# Investigation of vasculogenic mimicry in intracranial hemangiopericytoma

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**Abstract.** Vasculogenic mimicry (VM) has increasingly been recognized as a form of angiogenesis. Previous studies have shown that the existence of VM is associated with poor clinical prognosis in certain malignant tumors. However, whether VM is present and clinically significant in intracranial hemangiopericytoma (HPC) is unknown. The present study was therefore designed to examine the expression of VM in intracranial HPC and its correlation with matrix metalloprotease-2 (MMP-2) and vascular endothelial growth factor (VEGF). A total of 17 intracranial HPC samples, along with complete clinical and pathological data, were collected for our study. Immunohistochemistry was performed to stain tissue sections for CD34, periodic acid-Schiff, VEGF and MMP-2. The levels of VEGF and MMP-2 were compared between tumor samples with and without VM. The results showed that VM existed in 12 of 17 (70.6%) intracranial HPC samples. The presence of VM in tumors was associated with tumor recurrence ($P<0.05$) and expression of MMP-2 ($P<0.05$). However, there was no difference in the expression of VEGF between groups with and without VM.

## Introduction

Hemangiopericytoma (HPC) is a highly cellular and vascularized mesenchymal tumor that was thought to be derived from Zimmerman pericytes, cells surrounding capillaries and postcapillary venules. Although HPC may be found anywhere in the body, common sites of occurrence include the musculoskeletal system and skin (1,2). Intracranial HPC is a rare aggressive malignancy, which accounts for less than 1% of all CNS tumors (3,4). Intracranial HPC was previously described as an angioblastic meningioma. However, the World Health Organization (WHO) currently defines HPC as a 'mesenchymal, non-meningothelial tumor', which exhibits different clinical behaviors, immunohistochemical characteristics and ultrastructural features from meningioma (5).

Angiogenesis is one of the most essential processes required for invasive tumor growth, recurrence and metastasis (6). The mechanisms of tumor angiogenesis include intussusceptive angiogenesis, sprouting angiogenesis, co-opted vasculature, recruitment of endothelial progenitor cells and vasculogenic mimicry (VM).

VM is a recently described pattern of tumor angiogenesis, which differs from normal angiogenesis markedly. Tumors containing VM show such biological behaviors as higher malignancy, non-directional or bi-directional activity, rapid proliferation and high incidence of metastasis by a vascular route (7). VM was first discovered in a human uveal malignant melanoma by Maniotis *et al* in 1999 (8). In recent years, VM has been described in numerous tumors, including ovarian carcinoma (9), melanoma (10), and inflammatory and ductal breast carcinoma (11). VM has been considered to be a marker of poor clinical prognosis due to its close association with more aggressive tumors and increased tumor-related mortality (8).

To the best of our knowledge, whether VM is present and clinically significant in intracranial HPC remains unknown. In this study, we focused on an immunohistochemical analysis of intracranial HPC samples to identify the existence of VM. We then compared the VM status with the clinical data and expression of vascular endothelial growth factor (VEGF) and matrix metalloprotease-2 (MMP-2) to determine whether VM was associated with tumor location, recurrence and expression of VEGF and MMP-2.

## Materials and methods

*Tissue samples*. From 1997 to 2010, 21 paraffin-embedded tissue samples of intracranial HPC were obtained from the Department of Neurosurgery, Provincial Hospital affiliated to Shandong University (Shandong, China). Seventeen of 21 cases with integrated follow-up documents were enrolled in the study. Detailed clinical and pathological data including age, gender, tumor location and recurrence were collected. All

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of the 17 patients had not undergone therapy prior to tumor surgery. Diagnosis of these samples was established by two independent pathologists depending on the clinical and pathological features. Serial 4-$\mu$m sections were obtained from paraffin-embedded tumor tissues, and at least 4 sections were collected for each sample.

*Main reagents*. The primary antibodies used in this study were rabbit monoclonal antiserum raised to human CD34 (dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and mouse monoclonal antibodies raised to VEGF (dilution 1:50; Santa Cruz) and MMP-2 (dilution 1:50; Dako Cytomation, Glostrup, Denmark). These primary antibodies were purchased from Beijing Zhongshan BioTechnologies Co., Ltd. (Beijing, China). The 0.5% periodic acid-Schiff (PAS) solutions were made in the Central Laboratory of the Provincial Hospital affiliated to Shandong University.

*Immunohistochemistry*. Standard immunohistochemical staining was performed on paraffin-embedded tumor tissues for VEGF and MMP-2. For CD34 and PAS double staining, slides were treated with immunohistochemical staining for CD34 and then with 0.5% PAS for 10 min and rinsed with distilled water for 5 min. Sections were then treated with Schiff solution for 15 min avoiding light and finally counterstained with hematoxylin.

