Histol Histopathol (2002) 17: 301-321

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Angiogenesis in the central nervous system: a role for vascular endothelial growth factor/vascular permeability factor and tenascin-C. Common molecular effectors in cerebral neoplastic and non-neoplastic "angiogenic diseases"

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Summary. Human pathological conditions of the central nervous system (CNS) associated with angiogenesis (i.e. neovascularization) include neoplastic, as well as infectious, ischemic, and traumatic processes. Upregulation of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and tenascin-C (TN-C) is spatially and temporally related to neovascularization. Spatially, VEGF/VPF and TN-C are both found at the site of neovascularization, but they are not detected in areas of normal brain or in areas without neovascularization. Temporally, VEGF/VPF and TN-C are found at the peak of angiogenesis and are not detected when angiogenesis had ceased.

Key words: Angiogenesis, Central nervous system, Tenascin, Vascular endothelial growth factor/vascular permeability factor

Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF)

Angiogenesis, i.e. neovascularization, is a complex biological process whose regulatory mechanisms are not completely understood. Formation of new vessels occurs not only during embryogenesis, wound healing and regeneration, but also in pathological processes, e.g. neoplasia, diabetic retinopathy and arthritis (Folkman, 1995; Yancopoulos et al., 1998). VEGF/VPF (Senger et al., 1983; Nicosia, 1998) is a hypoxia-inducible, (Shweiki et al., 1992) secreted endothelial cell mitogen, (Nicosia, 1998) which has been shown to increase microvascular permeability and endothelial fenestration (Senger et al., 1983; Roberts and Palade, 1995). This ~45 kDa heparin-binding glycoprotein dimer contains two subunits of equivalent mass and is structurally homologous to platelet-derived growth factor (Nicosia, 1998). Of the four different isoforms arising from alternative mRNA splicing (VEGF/$VPF_{121, 165,189,206}$), $VEGF/VPF_{165}$ is predominantly expressed (Ferrara et al., 1991). The shorter forms are diffusible whereas the longer ones are bound to the extracellular matrix (ECM) (Ferrara et al., 1991). VEGF/VPF is secreted by a variety of cell types and is angiogenic *in vivo* (Ferrara et al., 1991; Claffey and Robinson, 1996; Nicosia, 1998). Although VEGF/VPF is thought to be a specific endothelial cell mitogen, receptors for VEGF/VPF have been demonstrated on smooth muscle cells (Brown et al., 1997). The two human VEGF/VPF receptors: flt-1/VEGFR-1 (De Vries et al., 1992) and KDR/VEGFR-2 (Terman et al., 1992) are widely distributed on endothelial cells (Millauer et al., 1993) while VEGFR-3 is specifically distributed on lymphatic endothelial cells (Jeltsch et al., 1997). Moreover, a novel receptor that binds $VEGF/VPF_{165}$ but not $VEGF/VPF_{121}$ was described and found to be identical to human neuropilin-1, a receptor for the collapsin/semaphorin family that mediates neuronal cell guidance (Soker et al., 1998). VEGF/VPF currently appears to be the principal mediator and a potent inducer of angiogenesis during normal physiological processes such as vascular development (Nicosia, 1988; Ferrara et al., 1991; Plate et al., 1992; Jakeman et al., 1993; Millauer et al., 1993; Breier et al., 1995; Zagzag, 1995; Claffey and Robinson, 1996; Brown et al., 1997; Nicosia, 1998) and in

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inflammatory and neoplastic pathologies outside the CNS (Jakeman et al., 1993; Millauer et al., 1993; Breier et al., 1995; Dvorak et al., 1995). Hypoxia-inducible factor-1 (HIF-1), a heterodimeric basic-helix-loop-helix-PAS (bHLH-PAS) transcription factor composed of HIF-1α and HIF-1ß subunits, plays an essential role in oxygen homeostasis (Wang and Semenza, 1995; Wang et al., 1995; Iyer et al., 1998). HIF-1ß, which is also known as the aryl hydrocarbon receptor nuclear translocator (Hoffman et al., 1991), can dimerize with several different bHLH-PAS transcription factors. In contrast the HIF-1α subunit is unique to HIF-1. Its expression increases as cellular O_2 concentration decreases, and determines the level of HIF-1 activity (Wang et al., 1995; Jiang et al., 1996; Semenza et al., 1996). HIF-1 activates a large battery of genes whose protein products function either to increase O_2 availability or to allow metabolic adaptation to O_2 deprivation (Semenza, 1998). Included among these are genes encoding VEGF/VPF erythropoietin, glucose transporters, glycolytic enzymes, insulin-like growth factor 2 (IGF2), and IGF binding proteins-1,-2, and -3 (Semenza et al., 1996; Iyer et al., 1998; Semenza, 1998; Tazuke et al., 1998; Feldser et al., 1999). Such genes share the presence of hypoxia-response elements, which contain binding sites for HIF-1. Upon reoxygenation, HIF-1α is rapidly degraded, both in cultured cells and *in vivo* (Wang et al., 1995; Huang et al., 1996). Hypoxia or iron chelation prevents ubiquitination of HIF-1α. The interaction between pVHL and HIF-1α is regulated the binding of pVHL to a hydroxylated proline residue of HIF-1α (Ivan et al., 2001; Jaakkola et al., 2001). In addition to hypoxia, the regulation of VEGF/VPF expression may involve diverse mechanisms including activated oncogenes, mutant or deleted tumor suppressor genes, and cytokine activation (Claffey and Robinson, 1996).

Tenascin-C

TN-C is a large complex secreted protein of the ECM which is expressed in developing brain, cartilage and mesenchyme and is re-expressed in tumors, wound healing and inflammation (Erickson, 1993; Redick and Schwarzbauer, 1995) where there is remodeling of the ECM (Erickson, 1993). It has a characteristic six-armed quaternary structure (hexabrachion) linked to a central

Table 1. VEGF/VPF and TN-C expression in neoplastic angiogenesis.

	AGE (years)	SEX	SITE	VEGF/VPF		TENASCIN-C			
				ISH		ISH		IHC	
				Vasc Cells	Tumor Cells	Vasc Cells	Tumor Cells	PV	IC
GBM									
	2	F	L. Frontal Lobe	-/-	+/+++	+/+++	+/+++	+/+++	+/+++
	39	F	L. Parietal Lobe	-/-	++/+++	+/+++	-/+	+/+	-/+
	47	M	L. Parietal Lobe	-/-	+/+++	+/+++	+/+	+/+++	+/++
	48	F	R. Occipital Lobe	-/-	+/+++	+/+++	-/+++	+/+++	-/+++
	55	M	R. Frontal Lobe	-/-	++/+++	+/+	+/+++	+/+++	+/+++
JPA									
	4	M	Cerebellum	-/-	+/++	+/+++	-/+	+/+++	-/+
	5	F	Cerebellum	-/-	-/++	-/+	-	-/+	-/-
	8	M	Cerebellum	-/-	-/-	-/+++	-/+	-/+++	-/+
	8	F	Cerebellum	-/-	-/-	-/+	-	-/+++	+/+
	12	F	Cerebellum	-/-	+/+++	-/+	-	-/+	-/+
	13	F	Lateral Ventricle	-/-	+/+	-	-	-/+	-/-
HB									
	15^~	M	Cerebellum	-/-	+/+++	+/+++	-/+	+/+++	-/+
	17^	M	Spinal Cord	-/-	++/+++	+/+++	-/+	++/+++	-/+
	18~	F	Cerebellum	-/-	++/+++	+/+++	-/+	+/+++	-/+
	28	F	Cerebellum	-/-	+/+++	-/+++	-	-/++	+/++
	38	F	Spinal Cord	-/-	++/+++	+/+++	-/+	++/+++	-/++
	39	M	Cerebellum	-/-	++/+++	+/+++	-/+	+/+++	-/+
	45	M	Cerebellum	-/-	++/+++	+/+++	-/+	+/++	+/++

