localization of stem cell markers (green) in hemangioblastoma specimens. Endothelial cells were positive for CD133, Oct4, Nestin, and Bry. Perivascular cells sparsely expressed CD133, Oct4, and Bry. Intense Oct4 positivity was seen in the stromal cells. Sox2 transcription factor appeared to be cytoplasmic (indicating no activity) and Nanog reactivity was completely absent in the specimens [images were taken at 20X; inserts show higher magnification; slides were counterstained with DAPI (blue)].*

Results

Hemangioblastoma endothelium and stromal cells express stem cell antigen, CD133

CD133 has been shown to be expressed by stem cells, endothelial progenitor cells, neural stem cells, and mesenchymal stem cells. CD133 has also been used to identify tumor-initiating cells from medulloblastomas, glioblastomas, and prostate and colon carcinomas[10-15]. Therefore, we first examined whether hemangioblastoma specimens contain CD133+ stem cells. We analyzed all 13 samples for CD133 immunoreactivity and found robust expression of CD133 in hemangioblastoma endothelium (Figure 1). Interestingly, CD133 was also present on stromal cells and sparsely on perivascular cells. Besides cellularity, no significant differences were observed between sporadic hemangioblastomas and hemangioblastomas associated with vHL (Figure 1, a,b). These findings suggest an atypical precursor nature of the endothelial cells, the perivascular cells, and the stromal cells in hemangioblastoma. Next, we assayed for Oct4, a pluripotency marker that is expressed alongside Sox2 and Nanog in embryonic stem cells[16]. Similar to CD133, Oct4 also showed strong stromal positivity (Figure 1, c-f). Nuclear Oct4 (active form) was also observed in the some but not all endothelial cells (Figure 1, c,d) and the perivascular cells (Figure 1, e,f).

Lack of true pluripotency in hemangioblastoma

In order to confirm whether the stromal cells and possibly the vascular cells are pluripotent (as suggested by Oct4 positivity), we tested for the expression of Nanog. If the cells are truly pluripotent, Nanog should be positive and exhibit a nuclear localization. Interestingly, our results show complete lack of Nanog expression in the hemangioblastoma specimens (Figure 2a). Similarly, c-kit (commonly used as a marker of bone marrow-derived stem cells) was negative in hemangioblastoma specimens (Figure 2b).

To confirm these findings, including the nuclear localization of Oct4, we performed immunofluorescence staining of hemangioblastoma specimens. CD133 was positive in vascular endothelial cells and stromal cells (Figure 3). Endothelial cells also showed positivity for Nestin and mesodermal marker Bry. Oct4 was present in the nuclei of cells (complete overlap with DAPI). When we assayed for Sox2, we found cytoplasmic but not nuclear reactivity of the stem cell transcription factor.

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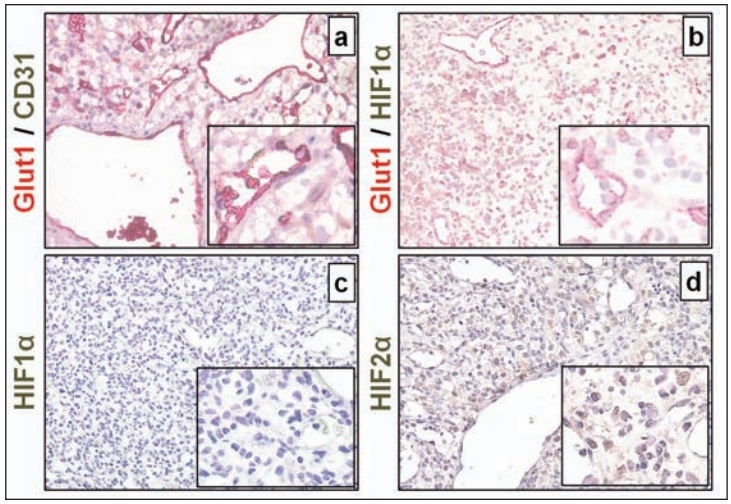