Slides were examined by two independent investigators who were blinded to the outcome. VM was defined as the presence of a PAS-positive and CD34-negative vascular-like channel. The channel consisted of tumor cells secreting PAS-positive materials, and the cells lining the channel were negative for CD34, which indicated that they were not endothelial cells. Based on the presence of VM structures, the samples were divided into VM-positive or -negative groups.

*Assessment methods*. VEGF and MMP-2 levels were quantified according to the method described by Mattern *et al* (12). Staining intensity and the percentage of positive cells were measured. At least 10 microscopic fields in one section were observed under x200 magnification, in which positive cells were counted in 100 tumor cells/field, 10 fields in each section. Positive cells were visually evaluated and cell expression was stratified as follows: 0, 0-10% positive cells; 1, 11-30% positive cells; 2, 31-70% positive cells; and 3, 71-100% positive cells. The sum (staining index) of the staining intensity and positive cell scores was used to determine the final result for each section. The number of microvessels was carefully counted in 5 high power fields (x200). The microvessel density (MVD) was calculated as the average vessel counts of these fields.

*Statistical analysis*. The following statistical analysis methods were used: the Fisher's exact test, the Student's t-test and the Mann-Whitney U test for non-normal distributive data. $P<0.05$ was considered to be statistically significant.

## Results

*VM in intracranial HPC*. Twelve of 17 (70.6%) intracranial HPC samples were observed to have VM (Fig. 1). The clinical data of the 17 patients are summarized in Table I. The incidence of VM was significantly higher in patients with recurrence (11/12, 91.7%) than in patients without recurrence (1/5, 20%; $P<0.05$). However, VM incidence did not differ with respect to patient gender, age or tumor location (Table II).

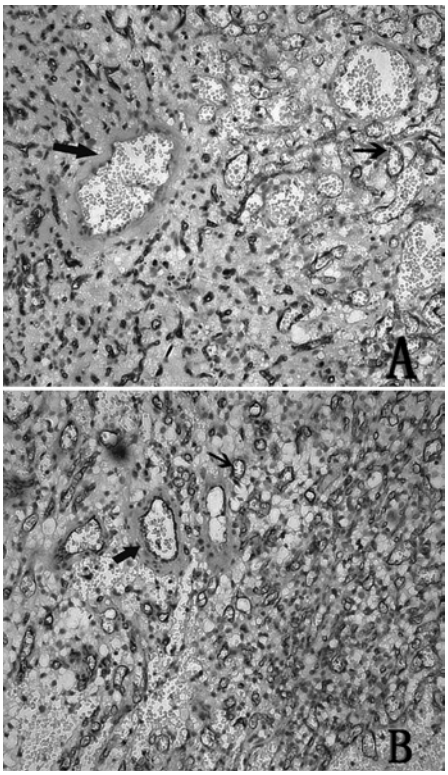


Figure 1. Identification of vasculogenic mimicry (VM) in intracranial hemangiopericytoma (HPC). (A) VM-positive (thick black arrow), the channel was periodic acid Schiff (PAS) positive and CD34 negative; microvessels (thin black arrow) in a lesion with VM. (B) VM-negative (thick black arrow); microvessels (thin black arrow) in a lesion without VM. (CD34/PAS double staining; original magnification, x200). The number of microvessels in A was significantly less than in B, $P<0.01$.

Table I. Clinicopathological data of the 17 patients with intracranial HPC.

| | |
|---|---|
| Gender | |
| Male:female | 10:7 |
| Mean age (years) | 53.6±2.9 |
| Recurrence | |
| Positive:negative | 12:5 |
| Tumor location | |
| Hemisphere:basicranial | 10:7 |

HPC, hemangiopericytoma.

*Correlation between VM and MVD*. Existence of microvessels was observed in VM-positive and -negative tumors. The number of microvessels in VM-positive tumors was significantly less than that in the VM-negative tumors ($P<0.01$; Table III).

*Correlation between VM and expression of VEGF and MMP-2*. VEGF and MMP-2 were expressed in all of the intra-

Table II. Relationship of VM to clinicopathological data.

| Factor | VM | | P-value[a] |
|---|---|---|---|
| | Positive | Negative | |
| Gender | | | |
| Male | 7 | 3 | 0.407 |
| Female | 5 | 2 | |
| Age (years) | | | |
| ≥50 | 6 | 3 | 0.380 |
| <50 | 6 | 2 | |
| Recurrence | | | |
| Positive | 11 | 1 | 0.010 |
| Negative | 1 | 4 | |
| Tumor location | | | |
| Hemisphere | 7 | 3 | 0.407 |
| Basicranial | 5 | 2 | |

VM, vasculogenic mimicry. [a]Fisher's exact test.

