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Laboratory Investigation

Possible pathophysiological role of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) in metastatic brain tumor-associated intracerebral hemorrhage

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Summary

Background: Intratumoral hemorrhage, as one of the cerebrovascular complications in various tumor-related conditions, occurs mainly in malignant brain tumors. Recent studies have shown that the overexpression of vascular endothelial growth factor (VEGF) and metalloproteinase (MMP) may play a role for the loss of vascular integrity and the subsequent hemorrhage in several instances, in addition to their well-known properties in tumor development and metastasis.

Methods: To investigate the potential role of VEGF and MMP in hemorrhagic complication of metastatic brain tumor, we estimated the expression of VEGF, MMP-2 & -9 by immunohistochemical studies in pathological specimens of metastatic brain tumors obtained from 16 patients, 7 in hemorrhagic and 9 in non-hemorrhagic group. We also examined the expression of collagen type IV, CD34, Factor VIII in order to evaluate the status of tumor vasculature.

Results: Patients in hemorrhagic group showed a higher VEGF expression with neovascularization than those in non-hemorrhagic group. The basement membranes of newly formed vessels were disrupted in cases with high expression in both MMP-2 and -9. These results indicate that rapid growing nascent blood vessels, responding vigorously to VEGF, are concentrated around the hemorrhagic tumors. Besides, these results suggest a possibility that the basement membranes of these nascent vessels could be disrupted proteolytically by MMP.

Conclusion: We conclude that overexpression of VEGF and MMP may play a role in metastatic brain tumor-associated hemorrhage. Presumably, the underlying pathophysiological mechanisms are through rapid growth and breakdown of vessels around the tumors caused by overexpression of VEGF and MMP of tumor cells.

Introduction

Spontaneous intracranial hemorrhage occurs in 3.9% of all brain tumors, mostly metastatic tumors or malignant gliomas [1]. Although hemorrhage from metastatic brain tumors has often been observed, the pathogenesis of intracerebral hemorrhagic occurrence in metastatic brain tumor has not been fully understood.

It has well known that vascular endothelial growth factor (VEGF), also called vascular permeability factor, plays an important role in developmental, physiological, and pathological neovasculization. Overexpression of VEGF in tumor cells enhances tumor growth and metastasis by stimulating neovasculization, thereby increasing microvessel density [2–4]. Previous animal model studies have shown that the fine structure of newly formed blood vessels induced by VEGF tend to be permeable [5] and fragile to cause hemorrhagic event [2]. Recent studies have also shown that the level of VEGF expression is correlated with a propensity for hemorrhage in other tumor cases [6–8]. Hemorrhage is also apparent in cerebral infarctions that often developed from ischemic stroke [9]. The fact that brain ischemia induces increased VEGF expression [10] and hemorrhagic transformation usually occurs within 2–3 days after an ischemic stroke suggests that two events, increased VEGF expression and hemorrhagic transformation may be mechanistically related.

Metalloproteinases (MMPs), a family of proteolytic enzymes, degrade extracellular matrix (ECM) proteins, cell surface molecules, and other pericellular substances. In cancer development and metastasis, tumor cells produce these enzymes that destroy the matrix barriers surrounding the tumors, thereby permitting invasion into surrounding connective tissues, and metastasis to distant organs [11]. Excessive degradation of the vascular matrix by MMPs in various aneurysmal diseases results in the destabilization of vessels, which potentially leads to weakening of the vessel wall, passive dilatation, and rupture [12–17]. Abnormal balance between MMP-9 and TIMPs may contribute to vascular instability of brain AVMs [18]. MMPs may play a primary role in basal lamina degradation, partly responsible for the loss of vascular integrity during focal cerebral ischemia

[19–24]. Hemorrhagic MMPs, isolated from the venom, degrade ECM proteins which maintain basement membrane structural/functional integrity, and lead to the loss of capillary integrity resulting in hemorrhage [25].