Figure 4: *Examining Glut1 and HIF expression in hemangioblastoma showed uniform Glut1 reactivity (red; upper panels) in the vascular endothelial cells. However, no HIF1α positivity was seen in the endothelial cells or the stromal cells (b and c). Hemangioblastoma specimens associated with vHL were also negative for HIF1α (data not shown). (d) Staining of HIF2α in hemangioblastoma specimen showing nuclear reactivity in stromal cells. [images taken at 20X; inserts show high magnification].*

Uniform expression of glucose transporter-1 (Glut1) in hemangioblastoma endothelium

The association of hemangioblastomas with vHL has suggested a role for the vHL gene in tumor development[1,17,18]. A mutation in the vHL gene is suggested to give rise to the tumor[18]. Since the product of the vHL gene targets the hypoxia-inducible factor (HIF; a transcription factor) for proteasomal degradation[18], we tested for the expression of HIF1α and the downstream target gene glucose transporter-1 (Glut1) in hemangioblastoma specimens. Immunohistochemical analysis of Glut1 showed intense reactivity in the hemangioblastoma endothelial cells (Figure 4a). Stromal and perivascular cells displayed no discernible positivity. We then determined whether HIF1α co-localizes to Glut1+ endothelial cells. Our results show no expression of HIF1α in endothelial cells, perivascular cells, or the stromal cells (Figure 4b,c). This surprising result prompted us to study whether HIF2α is expressed in hemangioblastomas. HIF2α is structurally similar to HIF1α but differs in target genes and function[19]. Our results show robust expression of HIF2α in stromal cells with little or no immunoreactivity in endothelial or perivascular cells (Figure 4d).

Discussion

We hypothesized that hemangioblastomas express many stem/progenitor cell markers, and that hemangioblastoma vasculature is derived from these atypical stem cells. The salient findings of our study include the novel characterization of hemangioblastoma stromal cells, which exhibit a committed

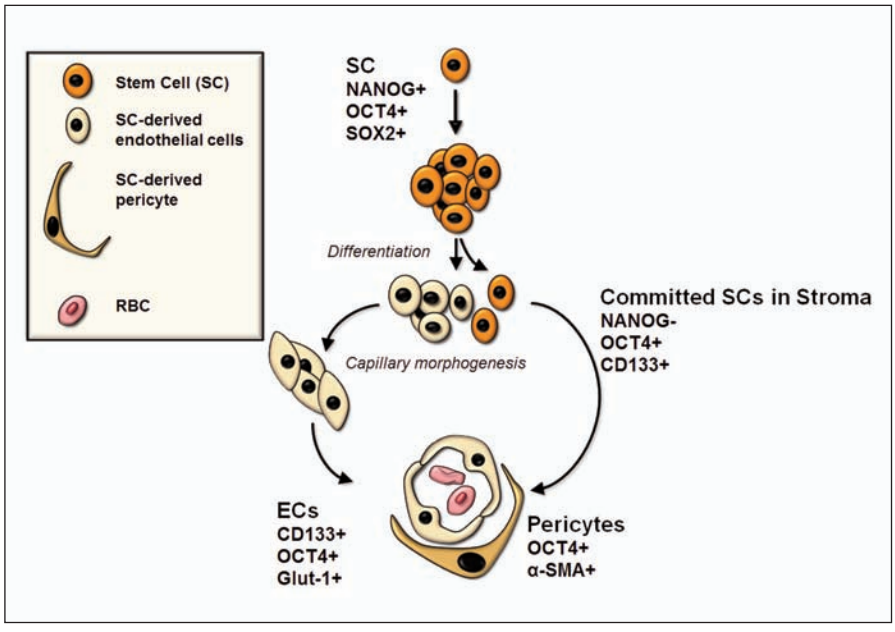

Figure 5: *Schematic illustration of the hypothesized origin of hemangioblastomas. We hypothesize that hemangioblastomas arise from committed mesodermal stem cells. These SCs may differentiate into atypical Glut1+ endothelial cells that retain CD133 and Oct4 positivity. Pericytes may also be derived from these stem cells upon differentiation and loss of CD133. Cells in the stroma that fail to undergo differentiation become highly vacuolated and lipid-laden.*

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stem cell phenotype. The presence of these committed stem cells supports the notion that tumor microvasculature originates *in situ* (Figure 5), rather than by recruitment processes.