ISH: in situ hybridization; IHC: immunohistochemistry; vasc: vascular; PV: perivascular; IC: intercellular; GBM: glioblastoma multiforme; JPA: juvenile pilocytic astrocytoma; HB: hemangioblastoma; ^: same patient; ~:patient with Von Hippel-Lindau; -: not detected; +: weak; ++: moderate; +++: strong. The tumors included 5 GBMs (astrocytoma, WHO Grade IV/IV), 6 JPAs (astrocytoma WHO Grade I/IV (Kleihues et al., 1993)), and 7 hemangioblastomas. We assessed the presence of vascular hyperplasia in each case, taking into account the following three histological criteria: 1) increased vascular density, 2) increased number of vascular cell layers, and 3) plump endothelial cells (Brem et al., 1972). Three out of 6 JPAs and 4 out 5 GBMs showed glomeruloid vascular complexes. One GBM without vascular hyperplasia was classified as such because of the presence of necrosis. Two JPAs cases showed tumor infarction (Giannini and Scheithauer, 1997). The 7 hemangioblastomas were highly vascular but had variable cell density with highly cellular and paucicellular regions. Four samples of histologically normal brain removed in the course of surgical exposure were used as controls. When present, normal tissue adjacent to the lesions was used as internal controls. There was no VEGF/VPF mRNA in normal brain vasculature and scant signal was detected in normal cerebral cortex but not in white matter in the 4 normal controls.

knob formed by disulfide links of cysteines in the N-terminal ends of the six polypeptide arms (Erickson, 1993). In addition, TN-C consists of epidermal growth factor-like and fibronectin-type III repeats, and a fibrinogen-like region at the carboxyl terminus. At least 2 structurally and functionally different human TN isoforms (~200 and 300kDa) are generated by alternative splicing, with seven type III repeats being included or omitted in the mRNA (Erickson, 1993). Knockout of TN-C expression in mice had no major phenotypic

Table 2. VEGF/VPF and TN-C expression in non-neoplastic angiogenesis.

	AGE (years)	SEX	SITE (LOBE)	INTERVAL^	VEGF/VPF		Tenascin-C			
					ISH		ISH		IHC	
					Vascular Cells	Nonvascular Cells	Vascular Cells	Nonvascular Cells	PV	IC
Abscess[1]										
	69	F	R. temoro-parietal	1day	-/+++	-/+++	+/++	+/+	+/+++	+/++
	9	F	L. temporo-parietal	7 days	+/++	+/+++	+/+++	-/+	-/++	-/+
	45	M	R. occipital	14 days	-/+	+/+++	++/+++	-/++	-/+++	-/++
	56	M	L. lateral ventricle	21 days	-/+	+/+++	+/++	-/+	+/++	-/+
	12	M	L. frontal	30 days	-/++	-/++	++/+++	-/++	+/+++	+/++
	32	M	L. frontal	34 days	-/-	+/+	-/++	-/+	-/+++	-/++
Infarcts[2]										
	33	M	R. temporal	1 day	-/+	-/+	+/+	-/+	+/++	-/+
	23	M	R. temporal	3 days	+/++	-/+	+/+	+/++	+/+	-/+
	47	F	L. frontal	5 days	+/+++	+/+++	+/++	+/+++	-/++	-/++
	61	M	L. occipital	7 days	+/++	-/+++	+/+++	+/+++	-/+++	-/++
Trauma[3]										
	35	M	L. temporo-parietal	2 days	-/+	-/+	+/+	+/+	-/+	-/-
	40	M	R. frontal	7 days	-/++	+/+++	+/+++	+/++	+/++	+/+
	53	M	R. frontal	9 days	+/++	+/+++	+/+++	+/++	+/++	+/++
	57	M	L. frontal	12 days	+/+++	+/+++	+/++	+/++	+/+++	+/++
	25	M	L. frontal	14 days	+/++	-/+	-/+	+/++	+/+++	+/++
	6	F	R. frontal	1825 days	-/-	-/-	-/-	-/-	-/+	-/+
SDH[4]										
	73	M	Bilateral convexity	1 day	-/+	-	+/+++	+/+	-/++	-/+
	70	M	R.frontal-parietal	14 days	-/+	+/+++	+/++	-/+	-/++	-/++
	92	F	L. frontal	42 days	-/++	-/+	+/+	-/+	+/+++	+/++
	79	M	Bilateral convexity	84 days	+/+	-/+	+/+	-/+	+/++	-/+
	50	M	L. parietal	120 days	-/-	-/-	-/	-/-	-/-	-/-

^: for abscesses and subdural hematomas (SDH) the interval is the time between the onset of symptoms and the surgical procedure. By contrast, for infarcts and traumas, it indicates the time between the onset of the clinical symptoms or head injury and the surgical procedure; ISH: in situ hybridization; IHC: immunohistochemistry; PV: perivascular; IC; intercellular; -: not detected; +; weak; ++: moderate; +++: strong pathological event and the surgical procedure; ie vascular occlusion or head injury; ISH: in situ hybridization; IHC: immunohistochemistry; PV: perivascular; IC; intercellular; -: not detected; +; weak; ++: moderate; +++:strong

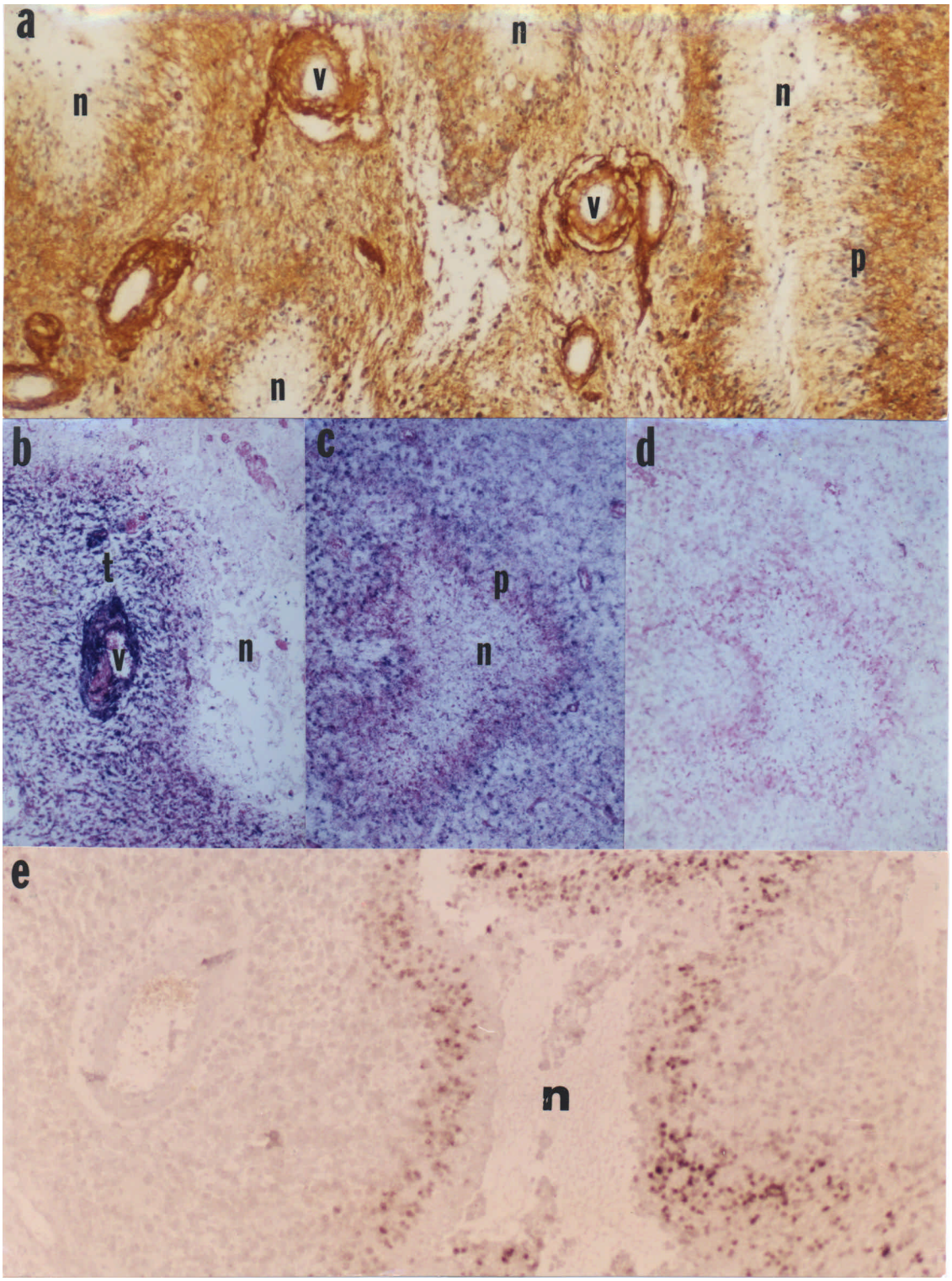
[1]: In each case, the wall of an organizing cerebral abscess i.e. inflamed “granulation tissue” with variable matrix deposition around a necrotic center with marked neovascularization was seen (Hardman, 1979). Organisms identified by gram stain and culture were *Nocardia* spp (two cases), *Streptococcus* intermedius and *Acinetobacter* in one. In 2 cases no organisms were found.

