Published in final edited form as:

Ophthalmology. 2007 January ; 114(1): 147–156.

Molecular Pathology and CXCR4 Expression in Surgically Excised Retinal Hemangioblastomas Associated with von Hippel-Lindau Disease

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Abstract

Purpose— The surgical excision of retinal vascular lesions including hemangioblastomas is rarely practiced. This study investigates the pathological characteristics of 4 patients (3 with von Hippel-Lindau [VHL] disease and 1 with a vasoproliferative retinal tumor) who underwent ocular tumor resection, von Hippel-Lindau is an autosomal dominant disease caused by a defect of the *VHL* tumor suppressor gene. The *VHL* protein is required for oxygen-dependent degradation of hypoxia-inducible factor 1 α . This factor regulates vascular endothelial growth factor (VEGF) and the chemokine receptor CXCR4. Retinal hemangioblastoma is the most common tumor observed in VHL disease. We investigated the expression of CXCR4; its ligand, CXCL12/SDF-1 α ; VEGF; and the *VHL* gene in VHL disease-associated retinal hemangioblastomas.

Design— Interventional case series with immunohistological and molecular pathological analyses.

Participants— Immunohistochemistry and molecular pathology of the surgically excised retinal lesions were performed.

Intervention— Large retinal hemangioblastomas (1–3 disc diameters) and vasoproliferative tumors were resected surgically after laser photocoagulation in 4 patients. The excised tissues were snap frozen and evaluated by histology. Molecular pathology was performed for the *VHL* gene, and immunohistochemistry and molecular detection (reverse transcription polymerase chain reaction) were carried out for *VEGF*, *CXCR4*, and *CXCL12*.

Main Outcome Measures— Evaluation of clinical presentations and molecular pathology of the excised retinal lesions.

Results— Large retinal hemangioblastomas were resected successfully from the 3 VHL cases. Postoperatively, all patients were stable. Molecular analyses disclosed the loss of heterozygosity at the *VHL* allele locus in the VHL retinal hemangioblastomas but not in the vasoproliferative tumor. High levels of transcript and protein were found for VEGF and CXCR4, whereas low levels of *CXCL12* mRNA were expressed in the retinal hemangioblastomas associated with VHL disease. In

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Financial support: National Eye Institute Intramural Research Program (DS, C-CC); Natural Science Foundation of Guangdong, Guangdong, China (grant no.: 036653 [XL]); Natural Science Foundation of China, Beijing, China (grant no.: 30471848 [XL]); and Ministry of Education Foundation of China, Beijing, China (grant no.: 2004527 [XL, YH]).

contrast, very low levels of *VEGF* and *CXCR4* mRNA were detected in the vasoproliferative tumor. Furthermore, increased expression of VEGF and CXCR4 was detected in more active hemangioblastomas.

Conclusions— Surgical resection of large retinal hemangioblastomas may be useful for therapy in selected VHL patients. Activated VHL lesions produce more VEGF. This is the first demonstration of CXCR4 expression in VHL disease–associated retinal hemangioblastomas. We suggest targeting CXCR4 as an additional therapeutic strategy for VHL disease–associated retinal hemangioblastomas.

von Hippel-Lindau (VHL) disease is an autosomal dominant multisystem neoplastic syndrome caused primarily by a germ-line mutation within the *VHL* gene.^{1,2} von Hippel-Lindau disease has an incidence of 1 in 36 000 in newborns and a penetrance of more than 90% by the age of 65 years. Germline mutations in the *VHL* gene lead to the development of several tumors and cysts in multiple organs. Affected individuals are at risk of developing retinal and CNS hemangioblastomas, endolymphatic sac tumors, renal cysts, renal clear cell carcinomas, pheochromocytomas, pancreatic cysts, neuroendocrine tumors, and epididymal and broad ligament cystadenomas.^{3,4} Retinal and CNS hemangioblastomas are the most common manifestations, and are frequently the earliest signs of VHL disease.⁵ Treatments for retinal hemangioblastomas include observation, laser photocoagulation, cryotherapy, plaque radiotherapy, and antiangiogenic therapy.^{6,7}