Nanog, Oct4 and Sox2 are transcription factors that play an important role in self-renewal of undifferentiated/uncommitted stem cells. Interestingly, of this triad of transcription factors, we only observed well-demarcated expression of Oct4. Positivity was confined to the nuclei of stromal cells, confirming their role as tumor stem cells. Less abundant expression was seen in some endothelial and perivascular cells, possibly owing to the atypical precursor nature of the cells and the decrease in Oct4 expression upon differentiation into vascular cells. The complete lack of both Sox2 and Nanog in the nuclei of stromal cells indicates that these cells are not pluripotent, but are maintained in a committed stem cell state. Two recent studies have also shown expression of stage-specific embryonic antigen-1 (SSEA-1) in a small population of hemangioblastoma stromal cells[20,21]. SSEA-1 is well established in mouse embryonic stem cells and denotes state of pluripotency. However, in human cells, SSEA-1 is expressed only upon differentiation of embryonic stem cells (time coinciding with decreased expression of SSEA-3 and -4)[22,23]. Taken together, these recent studies and our findings show a committed state of the hemangioblastoma stromal cells.

An insight into the atypical nature of the hemangioblastoma stem cells can also be gained by examining CD133+ cells. As noted by other studies and shown here, hemangioblastomas are positive for CD133[8,24]. However, with its expression previously being reported only as "scattered", our group is the first to describe well-demarcated endothelial localization of CD133 in hemangioblastoma. CD133 localization to vascular cells has been documented in highly angiogenic tumors, such as non-small cell lung cancer, infantile hemangioma, malignant glioma, and breast cancer[25-28]. Further, it was shown that CD133 positive glioma cell subpopulations (compared to CD133 negative) are responsible for enhanced angiogenesis, endothelial cell migration, and vessel tube formation[29], indicating that this cell type is involved in (or representative of) maintaining the angiogenic features of a tumor. CD133 positivity was also seen within the stromal cells of the tumor, and less abundantly in perivascular cells. Recently, adipose-derived mesenchymal stem cells were shown to express Oct4 but not Sox2[30]. However, these mesenchymal stem cells lacked the expression of CD133 and markers of endothelial/perivascular cells. Together, this suggests that the stromal cells in hemangioblastoma are unique in that they express Oct4 and CD133.

We studied the expression and localization of several other proteins, including c-kit (CD117), a cytokine receptor typically expressed on the surface of bone marrow-derived progenitor cells[31]. The apparent lack of c-kit positive cells across all hemangioblastoma samples analyzed suggests that bone marrow-derived progenitor recruitment may not be responsible for tumor angiogenesis. Another protein whose expression would substantiate these notions is Glut1. The expression of Glut1 is ubiquitous in most fetal endothelial cells during early development. This level of expression is selectively lost as the cells differentiate, and in adults, Glut1 expression is limited to erythrocytes and endothelia with blood-tissue barrier function[32]. If recruitment were the major contributor to the endothelial cells in hemangioblastoma, we would expect to find mosaic vessels and/or Glut1 negative vessels. Our results showed intense Glut1 reactivity in the endothelial cells (in the absence of HIF1α), demonstrating vasculogenesis as the main process involved. Negative HIF1α was an unexpected finding as VHL product has been shown to cause ubiquitination and degradation of HIF1α[18]. HIF1α also marks lesions in VHL kidneys[33]. Furthermore, vascular endothelial growth factor (VEGF), downstream target of HIF1α, has been shown to be highly expressed in hemangioblastomas[34,35]. Interestingly, VEGF levels correlate with HIF2α but not with HIF1α protein levels in hemangioblastomas[36]. To probe this further, we analyzed hemangioblastoma specimens for HIF2α and show robust expression of HIF2α in stromal cells. HIF2α has recently been shown to be highly expressed in glioma stem cells[37,38]. HIF2α also marks neural stem cells but not progenitor cells[37,39,40]. This suggests that HIF2α positive stromal cells in hemangioblastoma may be the tumor-initiating cells.