Although some studies reported that the expression of VEGF and MMP may reveal a possible explanation of hemorrhagic event in some other conditions [26,27], the role of these factors in metastatic brain tumor-associated hemorrhage has not been fully understood due to the lack of relevant data. In this study, we investigated the relationship between the expression level of VEGF and MMP-2 & -9 and the occurrence of hemorrhage in metastatic tumor specimens using immunohistochemistry. We also analyzed the correlation between their expressions and abnormalities in vascular structure of tumor specimens.

Materials and methods

Specimen selection and preparation of tissue sections

Seven hemorrhagic (Group 1; hemorrhagic brain metastases) and 9 non-hemorrhagic (Group 2; non-hemorrhagic brain metastases) metastatic brain tumors were selected from a pool of paraffinized tumor specimens from patients who underwent surgery at Chonnam National University Hospital in previous 2 years. To assess hemorrhagic events more accurately, we classified those tumors with overt hemorrhagic evidence on radiological studies as hemorrhagic (Figure 1).

In all selected patients, the metastatic tumors were completely removed. The specimens obtained by surgical resection were fixed in 4% buffered paraformaldehyde and embedded in paraffin, and 5 μm-thick sections were cut. To confirm hemorrhagic changes in metastatic tumor specimens, paraffin sections were stained with hematoxylin and eosin. All specimens were stained immunohistochemically with antibodies for VEGF and MMP-2 & -9. We also examined the expression of collagen type IV, CD 34, Factor VIII for evaluation of the status of tumor vasculature. Only central area of specimen was examined in order to eliminate the potential environmental effects.

Immunohistochemistry

Immunohistochemical stains for VEGF (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), MMP-2 & -9 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), collagen type IV (Zymed Laboratoies Inc., San Francisco, CA, USA), CD 34 (Dako, Denmark), Factor VIII (Dako, Denmark) were performed by standard immunohistochemical staining techniques. The paraffin-embedded sections of metastatic brain tumor tissues were deparaffinized and incubated in Pepsin solution (Research Genetics, Huntsville, AL, USA) for 3 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in methanol, and non-specific

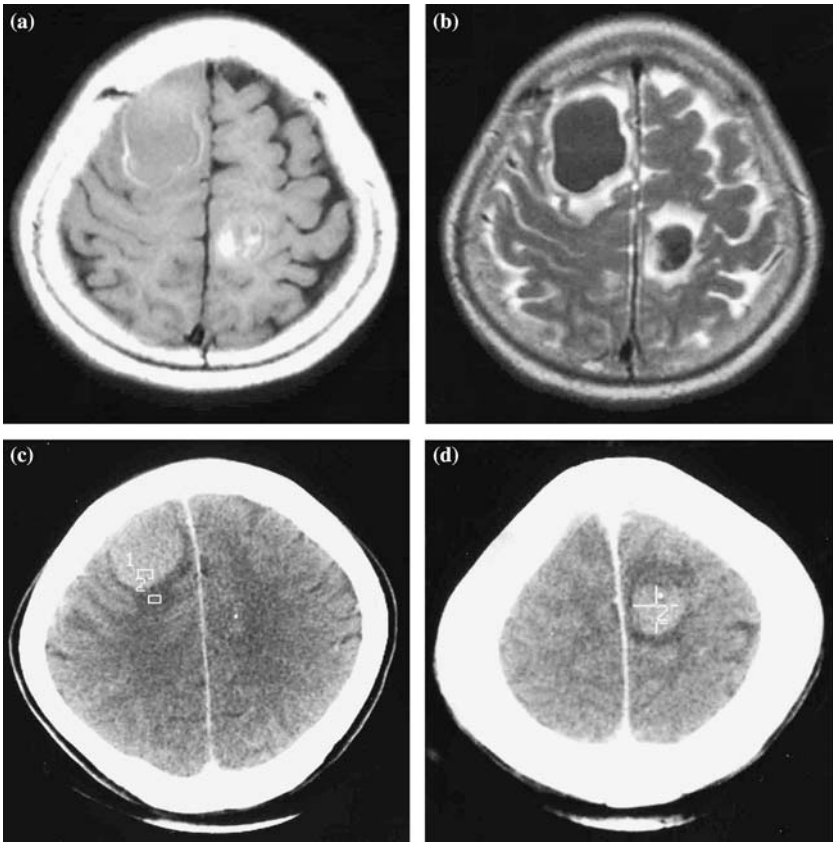

Figure 1. Radiological findings of multiple hemorrhagic brain metastases in case 6 (a & b; Brain MRI, c & d; Brain CT). The simultaneous occurrence of hemorrhagic events can lead to assume that some metastatic brain tumors have a tendency to bleed as basic characteristics.