The *VHL* gene is a tumor suppressor gene located on chromosome 3p25–26.^{8,9} On a molecular level, the VHL protein plays an important role in hypoxia sensing.^{10,11} Mutant VHL protein products induce the cell to upregulate the hypoxia-inducible factor (HIF) signaling pathway, thereby resulting in increased hypoxia-induced genes and proteins such as vascular endothelial growth factor (VEGF), platelet-derived growth factor, transforming growth factor α , and erythropoietin.^{12,13} We have demonstrated abundant expression of VEGF, HIF, and erythropoietin in VHL disease–associated retinal and optic nerve hemangioblastomas.^{14–16}

Stromal cell-derived factor 1 α /CXCL12 (a chemokine) is the sole ligand of chemokine receptor CXCR4.^{17,18} CXCR4 and CXCL12 are required for normal embryonic development of the neural, hematopoietic, and cardiovascular systems. They also are involved in the trafficking of hematopoietic progenitor and stem cells. CXCR4 is reported to be downregulated by the VHL protein and upregulated by HIF 1 α .¹⁹ Recently, the coexpression of CXCR4 and CXCL12 has been demonstrated in VHL disease–associated CNS hemangioblastoma and in renal clear cell carcinoma.²⁰ These findings suggest that the loss of function of a single tumor suppressor gene can upregulate the expression of both the ligand and its receptor. This upregulation results in the initiation of an autocrine signaling pathway that may play an important role in the pathogenesis of VHL disease–associated lesions. Our current study examines VEGF, CXCR4, and CXCL12 in 3 excised VHL disease–associated retinal hemangioblastomas and 1 vascular lesion of a vasoproliferative (retinal) tumor.

Patients and Methods

Four patients from the Zhongshan Ophthalmic Center (ZOC), Sun Yat-sen University of Guangzhou, China were enrolled in the study. All surgeries were performed at the ZOC, and all specimens were analyzed at the United States National Eye Institute. The study followed the tenets stated in the Declaration of Helsinki and was approved by the institutional review boards of both the ZOC and the National Eye Institute for human subjects. Informed consent was obtained from each of the patients.

Case Reports

Case 1—A 35-year-old male with familial VHL disease presented with poor vision for 10 years. He developed hemangioblastomas in the spinal cord and cerebellum in 1997 and 2002, respectively. At the time of first examination, his visual acuities (VAs) were 0.3 (20/70), right eye, and no light perception (LP), left eye. In the superotemporal and inferionasal equators of the right eye, there were 4 large reddish retinal hemangioblastomas measuring 2 to 3 disc diameters (DD), with well-defined feeder vessels and tractional and exudative retinal detachments (RDs) (Fig 1). An area of shallow macular detachment also was observed. The left eye showed severe tractional RD extending to the macula, hemorrhages, and many retinal hemangioblastomas. His father and one sister also were diagnosed with CNS hemangioblastoma. Pars plana vitrectomy (PPV), argon endolasing of the feeder vessels, endodiathermy of the inferionasal hemangioblastomas, and resection of the other 3 large hemangioblastomas, followed by filling of the vitreous cavity with silicone oil, were performed in the right eye. The retinal specimens were snap frozen. Three months after the surgery, vision in his right eye improved from 0.3 (20/70) to 0.5 (20/40). The macula seemed to be attached at the 8-month follow-up when the silicone oil tamponade was still in place. Mild posterior capsular cataract developed 8 months after the operation.

Case 2—A 20-year-old female with familial VHL disease complained of progressive loss of VA and superior visual field loss in the left eye for 6 months. She had no systemic manifestations. Her father had retinal hemangioblastoma in both eyes; her paternal uncle and aunt had a history of cerebellar hemangioblastoma. Her vision was 1.2 (20/16) in the right eye and 0.4 (20/50) in the left eye. There were several small retinal hemangioblastomas in the superior nasal periphery of the right eye. Three large retinal hemangioblastomas (1–3 DD) with large feeder vessels surrounded by serous detachment, as well as multiple hard and soft exudates, were seen in the nasal and inferior peripheries of the left eye (Fig 2). A few tiny retinal hemangioblastomas also were noted in the periphery. Tractional and exudative RDs in the inferior periphery as well as macular edema were observed at the time of presentation. The small retinal hemangioblastomas in both eyes were treated with laser photocoagulation. Later, the patient successfully underwent PPV, resection of the largest nasal hemangioblastoma, endolaser photocoagulation, endodiathermy, and silicone oil tamponade in the left eye. The resected retinal tissue was snap frozen and sectioned. Due to the formation of epiretinal membrane and recurrent RD in the inferior retina, her macula remained edematous and her vision worsened to 0.2 (20/100) from 0.4 (20/50) 2 months after surgery. The lens remained clear.

