To the editor:

Hemangioblastoma-associated mast cells in von Hippel-Lindau disease are tumor derived

von Hippel-Lindau disease (VHL) is a tumor syndrome resulting from an inherited mutation in the *VHL* gene. The primary neoplastic manifestation in VHL are CNS hemangioblastomas[1] that develop after loss of the second wild-type *VHL* allele (loss of heterozygosity [LOH]). Histologically, hemangioblastomas are composed of stromal cells, blood vessels, and mast cells.[2,3] The VHL-deficient neoplastic stromal cell expresses markers reflective of pluripotent embryologic hemangioblasts.[4] These cells can differentiate into tumor-derived endothelial and hematopoietic progeny in vitro.[5] In vivo, tumor-derived extramedullary hematopoiesis

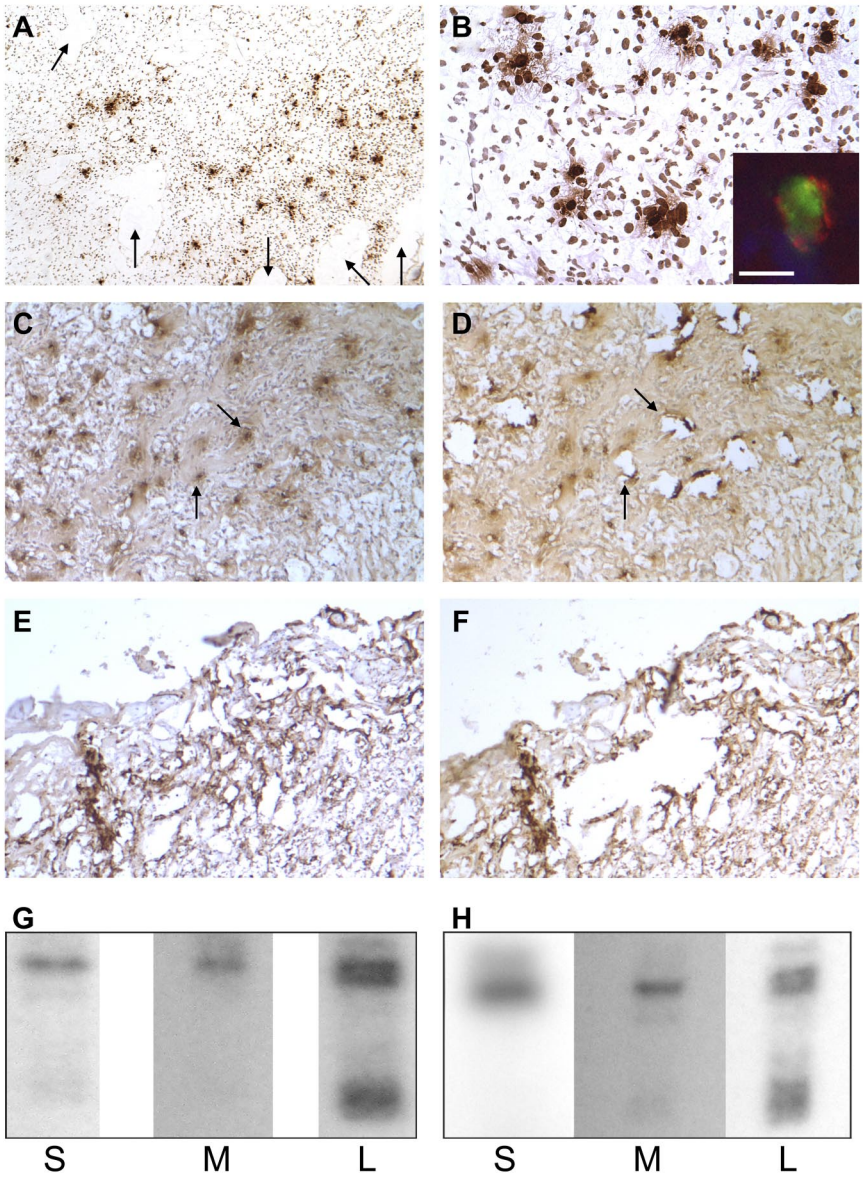

Figure 1. Analysis of LOH in mast cells in VHL disease hemangioblastomas. Low-power (A; 10× objective) and high-power (B; 40× objective) magnification of a hemangioblastoma stained for mast cell tryptase. Note the lack of any pattern or organization of mast cells in the tumor. Vessels are indicated by arrows (A). No perivascular cuffing can be observed. Note the tendency of mast cells to occur in clusters (B). Inset shows localization of mast cell tryptase (green) and KIT receptor (red) in the same cell. The scale bar indicates 10 μm. Mast or stromal cells, identified by tryptase (C) or inhibin-A (E) immunostaining, respectively, were microdissected from frozen sections of the hemangioblastomas. Sections stained for mast cell tryptase (C-D) or for inhibin-A (E-F) are shown before (C,E) and after (D,F) microdissection. Arrows (C-D) indicate the same location in the section before and after microdissection. Peripheral blood lymphocytes were acquired from the same patients. LOH analysis was performed as described previously[6,10] with minor modifications. PCR amplification products, generated using primers (D3S1038) from a microsatellite region associated with the VHL gene, are shown for a thoracic (G) and cerebellar (H) hemangioblastoma. Note the presence of allelic imbalance in the stromal (S) and mast cell (M) samples compared with the normal lymphocytes (L). Film exposures were selected to allow comparison of similar band intensities. For immunohistochemistry, primary antibodies (anti–mast cell tryptase [Thermo Fisher] and anti–inhibin-A [AbD Serotec]) were detected with MACH4 HRP-Polymer (Biocare Medical) and DAB (Vector Laboratories). Images were obtained with a Leica DMLB microscope using a spot-imaging camera and software. For the double immunofluorescence studies, anti-KIT (Sigma-Aldrich) and anti-tryptase antibodies were detected with rhodamine- and FITC-conjugated donkey secondary antibodies (Jackson ImmunoResearch). Images were obtained using Zeiss Axiophot2 microscope, Nikon Imaging Mono 12BIT camera, and Nikon NIS Elements BR software.

results in VHL-deficient erythrocytes.[6] Although the tumor origin of hemangioblastoma-associated stromal cells, reactive blood vessels, and intratumoral erythrocytes has been established, the origin of the abundant intratumoral mast cells is not known.