binding sites were blocked with normal goat serum for 15 min at room temperature. A primary polyclonal anti-VEGF antibody was then added at a previously determined optimum dilution of 1 : 200 and allowed to incubate at 45 °C for 15 min. Biotin-labeled secondary antibody (GAR-HRP, Sigma, St. Lois, MO, USA) was utilized at 45 °C for 7 min. The streptavidin-horseradish peroxidase (Zymed Laboratoies Inc., San Francisco, CA, USA) detection system was then applied to capillary channels, followed by 7 min of incubation at 45 °C. After drainage, the tissue sections were ready for chromogen reaction with 3-amino-9-ethyl carazole (AEC). Counterstaining was performed with Harris-hematoxylin. Control experiments were performed on all specimens at the time of immunostains using only secondary antibody. Immunostaining for other factors were performed in similar way above mentioned.

Microscopic examination of all specimens was conducted by two investigators simultaneously without prior knowledge of final pathological diagnosis or radiological findings. For VEGF and MMPs, the immunoreaction of the cytoplasm of tumor cells, endothelium and/or the perivascular tissue was judged. Immunohistochemical data were described semiquantitatively; no staining was recorded as −; very weak staining as ±; weak, patchy staining as +; strong, patchy staining as ++; strong, diffuse staining as +++.

Statistical analysis

Mann–Whitney *U* test was used to determine the significance of the difference in the level of expression of MMPs and VEGF between hemorrhagic and non-hemorrhagic groups. The difference was considered statistically significant when $P < 0.05$.

Results

Clinical and immunohistochemical profiles of the patients under study are summarized such as pathological diagnosis, primary origin site, presence or absence of hemorrhage, the presence of basement membrane disruption, VEGF expression, and MMP-2 & -9 expressions (Table 1). The mean age of group 1 (Hemorrhagic group) was 61 years (four men and three women) with origin sites of lung (three patients), uterus (2), thyroid (1), and skin (1). Histopathologic diagnosis was adenocarcinoma (1), large cell carcinoma (1), choriocarcinoma (2), papillary carcinoma (1), small cell carcinoma (1), and melanoma (1). Non-hemorrhagic group (Group 2) with mean age of 60 years (five men and four women) was developed from lung (5), gastrointestinal tract (2), breast (1), and unknown origin (1) at the time of surgery. Histopathological diagnosis was adenocarcinoma (7), squamous carcinoma (1), and infiltrating ductal carcinoma (1).

Two tumors in group 1 exhibited strong & diffuse staining pattern of VEGF and the remaining tumors showed strong patchy pattern. In group 2, all specimens except one case were stained with weak expression. The level of VEGF expression in hemorrhagic group tended to be higher than that in non-hemorrhagic group ($P < 0.005$). Among seven specimens in group 1, one case expressed strong & diffuse staining pattern of MMP-2 and three cases showed strong patchy pattern, whereas all specimens except one case in group 2 demonstrated weak expression. Furthermore, in immunohistochemical study for MMP-9, only three cases in group 2 exhibited weak staining pattern and the remaining tumors showed negative, contrary to group 1 showing similar patterns found in MMP-2 expressions. There was also higher expression of MMP-2 ($P < 0.05$) and MMP-9 ($P < 0.005$) in the hemorrhagic group than in the non-hemorrhagic group.