[2]Pathological examination (Garcia, 1992) of the four cerebral infarcts reveals hemorrhagic (3 cases) and non hemorrhagic “bland” (1 case) infarcts. Cases included a 24 hour old hemorrhagic infarct (due to cocaine abuse), a 3 day old arteriovenous malformation associated-hemorrhagic infarct, a 5 day old bland infarct and a 7 day old hemorrhagic infarct thought to be an intratumoral hemorrhage. This case showed luxurious vascular proliferation admixed with histiocytic cells. VEGF/VPF signal was strong in 5 and 7 day old infarcts. TN-C immunostaining was scant 1 and 3 days after the hypoxic injury, was more evident after 5 days and marked 7 days after onset of hypoxia/ischemia.

[3]: Surgical specimens were obtained 2, 7, 9, 12, and 14 days after blunt trauma to the head (Cancilla et al., 1979). Vascular proliferation was not detected in the lesion operated on 2 days after trauma, but was seen with increasing intensity on days 7 and 9 and was marked between days 12 and 14. The sixth patient was a 6 year old girl who suffered from medically refractory seizures 5 years after a car accident. The specimen obtained from this patient showed features consistent with old remote contusion with cavitation and gliosis. VEGF/VPF progressively increased in 7, 9, and 12 day old contusions but was less pronounced by day 14 and was not detected in the 5 year old injury. TN-C expression was stronger in the recent infarct as compared to TN-C expression accompanying the more chronic changes. Perivascular immunostaining was more pronounced in the areas of prominent neovascularization while the intervascular staining was variable. TN-C immunostaining increased from days 2 to 7, was maximal on day 9, was similar on days 12 and 14, and was not detected in the case obtained 5 years after injury. Thus, in cerebral contusions perivascular staining correlated with the extent of neovascularization and was not detected in the remote contusion.

[4]: Five patients, were operated upon to remove subdural hematomas from 1 to 120 days after onset of clinical symptoms. Pathological examination (Hardman, 1979) obtained 1 and 14 days after onset of clinical symptomatology showed well developed sinusoids within the clot. The specimens obtained 42 and 84 days after clinical onset showed recent hemorrhage within a more chronic membrane i.e. less abundant vascularization and more prominent matrix deposition. The case obtained over 4 months after beginning of symptoms revealed a hyalinized membrane with hemosiderin laden macrophages with no discernable vascular channels. In the case where tissue was obtained 5 years after injury no signal was found (data not shown).

abnormality (Saga et al., 1992; Forsberg et al., 1996) e.g. nerve regeneration and healing of cutaneous wounds is the same as in controls (Forsberg et al., 1996). Nevertheless, TN-C is believed to be important for several cellular processes including adhesion, migration, and proliferation of cells (Erickson, 1993). A variety of cell types including astrocytes (Bourdon et al., 1983; Grumet et al., 1985; Dorries et al., 1993; Brodkey et al.,



1995; Zagzag et al., 1995, 1996) and vascular cells (Schor et al., 1991; Mackie et al., 1992; Webersinke et al., 1992; Canfield and Schor, 1995; Hahn et al., 1995; Zagzag et al., 1996) express TN-C *in vitro* and *in vivo*.

Angiogenesis in the central nervous system

Angiogenesis plays a critical pathogenetic role in many pathological processes of the CNS. It is crucial for brain tumor growth (Zagzag et al., 1988; Cheng et al., 1996). The vascular proliferation associated with gliomas is well recognized (Burger et al., 1985) and is one of the criteria used for their grading (Daumas-Duport et al., 1988). Neovascularization often correlates with biological aggressiveness and degree of malignancy of brain tumors as well as clinical recurrence, and inversely with post-operative survival of patients with anaplastic astrocytomas (Burger et al., 1985; Daumas-Duport et al., 1988). Infiltration of malignant tumors in the brain can follow vascular channels (Scherer, 1940; Zagzag et al., 1988, 2000). Newly formed brain tumor blood vessels with defective blood-brain barrier (Zagzag et al., 1988, 1989; Del Maestro et al., 1990) are responsible for the contrast enhancement of brain tumors (Zagzag et al., 1989). They are associated with an increased risk of intratumoral hemorrhage (Liwnicz et al., 1987) and contribute to the pathogenesis of tumor-associated edema (Zagzag et al., 1998, 1989; Del Maestro et al., 1990). Like high grade gliomas, hemangioblastomas are highly vascular neoplasms. They are formed by two cellular components i.e. vascular cells and "stromal" cells. It has been suggested by several investigators that the stromal cells are the "main tumor cells" (Castaigne et al., 1968) and the vascular component is the result of an exuberant "reactive" vascular proliferation.

In cerebral abscesses, (Britt and Enzmann, 1983) two main stages exist. These are cerebritis and encapsulation. Each of these two stages can be subdivided in two, i.e. early and late substages. Early cerebritis (days 1-3) is associated with the spread of organisms across the injured vascular wall and with early necrosis, vascular congestion, petechial hemorrhages, microthromboses, perivascular fibrinous exudates and acute inflammation. Even at this early stage the endothelial cells swell. However, definite neovascularization is usually detected in the late cerebritis stage (days 4-9) when the necrotic purulent center is surrounded by a narrow irregular layer of granulation tissue infiltrated by neutrophils, lymphocytes and some macrophages often cuffing the perivascular spaces. At this stage, endothelial cells show marked hypertrophy and hyperplasia including mitoses and there is increased capillary density. Subsequently (days 10-13), matrix deposition around numerous newly formed blood vessels results in an early poorly defined developing abscess wall. As time passes (day 14 and later), the wall becomes firmer and is well demarcated from the surrounding edematous brain. Thus, neovascularization plays a major role in the organization of the wall of the abscess from matrix deposition to encapsulation.

Cerebral infarcts (Liu, 1988; Garcia, 1992) and traumas (Mitchell et al., 1978; Cancilla et al., 1979; Hardman, 1979) are histologically similar. However, the molecular layer of the cortex, which is regularly spared in an infarct, is usually disrupted at the crown of the contused gyri. In both conditions, neurons in the affected region undergo necrosis as shown by the presence of ischemic cell changes (nuclear pyknosis and cytoplasmic hypereosinophilia). However, the earliest microscopical tissue alterations include white matter edema. Approximately 3 days after the original insult, early vascular proliferation can be detected at the edge of the

Fig. 1. GBM. Tissue blocks for immunohistochemistry (Zagzag et al., 1995) and ISH (Zagzag et al., 1996) were prepared as previously described. **a.** TN-C immunoreactivity was found to be variable and heterogeneous within individual tumors. Enhanced TN-C expression was also detected among tumor cells and around individual cells as a fine fibrillary network. Occasional tumor cells showed intracytoplasmic expression of TN-C. There is strong immunoreactivity especially in hyperplastic vessels (v) including the pseudopalisading areas (p) around areas of necrosis (n). Necrotic tumor tissue remains negative. Fine fibrillar extension of TN-C from blood vessels to the surrounding tumor cells was occasionally seen. TN-C expression helped to delineate the tumor margin against the surrounding gliotic brain tissue, where it was mainly seen around hyperplastic blood vessels as previously described (Zagzag et al., 1995). Normal brain distant from the tumors showed vascular TN-C expression that was similar to the 4 samples of normal control brain i.e. TN-C was weakly expressed in the media of small intraparenchymal arterioles and leptomeningeal arteries, as previously described (Zagzag et al., 1995). Immunoperoxidase and hematoxylin couterstain, x 50. **b.** ISH for TN-C mRNA. Strong signal of TN-C mRNA is demonstrated in a hyperplastic vessel (v) and tumor cells (t) including the edge of a necrotic area (n). TN-C mRNA is seen in vascular cells lining the vascular lumens and within the walls of the vascular complexes especially at the invasive edge of the GBMs. TN-C mRNA was detected in vessels beyond the tumor "margin" in the brain tissue adjacent to the tumor in 2 out of 5. NBT/BCIP, x 50. **c.** Upregulation of TN-C mRNA expression in pseudopalisading cells (p) around necrotic areas (n). NBT/BCIP, x 50. **d.** No staining is seen with the sense probe and no detectable TN-C mRNA staining in the 4 normal brains used as controls NBT/BCIP, x 50. **e.** ISH demonstrated strong VEGF/VPF mRNA in tumor cells especially in pseudopalisading cells around areas of necrosis (n) and in areas just adjacent to the infiltrating edge of the tumors as previously described (Plate et al., 1992; Shweiki et al., 1992). NBT/BCIP, x 100. In 3 GBMs where brain tissue more distant from the tumor was present, no detectable VEGF/VPF message was found in blood vessels. ISH for VEGF/VPF was performed using a probe of the whole published sequence of VEGF/VPF (980bp). The sequence product was introduced into pBluescript II SK (Stratagene Cloning Systems, La Jolla, California). Anti-sense and sense riboprobes were prepared using digoxigenin RNA Labeling Kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). ISH for VEGF/VPF was performed using a similar protocol as for TN-C (Zagzag et al., 1996) with a few modifications. The concentration VEGF/VPF probe was 6 ng/µl. Bakers yeast was added to the hybridization buffer. Hybridization was achieved by applying 125 µl of the probe with incubation at 56 °C. Washes following hybridization were done using 2 x SSC at 56∞C and 0.2 SSC at room temperature. The alkaline phosphatase was 1:5000 dilution. Incubation with NBT/BCIP was done at room temperature in the dark. Before mounting the slides were washed with tris-EDTA and counterstained with methylene green.