Normally associated with the allergic response in peripheral tissues, mast cells are not usually observed in the CNS, although they can be found there under certain inflammatory conditions.[7] However, inflammatory cellular infiltrate is not a feature of hemangioblastomas, leading to speculation about the origin of intratumoral mast cells in this setting. We hypothesized that these mast cells arise from VHL-deficient tumor cells in a manner analogous to the VHL-deficient erythrocytes identified in areas of intratumoral extramedullary hematopoiesis.[6] To determine whether mast cells in hemangioblastomas are derived from VHL-deficient tumor cells, we performed LOH analysis on microdissected intratumoral mast cells. We analyzed 4 confirmed hemangioblastomas (3 from the cerebellum and 1 from the spinal cord) resected from 4 VHL patients and procured according to National Institutes of Health guidelines.

Mast cell distribution occurred randomly throughout the tumors as single cells or in clusters (Figure 1A-B). No mast cell perivascular cuffing was observed around the tumor vasculature, as occurs with inflammatory infiltration. Mast cells also stained for KIT, the SCF receptor that is required for mast cell development.[8] Microdissection (Figure 1C-F) was performed on frozen sections that were immunostained for the mast cell marker tryptase (Figure 1C-D) or the stromal cell marker inhibin-A (Figure 1E-F). The distinct morphology and staining patterns of the mast and stromal cells allowed for clear identification of the cell populations under study. For each patient, LOH analysis in the *VHL* alleles was performed for tumor stromal cells, mast cells, and peripheral blood lymphocytes. Because hemangioblastomas are resected without removal of adjacent normal tissue, we were unable to microdissect mast cells from nontumor tissue as a control. Consistent with a tumor cell origin, mast cell samples from all 4 patients exhibited LOH in the *VHL* alleles compared with the peripheral blood lymphocytes (Figure 1G-H).

VHL-deficient tumor cells in hemangioblastomas are pluripotent.[5] Endothelial, erythrocyte, granulocyte, and now mast cell (this report) progeny can arise from VHL-deficient cells. Erythrocytes, granulocytes, and mast cells all derive from a common myeloid progenitor. KIT, a key receptor in mast cell development[8] and erythropoiesis,[9] has been observed in hemangioblastomas.[5] The Kit signaling pathway may contribute to the ability of some of the VHL-deficient pluripotent neoplastic cells to develop into more differentiated progeny, including mast cells and erythrocytes.

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Contribution: M.J.M. designed the study, interpreted the data, and wrote the manuscript; N.A.E. designed and performed the experiments and analyzed the data; and R.R.L. supervised the study, reviewed the data, and revised the manuscript for intellectual content.

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To the editor:

Comparison of the efficacy of rabbit and horse antithymocyte globulin for the treatment of severe aplastic anemia in children

Immunosuppressive therapy (IST) with horse antithymocyte globulin (h-ATG) and cyclosporine (CSA) is an effective therapy for aplastic anemia (AA), resulting in a response rate of 60%-70% and an excellent survival for responders. However, lymphoglobulin (h-ATG; Genzyme) was withdrawn from the market in 2007. Because of the unavailability of h-ATG in most countries, the evaluation of rabbit ATG (r-ATG) as first-line therapy was stimulated. Marsh et al recently showed a low response to IST at 6 months (37%) in 35 patients who were given r-ATG (thymoglobulin; Genzyme).[1] The overall survival at 2 years was significantly