To analyze the integrity of blood vessels in these specimens, we used several antibodies against the endothelial cell, collagen type IV, CD 34, and Factor VIII. Apart from the presence of hemorrhagic change, numerous newly formed blood vessels were stained in VEGF overexpressing specimens (Figure 2, 3). However, the breakdown of newly formed nascent vessels was observed in hemorrhagic case that showed both VEGF

Table 1. Summary of clinical data and immunohistochemical study

No	Age/sex	Origin	Tumor bleeding	Biopsy	BM breakdown	VEGF	MMP-2	MMP-9
1	73/M	Lung	Y	Adenoca	+	++	++	++
2	55/M	Lung	Y	Small cell ca	−	++	+	±
3	53/F	Uterus	Y	Chorioca	−	++	+	+
4	32/F	Uterus	Y	Chorioca	−	++	±	±
5	66/M	Thyroid	Y	Papillary ca	−	++	++	+
6	69/F	Lung	Y	Large cell ca	+++	+++	+++	+++
7	79/M	Skin	Y	Melanoma	+	+++	++	++
8	74/F	GI	N	Adenoca	−	+	+	±
9	38/F	Breast	N	Infiltrating ductal ca	−	±	+	−
10	60/M	Lung	N	Squamous cell ca	−	±	+	−
11	72/M	GI	N	Adenoca	−	+	++	+
12	72/M	−*	N	Adenoca	−	+	+	−
13	54/F	Lung	N	Adenoca	−	++	±	−
14	61/M	Lung	N	Adenoca	−	+	+	−
15	65/M	Lung	N	Adenoca	−	±	+	−
16	49/F	Lung	N	Adenoca	−	+	±	±

−* Unknown primary origin, BM; Basement membrane, VEGF; Vascular endothelial growth factor, MMP; Matrix metalloproteinase.

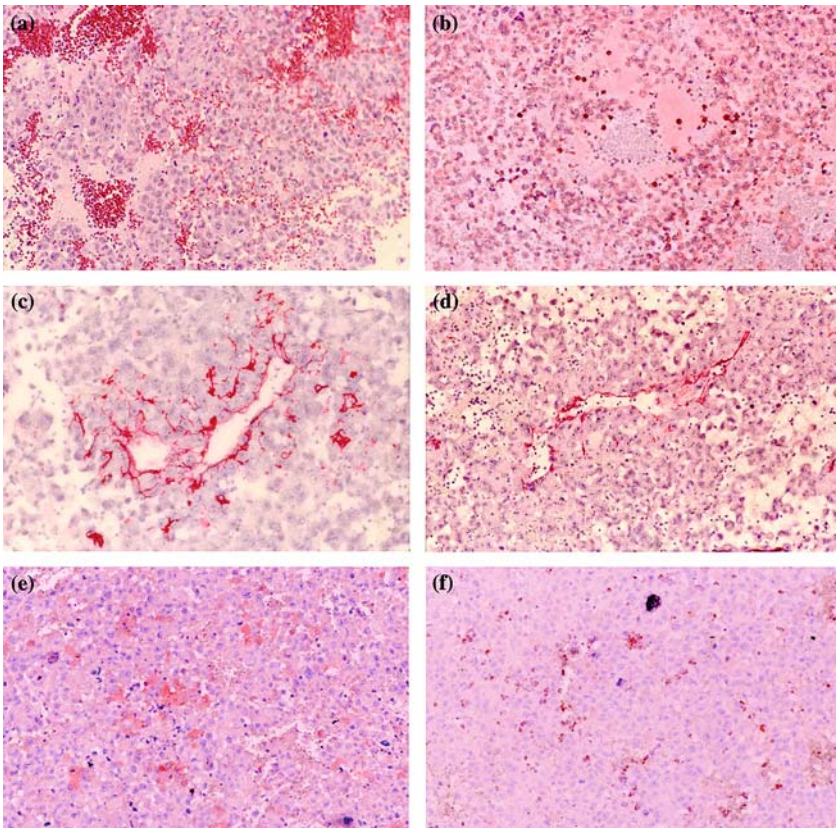

Figure 2. Photomicrographs of immunohistochemical study in case 6. (a; H&E, b; Immunostaining for VEGF, c; for Collagen type IV, d; for Factor VIII, e; for MMP-2, f; for MMP-9, Original magnification; a: ×40, b–f: ×200). Note that hemorrhagic metastatic brain tumor with overexpression of VEGF and MMP-2 & -9 demonstrates disruption of basement membrane in newly formed nascent vessels.