necrotizing process in both infarct and trauma. By days 5 to 7 capillaries proliferate at the margin of the necrosis. Thus, in cerebral infarct edema precedes angiogenesis (Liu, 1988). Over the next 2-3 weeks neovascularization increases with marked proliferation of capillaries associated with gliosis and microglial cell activation. Hyperplastic endothelial cells with mitotic figures can be detected.

In chronic subdural hematomas (SDH), angioblastic invasion of the clot starts within a week. The new capillaries originate almost entirely from the dural aspect, (Putnam and Cushing, 1925) (i.e. from the inner dural surface). They penetrate the clot and migrate around its outer surface and then follow its inner surface. Thus, the clot becomes enclosed by a highly vascular membrane. The membrane on the dural (outer) aspect of the clot is thicker and more vascular than the inner membrane. Both membranes have formed within 2 to 3 weeks. Small blood vessels located within the capsule of the hematoma have attenuated endothelial cells and wide endothelial gap junctions (Yamashima et al., 1983) and can either "spontaneously" or after minor trauma be the source of repeated and continuing bleeding and transudation of plasma (Markwalder, 1981; Yamashima and Yamamoto, 1984). These contribute to the enlargement of the chronic SDH, rendering it a slowly expanding space-occupying lesion. Therefore, angiogenesis within the subdural membrane plays an essential role in the organization of the chronic SDH and its enlargement.

Folkman and Klagsbrun introduced the concept of Angiogenic Diseases, and proposed to categorize as such, diseases where the dominant pathology is angiogenesis (Folkman and Klagsburn, 1987). There is precedent for regrouping diseases with common pathological features or pathogenesis but with different etiologies. For example, inflammatory myopathies (Heffner, 1993) include dermatomyositis which is a B cell-mediated process causing vascular damage and is often associated with cancer, polymyositis which is a T cell-mediated process and inclusion body myositis, a disease of unknown etiology. Inflammatory myopathies also include infectious myopathies (e.g. trichinosis) and granulomatous myopathies (e.g. sarcoidosis). Demyelinating diseases (Prineas and McDonald, 1997) include pathological conditions of diverse etiologies. For example, multiple sclerosis has an incompletely understood etiology involving genetic and environmental factors. Adrenoleukodystrophy, known in the past as Schilder's disease, is an X-linked condition associated with an abnormal excess of very long chain fatty acid esters due to an impaired capacity to form the coenzyme A derivative. Acute disseminated encephalomyelitis follows viral infections (measles,

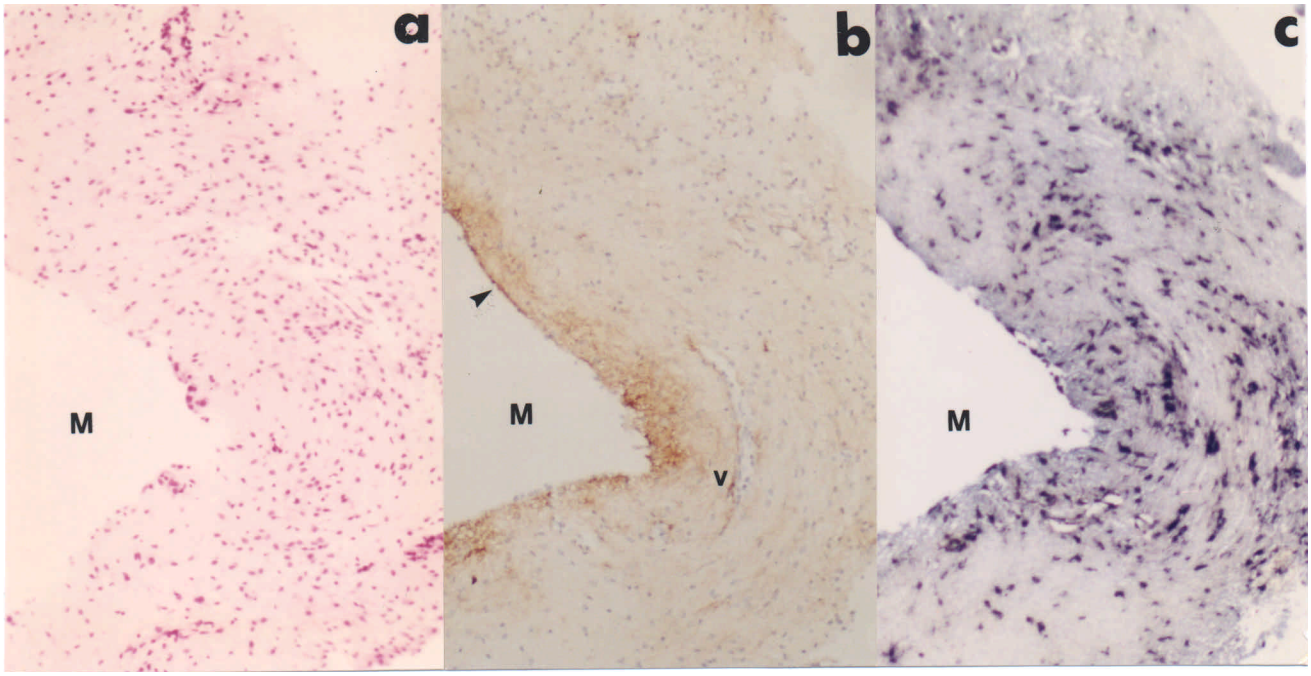


Fig. 2. Microcyst in a JPA. **a.** Moderate cellularity around a microcyst (M) in a JPA. H&E, x 100. **b.** Immunohistochemistry for TN-C of the same area as in (a) shows reactivity lining the microcyst (arrowhead) and around a vessel wall (v) adjacent to the microcyst. Immunoperoxidase and hematoxylin counterstain, x 100. TN-C was detected in hyperplastic vessels including in those lining cyst walls as previously described (Zagzag et al, 1995; Jallo et al., 1997). The immunostaining around hyperplastic vessels was either within the vascular wall or coating its outer surface. TN-C in or around vascular channels was consistently greater within and around the walls of hyperplastic vessels than non-hyperplastic blood vessels. TN-C expression was faint or focal among tumor cells but no message was detected in the tumor cells. **c.** ISH for VEGF/VPF mRNA of the same area as in (a) and (b) showing upregulation of VEGF/VPF around the microcyst. NBT/BCIP, x 100. VEGF/VPF mRNA was also detected in areas adjacent to vascular hyperplasia.

mumps, rubella, chicken pox) or vaccination (smallpox, rabies). Acute hemorrhagic leukoencephalitis (Hurst's disease) usually occurs after viral upper respiratory tract infection. Marchiafava-Bignami disease was originally described in crude red wine drinkers and is thought to be related to a vitamin deficiency. Progressive multifocal encephalopathy is due to cytopathic killing of oligodendrocytes infected with JC virus and usually occurs in immunocompromised patients. Central pontine myelinolysis is believed to be associated with the rapid correction of hyponatremia. Demyelination has also been associated with neoplasia (Peiffer, 1988). Finally, Balo's concentric sclerosis is of unknown etiology. All these conditions which have different causes are grouped together as inflammatory myopathies or demyelinating diseases because they all share a common pathological finding, i.e. inflammation or demyelination. Similarly, the pathological conditions in the CNS in which neovascularization plays a pivotal role and where VEGF/VPF and TN-C are both upregulated could be regrouped as "Angiogenic Diseases" of the CNS.