Overall, our findings indicate that the hemangioblastoma phenotype is regulated by a committed hemangioblastoma stem cell (Figure 5). These committed stem cells ($Nanog^-$, $Oct4^+$, $CD133^+$) may be derived from uncommitted stem cells ($Nanog^+$, $Sox2^+$, $Oct4^+$), and are found within the stroma of the tumor. Neovascularization then likely occurs by vessel formation *in situ* (vasculogenesis) upon upregulation of angiogenic factors (such as VEGF) that can induce differentiation of stem cells into vascular cells. The endothelial cells that comprise the vessels of hemangioblastoma are unique in that they are also relatively immature (able to maintain the expression of CD133), which is representative of the highly vasculogenic nature of this tumor.

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References

1. Hussein MR. Central nervous system capillary haemangioblastoma: the pathologist's viewpoint. Int J Exp Pathol. 2007 Oct;88(5):311-24.
2. Glasker S, Li J, Xia JB, et al. Hemangioblastomas share protein expression with embryonal hemangioblast progenitor cell. Cancer Res. 2006 Apr 15;66(8):4167-72.
3. Aldape KD, Plate KH, Vortmeyer AO, Zagzag D, Neumann HPH. World Health Organization: Classification of Tumours of the Central Nervous System. 4th ed: IARC; 2007. p. 184-6.
4. Atlas SW, Lavi E, Fisher PG. Intraxial brain tumours. In: Atlas SW, editor. Magnetic resonance imaging of the brain and spine. 3rd ed: Philadelphia. Lippincott, Williams & Wilkins; 2001. p. 389-94.
5. Lonser RR, Glenn GM, Walther M, et al. von Hippel-Lindau disease. Lancet. 2003 Jun 14;361(9374):2059-67.
6. Chaudhry AP, Montes M, Cohn GA. Ultrastructure of cerebellar hemangioblastoma. Cancer. 1978 Oct;42(4):1834-50.
7. Richard S, Campello C, Taillandier L, Parker F, Resche F. Haemangioblastoma of the central nervous system in von Hippel-Lindau disease. French VHL Study Group. J Intern Med. 1998 Jun;243(6):547-53.
8. Park DM, Zhuang Z, Chen L, et al. von Hippel-Lindau disease-associated hemangioblastomas are derived from embryologic multipotent cells. PLoS Med. 2007 Feb;4(2):e60.