and MMP-2 & -9 overexpressing specimens (Figure 2). The structures of nascent vessels in cases of only VEGF overexpresion were not disrupted (Figure 3). Taken together, these results indicate that rapid growing nascent blood vessels which respond vigorously to VEGF are concentrated around the hemorrhagic tumors during the development of hemorrhagic change in metastatic brain tumor. Additionally, the basement membranes of these fragile vessels could be disrupted proteolytically by MMP.

Discussion

Hemorrhage into metastatic brain tumor is commonly reported. According to one study, 50% of metastatic malignant melanoma patients showed intratumoral bleeding, while adenocarcinoma, squamous carcinoma, and anaplastic carcinoma patients showed significant lower bleeding rates [28]. Previous studies have also shown that in order to produce brain metastases, tumor cells must satisfy the following steps; (1) reach the vasculature of the brain, (2) attach to the endothelial cells of microvasculature, (3) extravasate into the parenchyma, (4) proliferate and induce the formation of new blood vessels [29].

The growth and spread of neoplasm depends on the establishment of an adequate blood supply. VEGF stimulates the proliferation and migration of endothelial cells and induces the expression of MMPs and plasminogen activity [30–33]. Accumulated evidence suggests that VEGF may be a major factor in hemorrhagic pathologic processes, in addition to its playing pivotal role in angiogenesis in tumor growth. For example, levels of VEGF were increased in hemorrhagic pleural fluids of patients with lung cancer [6], and in ocular fluids of patients with proliferative diabetic retinopathy, which often led to vitreous hemorrhage [34]. Bloody ascites due to malignant murine tumors contained a higher concentration of VEGF than non-bloody ascites, and anti-VEGF antibody treatment effectively reduced the leakage of erythrocytes into ascites [7]. Similarly, those cases of hemorrhage associated with perfusion of the cerebral vasculature after ischemic stroke reveal a significant role of VEGF in developing of hemorrhagic infarction [9,10]. Another example supporting the association of VEGF expression with hemorrhage comes from hemangioblastoma in von Hippel-Lindau patient [8]. Thus, VEGF expression is correlated with a propensity for hemorrhage in various pathologic conditions.

Although the molecular mechanism by which VEGF mediates the development of these hemorrhagic changes has remained obscure, VEGF-overexpressing glioblastoma cells implanted into mouse brain indicated that numerous nascent blood vessels and blood cells or substances were concentrated around the hemorrhagic tumors [2]. That is, during the development of such hemorrhage, the brain vasculature experiences rapid growth and breakdown of newly formed vessels as a result of response to VEGF. Cao et al. [5] also reported that VEGF stimulated formation of disorganized and nascent vasculatures that resulted in high permeability of blood vessels.