VEGF/VPF in CNS angiogenesis

In situ hybridization (ISH) for VEGF/VPF in glioblastomas multiforme (GBMs) (Fig. 1), juvenile pilocytic astrocytomas (JPAs) (Figs. 2-4), hemangioblastomas (Fig. 5), cerebral abscesses (Fig. 6), cerebral infarcts (Fig. 7), trauma-induced cerebral lesions (Fig. 8) including 5 chronic SDHs (Fig. 9) and 4 normal control brains, demonstrates the expression of VEGF in these conditions that are associated with

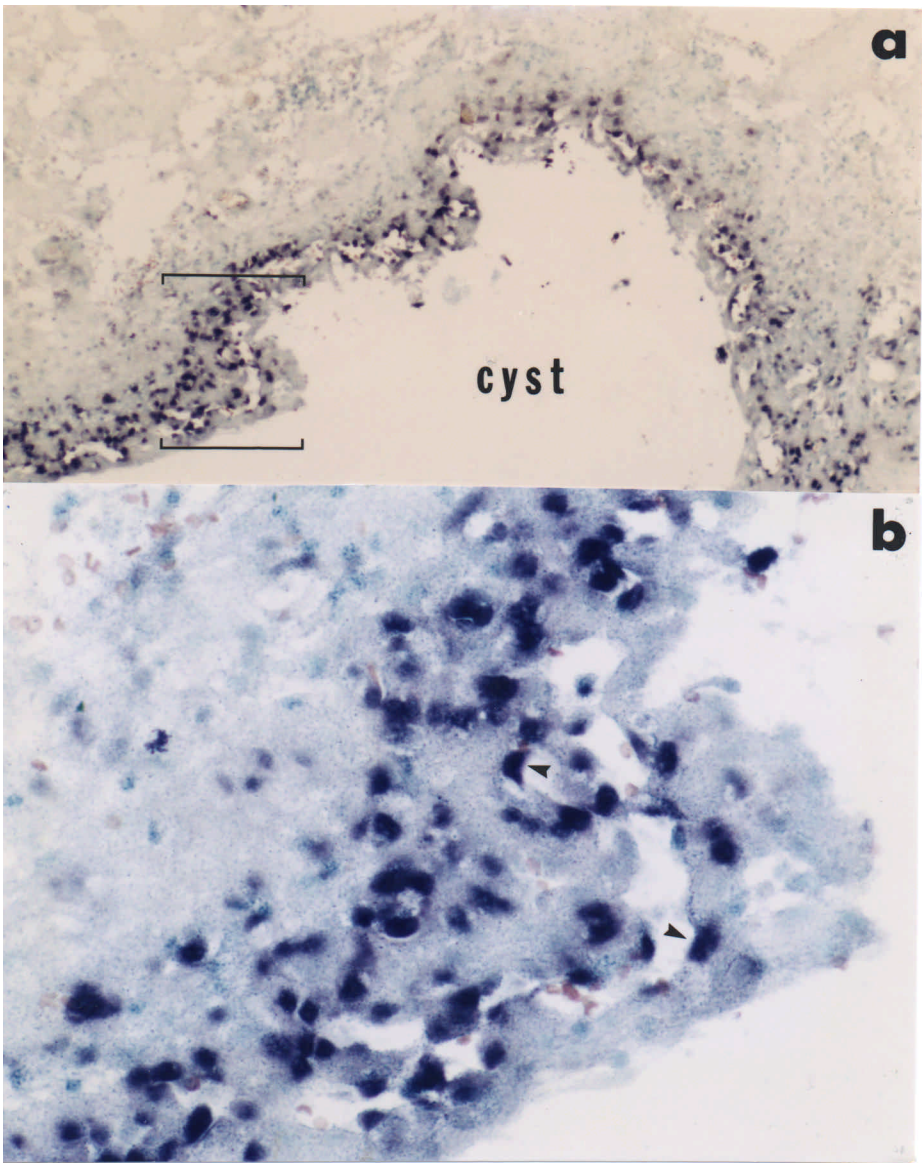


Fig. 3. ISH for VEGF/VPF in a cyst of a JPA. **a.** Wall of a large cyst showing strong signal for VEGF/VPF mRNA in the cells of hyperplastic vessels. NBT/BCIP, x 50. **b.** High magnification of the boxed area in (a). Note the strong signal for VEGF/VPF mRNA in vascular cells (arrowheads). NBT/BCIP, x 200. Only weak staining was observed in the tumor vasculature of JPAs which failed to show hyperplastic vessels. VEGF/VPF mRNA in tumor cells was focal and weak in one case and not detectable in 2 cases.

angiogenesis. Moreover, our findings demonstrate the spatial and temporal upregulation of VEGF/VPF in relation to neovascularization in both neoplastic and non-neoplastic pathological conditions of the CNS. Several lines of evidence suggest that VEGF/VPF is involved in brain tumor angiogenesis: 1) VEGF/VPF is produced by glioma cells *in vitro*; (Plate et al., 1992; Shweiki et al., 1992); 2) VEGF/VPF expression is dramatically up-regulated in various human brain tumors *in vivo*, such as highly vascularized GBMs (Plate et al., 1992; Shweiki et al., 1992), or von Hippel-Lindau disease-associated hemangioblastomas; (Stratmann et al., 1997); 3) receptors for VEGF/VPF have been demonstrated in both high and low grade gliomas (Plate et al., 1993; Weindel et al., 1994; Leung et al., 1997); and 4) experimentally induced angiogenesis and brain tumor growth in nude mice can be specifically inhibited by anti-VEGF/VPF monoclonal antibodies (Kim et al., 1993) or by a dominant-negative flk-1 mutant (Millauer et al., 1994). Moreover, VEGF/VPF plays a role in experimental animal models of cerebral trauma (Nag et al., 1997) and infarct (Kovacs et al., 1996; Plate et al., 1999), and has been demonstrated in a variety of non-neoplastic cell types. These include neurons (Kovacs et al., 1996), astrocytes (Ijichi et al., 1995), pericytes (Murata et al., 1996), smooth muscle cells (Li et al., 1995; Stavri et al., 1995), macrophages (Berse et al., 1992), lymphoid cells (Freeman et al., 1995), platelets (Mohle et al., 1997), and fibroblasts (Volpert et al., 1997). Endothelial cells isolated from a variety of organs including skin (Namiki et al., 1995; Detmar et al., 1997), umbilical cord (Namiki et al., 1995), brain (Fischer et al., 1995), lung (Liu et al., 1995), and kidney (Seghezzi et al., 1998), *in vitro* and in organotypic cultures (Fischer