Case 3—A 19-year-old female noticed progressive loss of vision in the right eye over the preceding 3 years. She was diagnosed with cerebellar hemangioblastoma. The hemangioblastoma was removed successfully the previous year. Her grandfather, father, and 4 uncles all had cerebellar hemangioblastomas. Her father and a sister also suffered from retinal hemangioblastomas. She was referred to the ZOC after being treated twice with laser photocoagulation elsewhere. Ocular examination disclosed VAs of LP, right eye, and 1.5 (20/15), left eye. A 3-DD retinal hemangioblastoma was seen in the right superior temporal periphery surrounded by exudates and small hemorrhages (Fig 3). Several tiny retinal hemangioblastomas also were detected in the temporal and inferior peripheries of the right eye. Vitreal hemorrhages and tractional RD were observed. The macula had lipid exudates in a star configuration (Fig 3). Laser photocoagulation was applied to the small retinal hemangioblastomas of the left eye. Subsequently, the large retinal hemangioblastoma with feeder vessels was excised after PPV, endolasing, endodiathermy, and silicone oil tamponade in the right eye. Three months later, the number of hard macular exudates decreased, and her vision improved from LP to 0.2 (20/100). The right eye maintained the same vision without cataracts 6 months after the operation without removal of the silicone oil tamponade.

Case 4—A 17-year-old male complained of progressive loss of vision in his right eye over the past 2 months. There was no history of ocular injury. Family history was negative. His VAs were 0.6 (20/33), right eye, and 1.2 (20/16), left eye. There was a round, 4-DD, orange, elevated vascular lesion in the superior nasal peripheral retina of the right eye (Fig 4). The vascular lesion was surrounded by exudates and serous RD without retinal or vitreal traction. Multiple lipid exudates also were observed in the equator and periphery. A few tiny exudates were seen in the macula. The differential diagnoses included VHL disease, vasoproliferative (retinal) tumor,²¹ and Coats' disease.^{22,23} The patient underwent PPV, resection of the retinal vascular lesion, endolaser photocoagulation, and silicone oil tamponade in the right eye. The retina was reattached, and the macula appeared normal. His VA recovered from 0.6 (20/33) to 0.9 (20/25) 3 months after surgery. The silicone oil was replaced by perfluoropropane in the vitreous. There were no complications such as hemorrhages, epiretinal membrane formation, RD, or cataract formation.

Immunohistochemistry

The avidin–biotin complex immunoperoxidase technique was applied to the frozen sections. The primary antibodies were against VEGF diluted to 1:100 (polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA), CXCR4 diluted to 1:50 (polyclonal antibody, Santa Cruz Biotechnology), and CXCL12 diluted to 1:50 (monoclonal antibody, R&D Systems, Minneapolis, MN). The secondary antibodies were biotinylated antirabbit or antimouse antibodies (Vector Laboratories, Burlingame, CA). Detection was carried out using avidin–biotin–peroxidase complex (Vector Laboratories) and 3,3'-diaminobenzidine as the chromogen. Control procedures included isotype-matched murine and rabbit immunoglobulin G of irrelevant specificity. An inflammatory granulation tissue was used for the positive control for the applied primary antibodies.

Microdissection and Detection of VHL Genetic Heterozygosity

The frozen sections were stained with hematoxylin-eosin without a cover slide. Under the microscope, retinal hemangioblastoma cells (avoiding vascular endothelium) or microaneurysm vascular cells were selected and microdissected carefully. These cells were placed immediately in DNA extraction buffer containing proteinase K and subjected to polymerase chain reaction (PCR), as described previously.¹⁴ Briefly, all samples were examined for loss of heterozygosity using the microsatellite markers D3S1038 and D3S1110, which flank the *VHL* gene (Research Genetics, Huntsville, AL). A case was considered informative for a polymorphic marker if normal tissue DNA showed 2 different alleles (heterozygosity). The criterion for loss of heterozygosity was complete or near complete absence of one allele in the tumor DNA as defined by direct visualization.