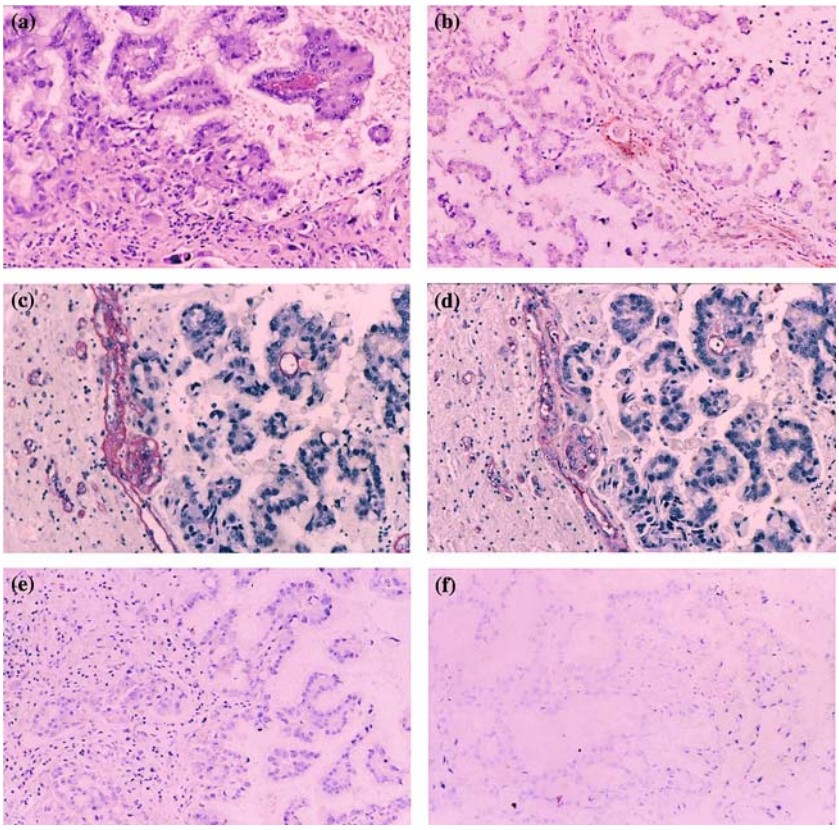

Figure 3. Photomicrographs of immunohistochemical study in case 13. (a; H&E, b; Immunostaining for VEGF, c; for Collagen type IV, d; for Factor VIII, e; for MMP-2, f; for MMP-9, Original magnification; ×200). Note that non-hemorrhagic metastatic brain tumor with only overexpression of VEGF demonstrates newly formed vessels without disruption of basement membrane.

The MMP family, initially classified as zinc-dependent proteinases capable of digesting the various structural components of the ECM, are generally expressed at high levels when tissues undergo proteolytic remodeling, such as in inflammation, wound healing, and cancer [11]. MMP-2 and MMP-9 specifically attack type IV collagen, laminin, and fibronectin, the major components of the basal lamina around cerebral blood vessels [35]. As a result of degradation of ECM components of the basal lamina, MMP may play a major role in loss of microvascular integrity leading to hemorrhagic transformation and secondary brain edema after ischemic and hemorrhagic brain injury [21,23,36]. The role of MMP expression in destruction of vascular integrity has been established since elevated serum level [13,14,17] and localized expression [12,15,18] of MMP contribute to degradation of EMC, leading to weakening of the vessel wall, passive dilatation, and rupture in various aneurysmal diseases and AVMs.

Some previous studies suggested that the expression of VEGF and MMP might be related to hemorrhagic event in some conditions. In polycystic kidney disease, neovasculization induced by VEGF and MMP may result in cyst expansion and aneurysmal formation responsible for the renal bleeding [26]. A case report for ovarian hemorrhagic leiomyosarcoma revealed positive staining for these factors [27].

Considering all these experimental evidence, it could be concluded that MMP and VEGF may have a role for the loss of vascular integrity and the onset of hemorrhage in several circumstances. In our study, we demonstrated that hemorrhagic metastatic brain tumors expressed significantly higher levels of VEGF and MMP-2 & -9 compared with non-hemorrhagic group. Although all of hemorrhagic group were not showing disruption of the basement membrane on the pathologic tissue specimen, the basement membranes were disrupted in cases with high expression in both MMP-2 & -9. These data may indicate that rapid growing nascent blood vessels, responding vigorously to VEGF, are concentrated around the hemorrhagic tumors. Additionally, the basement membranes of these fragile vessels could be disrupted proteolytically by MMP. The present study postulated MMP and VEGF as possible critical factors in metastatic brain tumor associated hemorrhage. The functional association between the overexpression of MMP and VEGF could not be investigated in our study. However, considering evidence that MMP may be direct transcriptional target of VEGF [33,37], anti-VEGF therapy can be implicated in prevention of hemorrhagic occurrence in metastatic brain tumor by reducing angiogenic property of VEGF, and by reducing MMP expression partly.