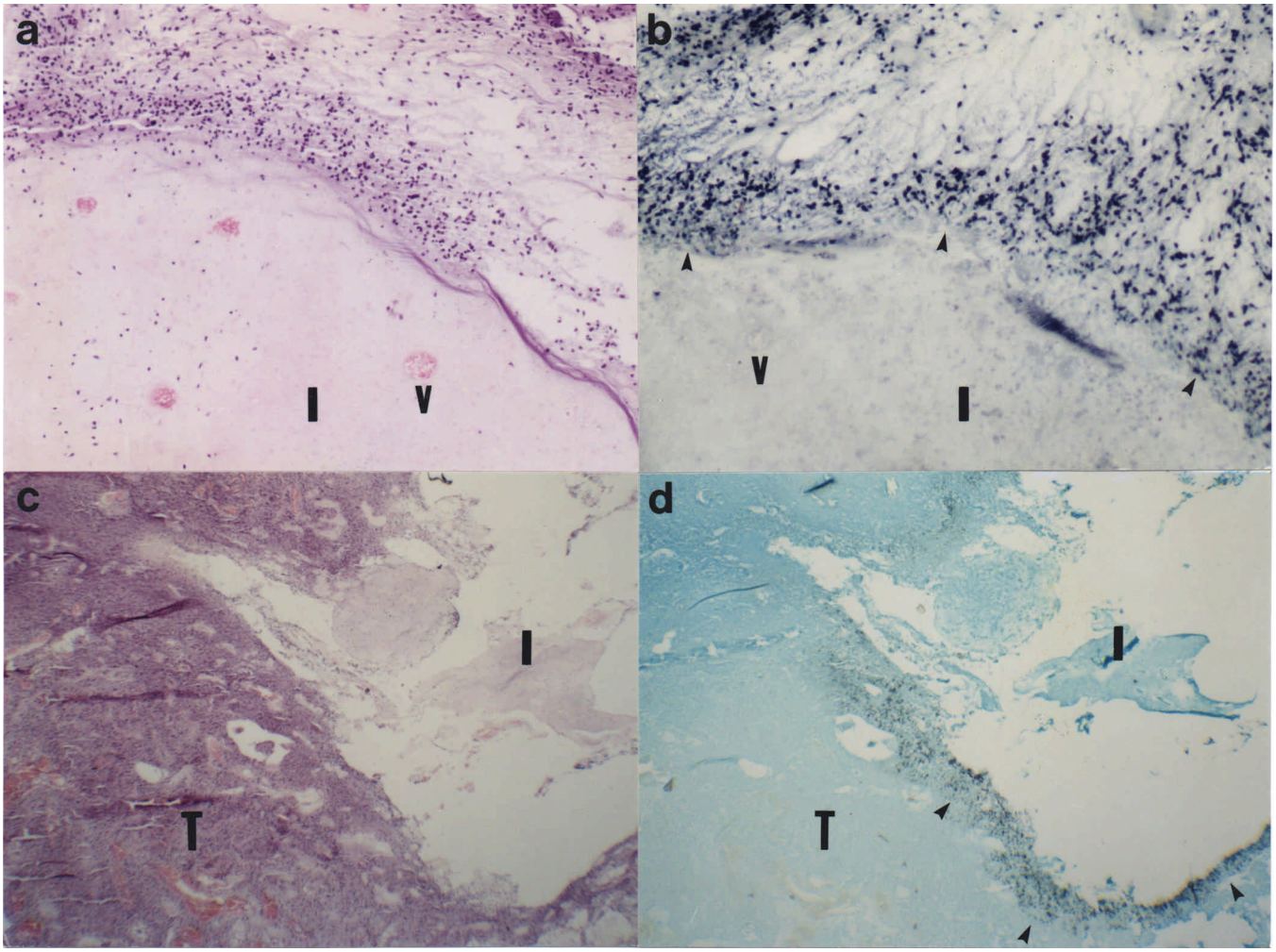


Fig. 4. ISH for VEGF/VPF mRNA in 2 JPAs with tumor infarction. High **(a)** and low **(c)** magnification of two different JPAs with tumor infarction (I). Ghost vessels can be seen in the infarcted zones (v). T: tumor adjacent to the infarct. HxE, a, x 100; c, x 50. High **(b)** and low **(d)** magnification of ISH for VEGF/VPF demonstrating mRNA in tumor cells around the infarcts (arrowheads). There is no VEGF/VPF in the rest of the tumor (T). NBT/BCIP, b, x 100; d, x 50. In addition in 2 out of 6 JPAs portions of the cerebellar granular layer were expressing VEGF/VPF mRNA as previously described in the normal adult rat brain (Monacci et al., 1993).

et al., 1995) have been shown to express VEGF/VPF. Our results demonstrate that under selected conditions, the role of endothelial cells in vascular growth extend beyond that of a target to involve contingency synthesis of VEGF/VPF and thus autocrine activation (Uchida et al., 1994). VEGF/VPF is angiogenic and increases microvascular permeability (Senger et al., 1983; Roberts and Palade, 1995) including that of cerebral microvessels (Wang et al., 1996). Because cerebral angiogenesis is associated with increased vascular permeability (Zagzag et al., 1988, 1989; Del Maestro et al., 1990) which plays a major role in the pathogenesis

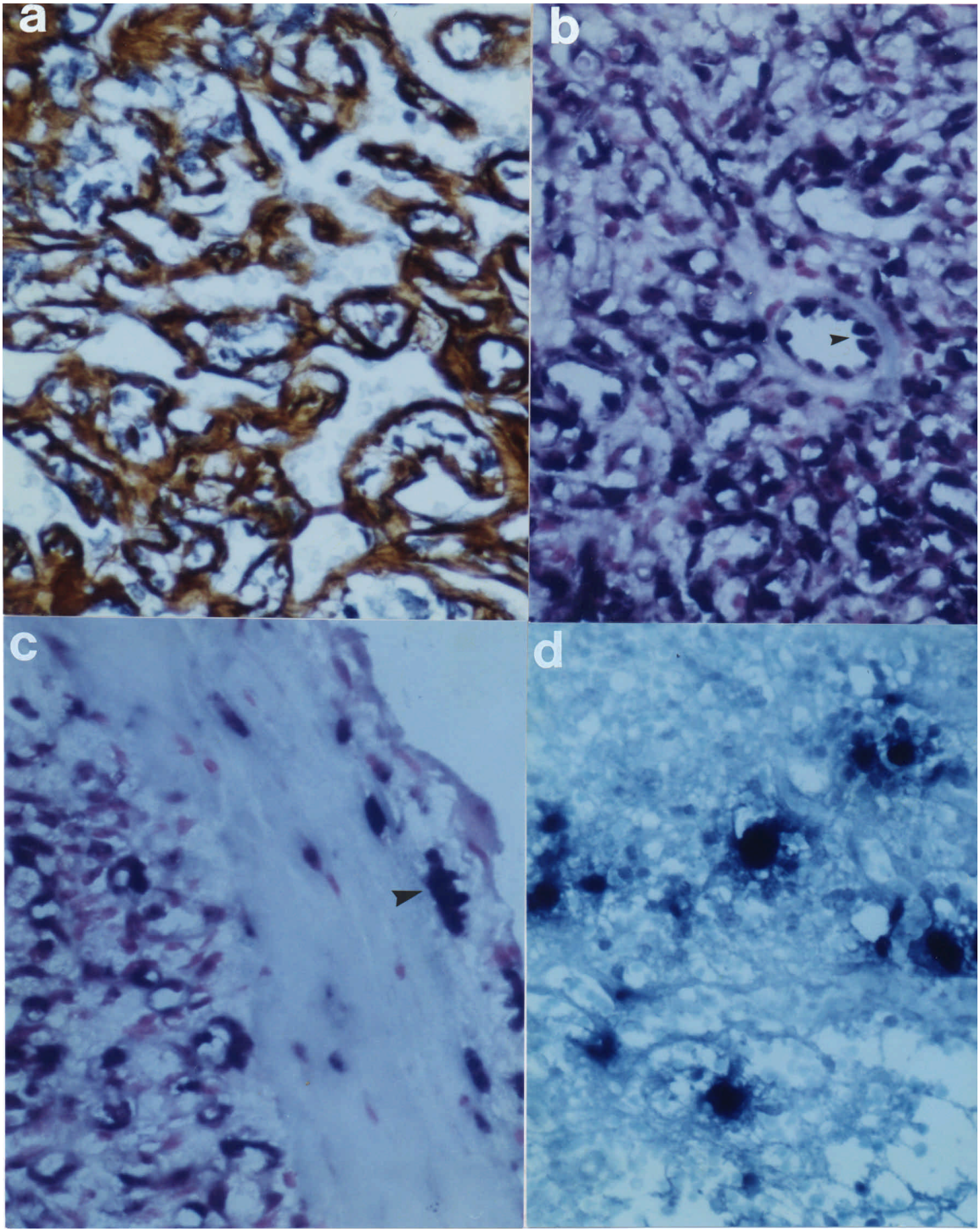
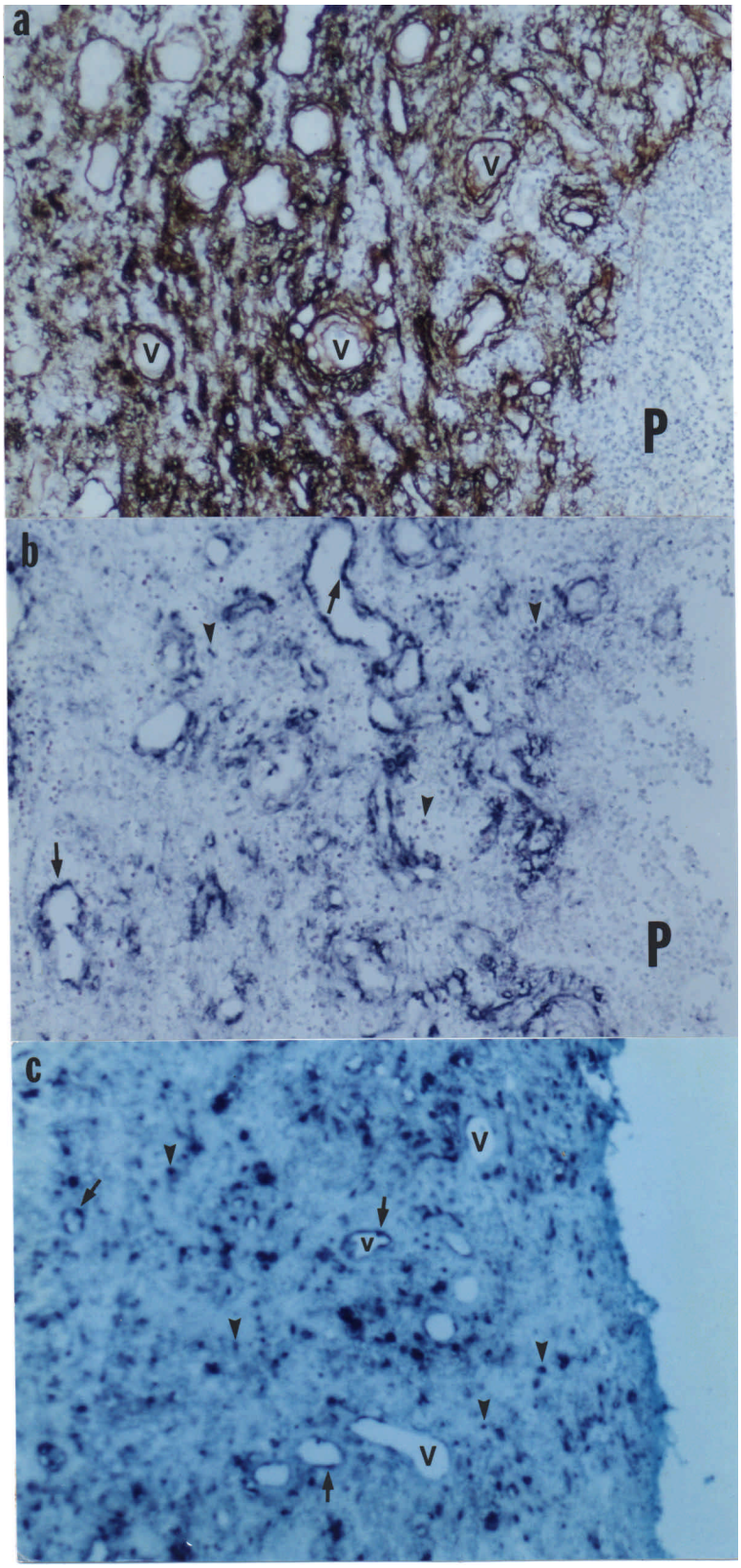