9. Vortmeyer AO, Frank S, Jeong SY, et al. Developmental arrest of angioblastic lineage initiates tumorigenesis in von Hippel-Lindau disease. Cancer Res. 2003 Nov 1;63(21):7051-5.
10. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007 Jan 4;445(7123):106-10.
11. Ricci-Vitiani L, Lombardi DG, Pilozzi E, et al. Identification and expansion of human colon-cancer-initiating cells. Nature. 2007 Jan 4;445(7123):111-5.
12. Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003 Sep 15;63 (18):5821-8.
13. Salmaggi A, Boiardi A, Gelati M, et al. Glioblastoma-derived tumorospheres identify a population of tumor stem-like cells with angiogenic potential and enhanced multidrug resistance phenotype. Glia. 2006 Dec;54(8):850-60.
14. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005 Dec 1;65(23):10946-51.
15. Richardson GD, Robson CN, Lang SH, Neal DE, Maitland NJ, Collins AT. CD133, a novel marker for human prostatic epithelial stem cells. J Cell Sci. 2004 Jul 15;117(Pt 16):3539-45.
16. Loh YH, Wu Q, Chew JL, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. Nat Genet. 2006 Apr;38(4):431-40.
17. Kaelin WG, Jr. The von Hippel-Lindau tumour suppressor protein: O2 sensing and cancer. Nat Rev Cancer. 2008 Nov;8(11):865-73.
18. Kaelin WG, Jr. Molecular basis of the VHL hereditary cancer syndrome. Nat Rev Cancer. 2002 Sep;2(9):673-82.
19. Pietras A, Johnsson AS, Pahlman S. The HIF-2alpha-driven pseudo-hypoxic phenotype in tumor aggressiveness, differentiation, and vascularization. Curr Top Microbiol Immunol. 2010;345:1-20.
20. Ma D, Zhu W, Zhang M, et al. Identification of tumorigenic cells and implication of their aberrant differentiation in human hemangioblastomas. Cancer Biol Ther. 2011 Oct 15;12(8): 727-36.
21. Ma D, Zhang M, Chen L, et al. Hemangioblastomas might derive from neoplastic transformation of neural stem cells/progenitors in the specific niche. Carcinogenesis. 2011 Jan;32(1):102-9.
22. Fox N, Damjanov I, Knowles BB, Solter D. Immunohistochemical localization of the mouse stage-specific embryonic antigen 1 in human tissues and tumors. Cancer Res. 1983 Feb;43(2):669-78.
23. Shamblott MJ, Axelman J, Wang S, et al. Derivation of pluripotent stem cells from cultured human primordial germ cells. Proc Natl Acad Sci U S A. 1998 Nov 10;95(23):13726-31.
24. Chan CC, Chew EY, Shen D, Hackett J, Zhuang Z. Expression of stem cells markers in ocular hemangioblastoma associated with von Hippel-Lindau (VHL) disease. Mol Vis. 2005;11:697-704.
25. Khan ZA, Boscolo E, Picard A, et al. Multipotential stem cells recapitulate human infantile hemangioma in immunodeficient mice. J Clin Invest. 2008 Jul;118(7):2592-9.
26. Hilbe W, Dirnhofer S, Oberwasserlechner F, et al. CD133 positive endothelial progenitor cells contribute to the tumour vasculature in non-small cell lung cancer. J Clin Pathol. 2004 Sep;57(9): 965-9.
27. Bao S, Wu Q, Sathornsumetee S, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res. 2006 Aug 15;66(16):7843-8.
28. Wright MH, Calcagno AM, Salcido CD, Carlson MD, Ambudkar SV, Varticovski L. Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics. Breast Cancer Res. 2008;10(1):R10.
29. Wang R, Chadalavada K, Wilshire J, et al. Glioblastoma stem-like cells give rise to tumour endothelium. Nature. 2010 Dec 9;468 (7325):829-33.
30. Lo Cicero V, Montelatici E, Cantarella G, et al. Do mesenchymal stem cells play a role in vocal fold fat graft survival? Cell Prolif. 2008 Jun;41(3):460-73.
31. Papayannopoulou T, Brice M, Broudy VC, Zsebo KM. Isolation of c-kit receptor-expressing cells from bone marrow, peripheral blood, and fetal liver: functional properties and composite antigenic profile. Blood. 1991 Sep 15;78(6):1403-12.
32. Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. Physiol Rev. 2003 Jan;83(1):183-252.
33. Mandriota SJ, Turner KJ, Davies DR, et al. HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. Cancer Cell. 2002 Jun;1(5): 459-68.
34. Wizigmann-Voos S, Breier G, Risau W, Plate KH. Up-regulation of vascular endothelial growth factor and its receptors in von Hippel-Lindau disease-associated and sporadic hemangioblastomas. Cancer Res. 1995 Mar 15;55(6):1358-64.
35. Bohling T, Hatva E, Kujala M, Claesson-Welsh L, Alitalo K, Haltia M. Expression of growth factors and growth factor receptors in capillary hemangioblastoma. J Neuropathol Exp Neurol. 1996 May;55(5):522-7.
36. Flamme I, Krieg M, Plate KH. Up-regulation of vascular endothelial growth factor in stromal cells of hemangioblastomas is correlated with up-regulation of the transcription factor HRF/HIF-2alpha. Am J Pathol. 1998 Jul;153(1):25-9.
37. Li Z, Bao S, Wu Q, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell. 2009 Jun 2;15(6):501-13.
38. McCord AM, Jamal M, Shankavaram UT, Lang FF, Camphausen K, Tofilon PJ. Physiologic oxygen concentration enhances the stem-like properties of CD133+ human glioblastoma cells in vitro. Mol Cancer Res. 2009 Apr;7(4):489-97.
39. Pietras A, Gisselsson D, Ora I, et al. High levels of HIF-2alpha highlight an immature neural crest-like neuroblastoma cell cohort located in a perivascular niche. J Pathol. 2008 Mar;214 (4):482-8.
40. Pietras A, Hansford LM, Johnsson AS, et al. HIF-2alpha maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells. Proc Natl Acad Sci USA. 2009 Sep 29;106(39):16805-10.