Conclusion

Abnormally high expression of VEGF and MMP detected in hemorrhagic brain metastases may lead to, in part, the structural instability of newly formed tumor vessels.

We conclude that overexpression of VEGF and MMP may play a role in metastatic brain tumor-associated hemorrhage. Our findings suggest that rapid growth and breakdown of vessels caused by overexpression of VEGF and MMP may be postulated as a possible mechanism in hemorrhagic change of metastatic brain tumor, although further studies should be required in a large number of patients.

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References

1. Salcman M: Intracranial hemorrhage caused by brain tumor. In: Kaufman HH (eds) Intracerebral Hematoma. Raven Press, New York, pp 95–106
2. Cheng SY, Nagane M, Huang HS, Cavenee WK: Intracerebral tumor-associated hemorrhage caused by overexpression of the vascular endothelial growth factor isoforms VEGF121 and VEGF165 but not VEGF189. Proc Natl Acad Sci USA 94: 12081–12087, 1997
3. Claffey KP, Brown LF, del Aguila LF, Tognazzi K, Yeo KT, Manseau EJ, Dvorak HF: Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res 56: 172–181, 1996
4. Oku T, Tjuvajev JG, Miyagawa T, Sasajima T, Joshi A, Joshi R, Finn R, Claffey KP, Blasberg RG: Tumor growth modulation by sense and antisense vascular endothelial growth factor gene expression: effects on angiogenesis, vascular permeability, blood volume, blood flow. Fluorodeoxyglucose uptake, and proliferation of human melanoma intracerebral xenografts. Cancer Res 58: 4185–4192, 1998
5. Cao R, Eriksson A, Kubo H, Alitalo K, Cao Y, Thyberg J: Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogesis, lymphangiogenesis, vascular fenestration, and permeability. Circ Res 94: 664–670, 2004
6. Ishimoto O, Saijo Y, Narumi K, Kimura Y, Ebina M, Matsubara N, Asou N, Nakai Y, Nukiwa T: High level of vascular endothelial growth factor in hemorrhagic pleural effusion of cancer. Oncology 63: 70–75, 2002
7. Luo JC, Toyoda M, Shibuya M: Differential inhibition of fluid accumulation and tumor growth in two mouse ascites tumors by an anti-vascular endothelial growth factor/permeability factor neutralizing antibody. Cancer Res 58: 2594–2600, 1998
8. Wizigmann-Voos S, Plate KH: Pathology, genetics and cell biology of hemangioblastomas. Histol Histopathol 11: 1049–1061, 1996
9. Schehr RS: New treatments for acute stroke. Nat Biotechnol 14: 1549–1554, 1996
10. Kovacs Z, Ikezaki K, Samoto K, Inamura T, Fukui M: VEGF and flt: Expression time kinetics in rat brain infarct. Stroke 27: 1865–1872, 1996
11. Coussens LM, Fingleton B, Matrisian LM: Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 295: 2387–2392, 2002
12. Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, Sicard GA, Thompson RW: Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. J Vasc Surg 31: 325–342, 2000
13. Gaetani P, Rodriguezy Baena R, Tartara F, Messina AL, Tancioni F, Schiavo R, Grazioli V: Metalloproteases and intracranial vascular lesions. Neurol Res 21: 385–390, 1999
14. Goodall S, Crowther M, Hemingway DM, Bell PR, Thompson MM: biquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms. Circulation 104: 304–309, 2001
15. Kim SC, Singh M, Huang J, Prestigiacomo CJ, Winfree CJ, Solomon RA, Connolly ES, Jr: Matrix metalloproteinase-9 in cerebral aneurysms. Neurosurgery 41: 642–666, 1997
16. Knox JB, Sukhova GK, Whittemore AD, Libby P: Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. Circulation 95: 205–212, 1997
17. Todor DR, Lewis I, Bruno G, Chyatte D: Identification of a serum gelatinase associated with the occurrence of cerebral aneurysms as pro-matrix metalloproteinase-2. Stroke 29: 1580–1583, 1998