Fig. 5. Hemangioblastoma. **a.** TN-C immunostaining was heterogenous and found around vascular channels, often as a fine network radiating out from larger size vessels. Immunoperoxidase and hematoxylin counterstain, x 100. **b.** TN-C mRNA signal is intense in vascular cells of the tumor including endothelial cells of capillaries (arrowheads). NBT/BCIP, x 200. **c.** Some vascular cells within the wall of larger vessels are also labeled for TN-C mRNA (arrowhead). NBT/BCIP, x 400. **d.** Strong VEGF/VPF mRNA is detected in stromal cells. NBT/BCIP, x 400. As previously described (Stratmann et al., 1997).



of cerebral edema (Del Maestro et al., 1990; Hariri, 1994), it is likely that the development of cerebral edema is intimately linked to angiogenesis. Moreover, in several conditions, edema formation precedes angiogenesis, e.g. in cerebral infarct (Liu, 1988). Our study suggests that VEGF/VPF could play an important role in the pathogenesis of angiogenesis associated-cerebral edema in the CNS which interestingly has been referred to as vasogenic edema (Klatzo, 1967). Ultrastructural cellular changes seen in cells exposed to VEGF/VPF include fenestrations (Roberts and Palade, 1995) and activated vesiculo-vacuolar organelles (Feng et al., 1996). Fenestrations (Long, 1970) and vesiculo-vacuolar organelles (Lossinsky et al., 1996) have been described in brain tumors vessels. Moreover, vesiculo-vacuolar organelles (Lossinsky et al., 1996) and capillary fenestrations have been linked to barrier permeability in brain tumors (Long, 1970) and in SDHs (Yamashima et al., 1983). It is interesting that among the 3 types of neoplasms we have studied, JPAs and hemangioblastomas which are often cystic are usually less likely to be associated with peritumoral edema. This suggests that the storage of VEGF/VPF primarily occurs in the tumor associated cyst (Weindel et al., 1994), rather than in the surrounding brain tissue as it probably occurs in most GBMs. In our study when VEGF/VPF mRNA was found just beyond the infiltrating edge of the GBMs.

Cerebral edema is one of the most important factors contributing to the morbidity and mortality associated with these edematogenic CNS diseases that we have

Fig. 6. Organizing wall of a cerebral abscess. **a.** Strong immunoreactivity for TN-C is seen around proliferating vascular channels (V). It was diffusely seen around vessels, delineating vascular channels by following the branches of the arborization pattern, it was also detected in the intervascular stroma and in the subendothelial matrix forming a thick band at the interphase between endothelium and stroma. Immunoperoxidase and hematoxylin counterstain, x 50. Necrotic tissue and areas composed mainly of neutrophils (p) were negative for TN-C immunostaining. **b.** ISH for TN-C mRNA shows signal within vascular cells (arrows), fibroblasts and inflammatory cells (arrowheads). Note lack of TN-C immunoreactivity and TN-C mRNA in necrotic regions within the purulent exudate (P). Perivascular staining was stronger than the intervascular staining and was diffusely expressed in the extracellular space as a discrete fibrillary network surrounding individual or groups of inflammatory cells; TN-C mRNA was also seen in reactive astrocytes, in brain tissue adjacent to the abscess. NBT/BCIP x 50. **c.** ISH for VEGF/VPF shows many inflammatory, fibroblastic and astrocytic cells (arrowheads) expressing VEGF/VPF in between vessels (v) some with labeled endothelial cells (arrows). VEGF/VPF mRNA. NBT/BCIP, x 50

studied. For example, it often complicates the post operative period of patients with brain tumors (Hariri, 1994). In cerebral abscesses, edema is often widespread (Klatzo, 1967; Nakagawa et al., 1990) and develops early, greatly increasing the mass effect of the local lesion. It is the major cause of early death in cerebral infarcts (White et al., 1979; Ropper and Shafren, 1984). In cerebral traumas, (Bruce et al., 1981) cerebral edema