Reverse Transcriptase Polymerase Chain Reaction

The microdissected hemangioblastoma cells or vascular cells were examined for *β-actin*, *VEGF*, *CXCR4*, and *CXCL12* mRNA using quantitative reverse transcriptase PCR. Total RNA was extracted from microdissected cells using the PicoPure RNA isolation kit (Arcturus, Mountain View, CA). The cDNA was primed using Random Primers (Promega, Madison, WI) and synthesized using the Superscript RNAs H⁻ Reverse Transcriptase kit (Invitrogen, Carlsbad, CA). Three microliters of cDNA were used for each gene expression assay. The quantitative PCR was calculated using Real-Time PCR MX 3000P (Stratagene, La Jolla, CA) with Brilliant SYBR Green QPCR master mix (Stratagene) and RT² PCR primer sets (SuperArray, Frederick, MD). The cDNA synthesized from 0.16 ng of Universal human RNA (BD Biosciences, Palo Alto, CA) was used as the normal control for each assay. The standard curves derived from Universal human RNA and $\Delta \Delta C_t$ methods were employed to quantify gene expression.

Results

Histology and Immunohistochemistry

The excised retinal hemangioblastomas from the 3 VHL cases were highly vascular (Figs 1–3). All lesions illustrated an anastomosing network of small capillarylike channels that separated groups of foamy stromal cells and a few glial cells. The hemangioblastoma in case 2 was composed mostly of stromal cells and less vascular channels. In contrast, the excised vascular retinal lesion seen in case 4 had only a few dilated small vessels and showed moderate retinal gliosis (Fig 4). A diagnosis of vasoproliferative tumor was made.

Positive VEGF expression was observed in the stromal cells and in some endothelial cells of all 3 VHL cases (Figs 2, 3), as described previously.¹⁴ There was no expression of VEGF in the vasoproliferative tumor case. CXCR4 immunoreactivity was detected in the cytoplasm and nuclei of the stromal and vascular cells in all 3 VHL cases (Fig 5). A small amount of vascular staining also was noted in some areas. The lack of adjacent normal retinal tissue precluded a definitive analysis of the expression of CXCR4 in cases 1 and 2; however, very weak CXCR4 staining was observed in the rare nonhemangioblastoma cells seen in case 3. There was no immunoreactivity against VEGF or CXCR4 in the vasoproliferative tumor (Fig 5). CXCL12 was negative in the retina of all 4 cases (Fig 5).

Molecular Pathology

All resected retinal hemangioblastomas of the 3 VHL cases detected a loss of heterozygosity in the *VHL* gene. Absent or very low autoradiographic intensity was seen in one allele in comparison to the other allele using the microsatellite marker D3S1110 (Fig 6). Retention of heterozygosity was shown in the normal blood cells of all 3 VHL cases as well as in the retinal vascular and gliotic lesions and blood cells of the vasoproliferative tumor case.

VEGF and *CXCR4* transcripts were expressed abundantly in all excised retinal hemangioblastomas (Fig 7). Interestingly, *VEGF* levels were significantly higher in case 2. This case presented with the most active disease stage, having a larger number of hemangioblastomas and more lipid exudates and hemorrhages observed clinically and composed of more stromal cells histopathologically. *CXCR4* mRNA was highly expressed in the hemangioblastomas of the 3 VHL cases (Fig 7). The highest level of *CXCR4* transcript was expressed in case 3. This case had the lowest VA. Although expression of the CXCR4 ligand (*CXCL12* mRNA) was detectable, the level was low (Fig 7). In contrast, the vasoproliferative tumor did not express *VEGF*, *CXCR4*, or *CXCL12* transcripts.