18. Hashimoto T, Wen G, Lawton MT, Boudreau NJ, Bollen AW, Yang GY, Barbaro NM, Higashida RT, Dowd CF, Halbach VV, Young WL: University of California, San Francisco BAVM Study Group: abnormal expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in brain arteriovenous malformations. Stroke 34: 925–931, 2003
19. Asahi M, Asahi K, Jung JC, del Zoppo GJ, Fini ME, Lo EH: Role of matrix metalloproteinase 9 after focal cerbral ischemia: effects of gene knockout and enzyme inhibition with BB-94. J Cereb Blood Flow Metab 20: 1681–1689, 2000
20. Clark AW, Krekoski CA, Bou SS, Chapman KR, Edwards DR: Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. Neurosci Lett 238: 53–56, 1997
21. Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ: Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab 19: 624–633, 1999
22. Montaner J, Alvarez-Sabin J, Molina C, Angles A, Abilleira S, Arenillas J, Gonzalez MA, Monasterio J: Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 32: 1759–1766, 2001
23. Montaner J, Alvarez-Sabin J, Molina CA, Angles A, Abilleira S, Arenillas J, Monasterio J: Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 32: 2762–2767, 2001
24. Rosenberg GA, Navratil M, Barone F, Feuerstein G: Proteolytic cascade enzymes increase in focal cerebral ischemia in rat. J Cereb Blood Flow Metab 16: 360–366, 1996
25. Baramova EN, Shannon JD, Bjarnason JB, Fox JW: Degradation of extracellular matrix proteins by hemorrhagic metalloproteinases. Arch Biochem Biophys Nov 15: 63–71, 1989
26. Bello-Reuss E, Holubec K, Rajaraman S: Angiogenesis in autosomal-dominant polycystic kidney disease. Kidney Int 60: 37–45, 2001
27. Mayerhofer K, Lozanov P, Bodner K, Bodner-Adler B, Mayerhofer-Gallenbacher N, Hudelist G, Czerwenka K: Immunohistochemical analysis of a primary ovarian leiomyosarcoma. Case report. Anticancer Res 23: 3433–3436, 2003
28. Kondziolka D, Bernstein M, Resch L, Tator CH, Fleming JF, Vanderlinden RG, Schutz H: Significance of hemorrhage into brain tumors: clinicopathological study. J Neurosurg 67: 852–857, 1987
29. Fidler IJ, Yano S, Zhang RD, Fujimaki T, Bucana CD: The seed and soil hypithesis: vasculization and brain metastases. Lancet Oncol 3: 53–57, 2002
30. Ferrara N, Henzel WJ: Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 161: 851–859, 1989
31. Kumar R, Yoneda J, Bucana CD, Fidler IJ: Regulation of distinct steps of angiogenesis by different angiogenic molecules. Int J Oncol 12: 749–757, 1998
32. Pepper MS, Ferrara N, Orci L, Montesano R: Vascular endothelial growth factor (VEGF) induces plasminogen activators and

plasminogen activator inhibitor-1 in microvascular endothelial cells. Biochem Biophys Res Commun 181: 902–906, 1991
33. Unemori EN, Ferrara N, Bauer EA, Amento EP: Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. J Cell Physiol 153: 557–562, 1992
34. Aiello LP, Avery RL, Arrigg PG, Keyt BA, Jampel HD, Shah ST, Pasquale LR, Thieme H, Iwamoto MA, Park JE, Nguyen HV, Aiello LM, Ferrara N, King GL: Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 331: 1480–1487, 1994
35. Mun-Bryce S, Rosenberg GA: Matrix metalloproteinases in cerebrovascular disease. J Cereb Blood Flow Metab 18: 1163–1172, 1998
36. Rosenberg GA: Matrix metalloproteinases in brain injury. J Neurotrauma 12: 833–842, 1995
37. Lamoreaux WJ, Fitzgerald ME, Reiner A, Hasty KA, Charles ST: Vascular endothelial growth factor increases release of gelatinase A and decreases release of tissue inhibitor of metalloproteinases by microvascular endothelial cells in vitro. Microvasc Res 55: 29–42, 1998

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