Table III. Differences among MVD, expression of VEGF and MMP-2 between the VM and non-VM groups.

| Stain | Tissue samples | | t/Z | P-value |
|---|---|---|---|---|
| | VM | Non-VM | | |
| MVD | 39.38±2.63 | 55.60±3.67 | -3.68 | 0.004 |
| MMP-2 | 5.68±0.41 | 3.14±0.26 | -2.95 | 0.003 |
| VEGF | 2.85±0.31 | 3.18±0.28 | -0.58 | 0.561 |

MVD, microvessel density; VEGF, vascular endothelial growth factor; MMP-2, matrix metalloprotease-2; VM, vasculogenic mimicry. Student's t-test for MVD; Mann-Whitney U test for MMP-2 and VEGF.

cranial HPC samples. The expression of MMP-2 was greater in the VM-positive than in the -negative samples (P<0.01; Fig. 2, Table III). There was no significant difference in the expression of VEGF between the two groups (P=0.561, Table III).

## Discussion

VM is a recently discovered pattern of tumor angiogenesis, which has been described as a marker of poor clinical prognosis in tumors. Our study provides the first evidence of VM in human intracranial HPC. Based on CD34 and PAS staining, the incidence of VM was observed to be significantly higher in patients with recurrence compared to those without recurrence. However, the number of microvessels was significantly lower in tumors with VM compared to tumors without VM. Such conditions indicate that VM may be a 'trick' pattern, which supplies enough nutrients and oxygen to tumor cells when they grow rapidly and lack food supplied by blood vessels (13). This is one possible reason to explain the fact that although MVD was decreased in tumors with VM compared to tumors without VM, no hemorrhage, necrosis or inflammatory cell infiltration was observed near these structures (14). Due to the presence of blood flow in VM and the VM-angiogenesis junction in the tumor (15), tumor cells are in direct contact with the bloodstream, and recurrence and metastasis are more likely to occur. These retrospective findings suggest that the existence of VM could have an unfavorable prognostic value in intracranial HPC.

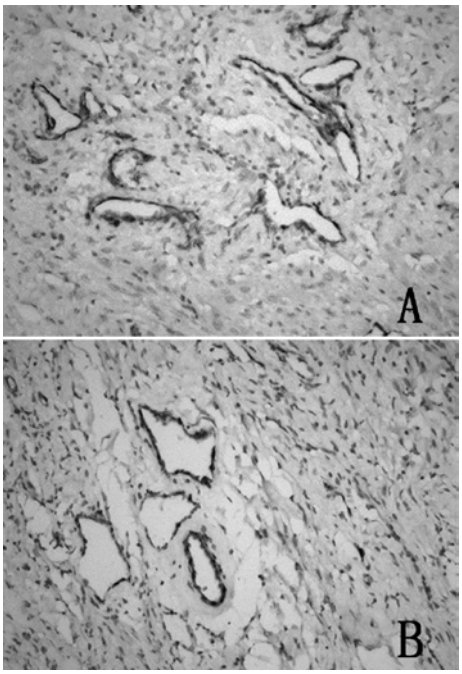


Figure 2. Expression of matrix metalloprotease-2 (MMP-2) in intracranial hemangiopericytoma (HPC). (A) Sample from the vasculogenic mimicry (VM)-positive group. (B) Sample from the VM-negative group. The positive staining range and the staining intensity in A were higher compared to those in B; P<0.01. (MMP-2 immunohistochemical staining; original magnification, x200).

Remodeling of the extracellular matrix (ECM) is one of the most significant factors governing VM channel formation. ECM remodeling provides the space required for VM and is associated with MMP (particularly MMP-2) secretion by tumor cells (9,16). Previous studies have suggested that MMP-2 protein contributes to the VM formation in melanomas (17,18). In the present study, the expression level of MMP-2 in the VM-positive group was significantly higher than that in the non-VM group, which demonstrated that a high level of expression of MMP-2 contributes to VM formation.

Although VEGF protein plays the most significant role in the process of angiogenesis inducing endothelial cell migration and vascular permeability (19), there were no significant associations observed in previous studies between VM formation and the expression of EGFR or the endothelial cell markers VEGF and CD31 (20). In this study, we found that there was no significant difference in VEGF expression between the VM-positive and -negative groups. These results demonstrate that VM is a different type of blood supplying model and is not dependent on the expression of VEGF in intracranial HPC.

In conclusion, we observed the presence of VM in intracranial HPC in this pilot study, which proved to be an unfavorable sign of prognosis. Moreover, we also provided evidence that

the formation of VM in intracranial HPC is associated with the expression of MMP-2. These findings may be significant in understanding the angiogenesis patterns in intracranial HPC and pose a major challenge to anti-angiogenesis treatment modalities. However, this study had a relatively small sample size as a result of the low incidence of intracranial HPC. For this reason, further studies containing a larger sample size are required to investigate the VM pattern in intracranial HPC.

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