Discussion

This study demonstrates that surgical resection of large retinal hemangioblastomas can be successfully performed in selected VHL patients by a skillful retinal surgeon. Peyman et al previously reported the successful removal of large retinal hemangioblastomas (>3 DD) by eye-wall resection in 2 VHL disease patients, one without significant operative and postoperative complications.²⁴ External excision of a retinal vascular lesion is a technically complicated procedure with risk of various complications. Therefore, this technique has not been established as a practical therapeutic approach for large retinal hemangioblastomas. At the 2005 annual meeting of the Association for Research in Vision and Ophthalmology, Schlesinger et al reported the successful excision of a retinal hemangioblastoma in a VHL disease patient (Invest Ophthalmol Vis Sci 46:abstract 3369, 2005). The tumor was resected by adapting standard PPV techniques to include the ligation of feeder vessels and endodiathermy to limit bleeding upon tumor removal. We propose that an internal resection in addition to modern vitreoretinal surgery is a viable option for the treatment of large tumors,

particularly for the large hemangioblastomas that may not respond to laser photocoagulation or cryotherapy.

Expected intraoperative or postoperative concerns include hemorrhage during resection, cataracts, and/or epiretinal membrane formation, as well as recurrent RD. In this study, short-term complications were minimal when the surgery was combined with PPV, argon endolasing of the feeder vessels, endodiathermy of the vascular lesion, and filling of the vitreous cavity with silicone oil. Postoperatively, visual outcome and general ophthalmic condition were relatively satisfactory. There were no excessive hemorrhages during the resection or after surgery in all 4 cases. Only 1 case (no. 2) developed epiretinal membrane formation and recurrent RD. One case (no. 1) developed cataracts. Although cataracts and recurrent RD may occur in the long term, the operation extends visual quality time to VHL disease patients who might otherwise become blind much sooner. This procedure not only is a sensible potential therapy for VHL disease and ocular hemangioblastomas, but also provides tissue for pathological, molecular, and genetic analyses, thus furthering our understanding of disease pathogenesis.

Retinal detachment is a serious concern associated with untreated large retinal hemangioblastomas.²⁵ In general, small retinal hemangioblastomas are treated by photocoagulation, whereas large hemangioblastomas with limited RD are treated by cryotherapy.²⁶ The therapeutic approach to retinal hemangioblastomas is to treat them early and at an asymptomatic stage. Modern treatment options for retinal hemangioblastomas include radiotherapy,²⁷ PPV,²⁸ photodynamic therapy,²⁹ and transpupillary thermotherapy³⁰ in addition to systemic treatment with a VEGF inhibitor.^{7,31,32} Laser photocoagulation and cryotherapy remain the primary therapeutic strategies for most cases.⁶ Again, early detection and treatment before the onset of severe visual loss are highly recommended. Although cataracts, epiretinal membrane formation, and recurrent RD may occur later, a successful surgery would provide a better quality of life for an extended time.

Vitreous surgery/PPV is recommended to treat large hemangioblastomas with traction RD or unabsorbed vitreous hemorrhage. In 1988, Machemer and Williams demonstrated the successful removal of vitreal and preretinal membranes with the destruction of leaking vessels for traction RD in 6 cases of retinal vasculopathies (including 2 VHL disease–associated retinal hemangioblastomas).²⁸ Later, several investigators also presented favorable results with vitreous surgery when applied to epiretinal membranes or traction RDs associated with hemangioblastomas.^{33–36} In this study, surgical resection of retinal hemangioblastomas via PPV combined with photocoagulation and intraocular tamponade was beneficial to all 3 VHL disease patients who exhibited large tumors, preretinal and vitreal membranes, and traction RD.

Retinal hemangioblastomas larger than 4 mm (2.5 DD) show a poor response to cryotherapy and laser photocoagulation.³⁷ However, hemangioblastomas up to 5 mm without preoperative exudative detachment have been treated successfully with plaque radiotherapy or brachytherapy.^{27,38} The most favorable outcomes were achieved using a combination of brachytherapy and surgical resection of the retinal angiomas, including vasoproliferative tumors.³⁹ Currently, plaque radiotherapy is not available in China; thus, surgical resection is the treatment of choice for large retinal hemangioblastomas that do not respond to photocoagulation and cryotherapy.

Despite endolaser photocoagulation and endodiathermy, the VHL disease–associated retinal hemangioblastomas in the 3 cases still exhibited a loss of heterozygosity at the *VHL* gene locus. Although coagulation might alter the morphology of the excised retinal tissue, it should not affect the *VHL* gene. We previously detected a *VHL* loss of heterozygosity in the retinal scar

of a VHL eye that had already received photocoagulation.^{14,40} Unlike the 3 VHL disease–associated retinal hemangioblastomas, the vasoproliferative tumor revealed normal *VHL* allele, VEGF, and CXCR4 expression. Prominent glial cell proliferation and clusters of small hyalinized vessels and capillaries are hallmarks of vasoproliferative retinal tumors.^{39,41} Small dilated vessels and gliosis were present in case 4, with a complete absence of foamy stromal cells (VHL disease tumor cells).

Overexpression of both CXCR4 protein and transcript was found in the stromal and vascular cells of the retinal hemangioblastomas associated with VHL disease. Chemokine receptors are involved in the trafficking of leukocyte chemoreceptors, including CXCR4. Chemokine receptor–ligand interactions have been implicated in the homing of various subsets of hematopoietic cells to specific anatomical sites.⁴² These interactions also determine the metastatic destination of malignant cells, as seen in breast carcinoma.¹⁸ Breast carcinoma cells express the chemokine receptor CXCR4 and commonly metastasize to organs that are an abundant source of the CXCR4-specific ligand CXCL12. In vivo, neutralizing the interactions of CXCR4/CXCL12 significantly impairs metastasis of breast carcinoma cells to regional lymph nodes and the lung. We also have demonstrated expression of CXCR4 and CXCR5 in the primary intraocular lymphoma cells, along with CXCL12 and CXCL13 expression on the retinal pigment epithelium (RPE). Therefore, the B-cell chemokine system (CXCR4/CXCL12 and CXCR5/CXCL13) may be one of the mechanisms responsible for attracting lymphoma cells to the RPE.⁴³ The expression of B-cell chemokine receptors also has been reported in primary CNS lymphoma cells.⁴⁴

Normal VHL protein is a recognition subunit of an E3 ubiquitin protein ligase complex that targets the α subunits of the DNA-binding transcription factor HIF for ubiquitin-mediated degradation in the presence of oxygen. This process is regulated by oxygen availability and blocked by disease–causing VHL protein mutations.⁴⁵ The gene encoding the G protein–coupled chemokine receptor CXCR4 is reported to be suppressed most strongly by functional VHL protein.¹⁹ Zagzag et al recently reported an overexpression of both CXCR4 and CXCL12 in VHL disease–associated cerebellar hemangioblastoma and renal cell carcinoma.²⁰ These authors suggest that chemotactic influences combined with the proliferative effects of VEGF may represent a powerful angiogenic signal in all tumors associated with VHL disease. Our findings of CXCR4 overexpression and expression of *CXCL12* transcripts in VHL disease–associated retinal hemangioblastomas are in accordance with the previous reports on other VHL disease–associated systemic tumors. In addition, in this study we displayed a positive correlation between the level of VEGF/CXCR4 and disease activity in a VHL disease retinal lesion. However, this association must be confirmed by analyzing additional cases.

Currently, CXCL12/CXCR4 is recognized to play an important and unique role in the homing and trafficking of tissue-specific stem cells expressing CXCR4 on their surface.^{46–48} This occurs during embryo/organogenesis and tissue/organ regeneration.⁴⁹ Functional CXCR4 is expressed on non-hematopoietic tissue-committed stem/progenitor cells such as primordial germ cells, neural stem cells, retinal pigment epithelial progenitors, and murine embryonic stem cells. Thus, CXCL12/CXCR4 emerges as a pivotal regulator in trafficking various types of stem cells in the body. Recently, we described stem cell components in VHL disease–associated retinal and optic nerve hemangioblastomas.¹⁶ Strong CXCR4 and weak CXCL12 expression in these tumors could affect the development, mobilization, and regulation of VHL disease cells within the retina.

Chemokines may have therapeutic potential as an adjuvant or treatment in antitumor immunotherapy.⁵⁰ Strategies aimed at modulating the CXCL12/CXCR4 axis may have clinical implications in regenerative medicine by delivering normal stem cells to desired tissues and organs. This therapy has been used in the clinical oncology field to inhibit metastasis of

neoplastic stem cells.⁵¹ Encouragingly, the first clinical trials in human immunodeficiency virus (HIV)-infected patients using a blocking agent to prevent binding of HIV to CXCR4 have been proven pharmacologically safe.⁵² Antisense oligodeoxynucleotides or double-stranded RNA-mediated interference could serve as downregulators of CXCR4 expression in targeted breast carcinoma cells.⁵³ Therefore, CXCR4/CXCL12 inhibitors should be considered possible novel therapeutic agents in the treatment of VHL disease-associated retinal hemangioblastomas.

In summary, skillful vitreoretinal surgeons are able to excise large isolated retinal hemangioblastomas effectively. The excised tissue can provide important information on the molecular pathology and pathogenesis of diseases such as VHL. von Hippel-Lindau disease-associated retinal hemangioblastomas were shown to express high levels of CXCR4, an important chemokine receptor that regulates the trafficking of various stem cells and certain tumor cells. As with VEGF, elevated levels of CXCR4 result from a mutated VHL protein and correlate to the activity of the retinal lesions found in VHL disease. These findings are in accordance with recently published data showing increased CXCR4 expression in both CNS and renal malignancies.²⁰ We propose that CXCR4 expression, along with VEGF, may play an important role in the development of ocular hemangioblastomas associated with VHL disease. This finding also suggests that anti-CXCR4 therapy may be a useful approach to treating VHL disease-associated hemangioblastomas.

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Figure 1.

Case 1. Clinical photographs illustrating 2 large retinal vascular lesions in the temporal superior and nasal inferior quadrants (**upper left**). **Upper right, lower left, lower right**, The surgical and laser scars healed gradually within 3 months (mon) after the surgery. Histology of the excised tissue shows capillarylike channels and vacuolated cells (stain, hematoxylin–eosin; original magnification, $\times 200$). Pre-op = preoperative.

Figure 2.
 Case 2. Clinical photographs illustrating multiple typical retinal hemangioblastomas, hemorrhages, and lipid exudates in the left eye before (**upper left**) and after (**upper right**) the surgery. **Lower left**, The right eye has tiny retinal hemangioblastomas in the far periphery. **Lower right**, Immunohistochemistry shows positive vascular endothelial growth factor (VEGF) in the excised hemangioblastoma (avidin–biotin–peroxidase complex immunostaining; original magnification, $\times 400$).

Figure 3.

Case 3. Clinical photographs illustrating a large retinal hemangioblastoma with well-defined feeder vessels in the temporal superior retina and macular star before (**upper left, upper middle**) and after (**upper right**) the surgery. Histology shows the tumor admixed with many small vascular channels (**lower left**) (stain, hematoxylin–eosin, original magnification, $\times 100$) and expressed vascular endothelial growth factor (VEGF) (**lower right**) (avidin–biotin–peroxidase complex immunostaining; original magnification, $\times 400$).

Figure 4. Case 4. Clinical photographs illustrating a large vascular lesion without prominent feeder vessels in the superior nasal retina before (**left**) and after (**right**) the surgery. Exudates and subretinal fluid are also observed. Histology shows few dilated vessels in the gliotic retina (stain, hematoxylin-eosin; original magnification, $\times 100$).

Figure 5. CXCR4 is positive in retinal hemangioblastomas of the 3 von Hippel-Lindau disease cases (a–c) but negative in the vasoproliferative retinal tumor case (d). e, f, CXCL12 stains negative in all cases (stain, avidin-biotin immunoperoxidase; original magnification, $\times 100$).

Figure 6.

Loss of heterozygosity of von Hippel-Lindau disease (*VHL*) genes is detected with microsatellite marker D3S1110 in cases 1 to 3 of the von Hippel-Lindau disease cases but not in case 4, with a vasoproliferative retinal tumor. Odd numbered lanes, white blood cells; lanes 2, 4, and 6, retinal hemangioblastoma cells; lane 8, vasoproliferative tumor cells.

Figure 7.

Quantitative reverse transcription polymerase chain reaction shows high levels (folds) of vascular endothelial growth factor (*VEGF*) and *CXCR4* mRNA as well as low levels of *CXCL12* mRNA in cases 1 to 3; in contrast, only low transcript levels of *VEGF* and *CXCR4* are seen in case 4.