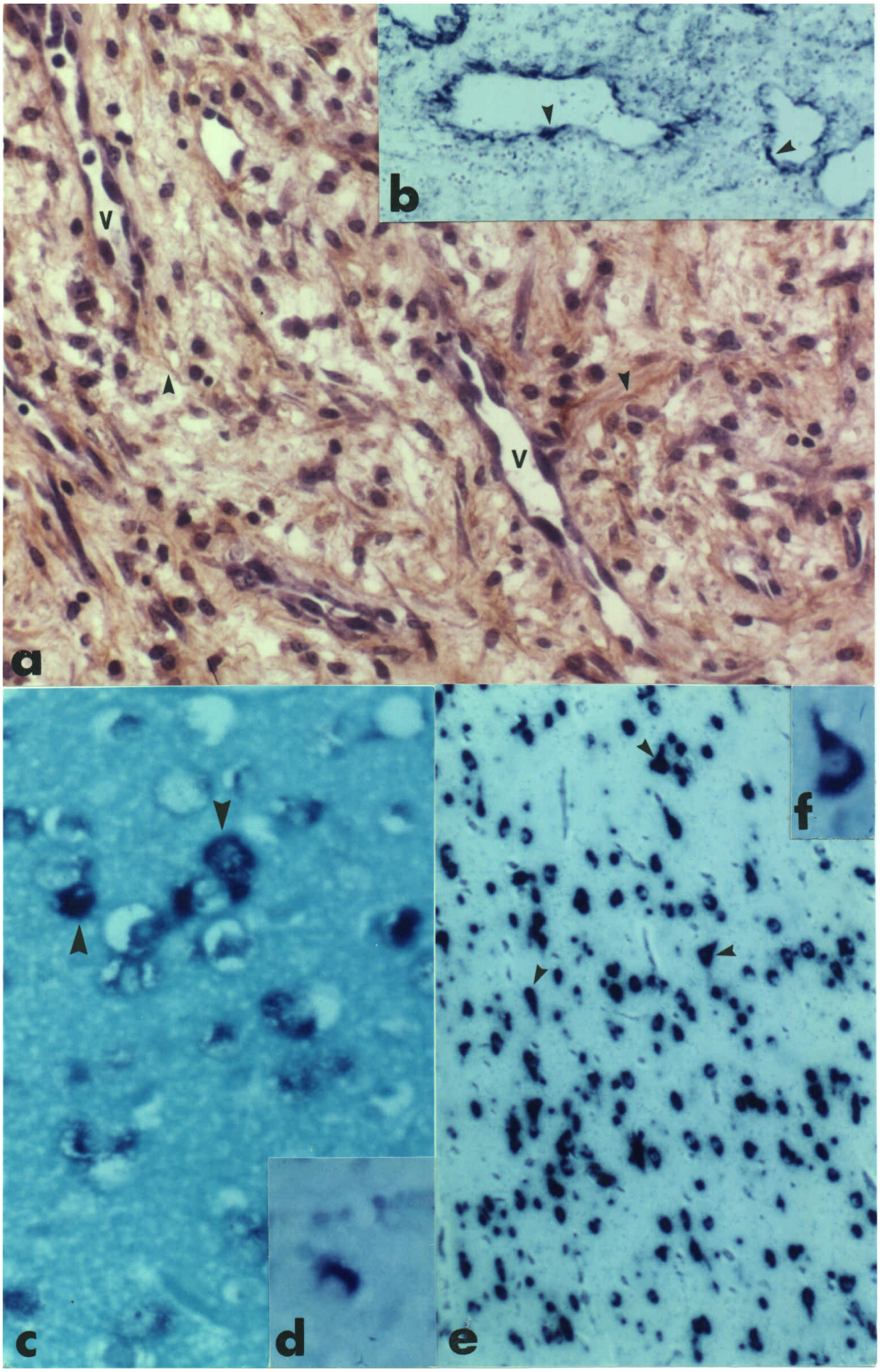


Fig. 7. Cerebral infarct. **a.** Fibrillary TN-C immunostaining (arrowheads) at the edge of an infarct where numerous new blood vessels are present (v). TN-C expression was minimal or undetectable in the center of the infarct, with enhanced fibrillary staining at the periphery of the lesion primarily around vascular channels and was more abundant in areas where lumens were discernible and plump endothelial cells were seen. TN-C mRNA was primarily seen in capillaries and in reactive astrocytes. Immunoperoxidase and hematoxylin counterstain, x 100. **b.** ISH for TN-C shows strong signal within vascular cells (arrowheads). NBT/BCIP, x 50. **c.** ISH for VEGF/VPF shows signal within macrophages (arrowheads) and vascular cells. NBT/BCIP, x 200. **d.** Irregular nuclei resembling microglial cells were also labeled. **e.** Cortex adjacent to an infarcted area of brain tissue showing upregulation of VEGF/VPF within neurons (arrowheads). NBT/BCIP; x 50. **f.** Higher magnification of a pyramidal neuron labeled for VEGF/VPF mRNA. NBT/BCIP, x 200.

is variable. However, even in a patient with a small cerebral contusion, edema may involve the majority of white matter of the hemisphere bearing a focal injury. In all these processes it adds to the increased intracranial pressure caused by the primary lesion by superimposing a significantly larger mass on the brain. It may worsen the neurological condition with the development of hemiparesis, speech dysfunction, and convulsions, and in more severe cases, brain swelling may cause a fatal cerebral herniation syndrome with secondary damage to the brainstem. Cerebral edema is therefore a key component in determining prognosis and clinical outcome. The potentially lethal aspect of cerebral edema is especially well illustrated by the significant decline in

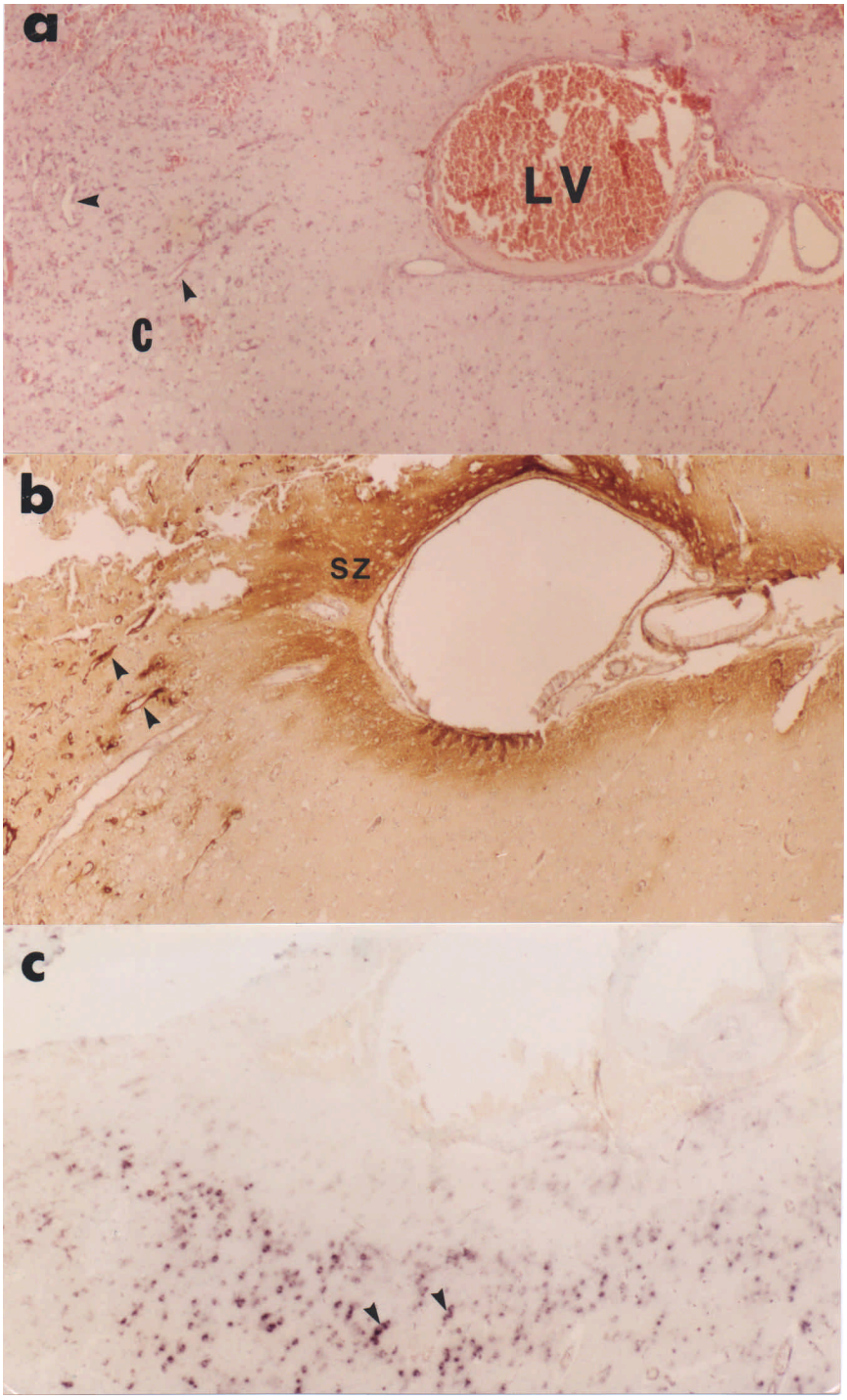


Fig. 8. Cerebral contusion. **a.** Histological section of cortex and subarachnoid space with distented leptomeningeal vessel (LV) at the edge of a contused area (c) showing many blood vessels (arrowheads). H&E, x 50. **b.** Immunohistochemistry for TN-C of the same area described in **(a)**. Note the strong reactivity around vessel (arrowheads) in the contused region and in the subpial zone (SZ). In blood vessels with flat endothelial cells and areas without obvious vascular lumens, TN-C expression was weak. Intracellular TN-C was seen in rare reactive astrocytes. Immunoperoxidase and hematoxylin counterstain, x 50. **c.** ISH for VEGF/VPF mRNA of the same area described in **(a)** and **(b)**. Upregulation for VEGF/VPF is seen adjacent to the contused area primarily in neurons (arrowhead). Macrophages and vascular cells are also labeled. NBT/BCIP; x 50

neurosurgical operative mortality and by the dramatic neurological improvement in non-surgical patients associated with the use of corticosteroid antiedematous therapy (Jelsma and Bucy, 1967) shown to downregulate VEGF/VPF expression *in vitro* (Criscuolo et al., 1988). Despite progress in understanding the nature, pathophysiology and therapy of cerebral edema, still, it remains a common and ongoing problem for many patients with brain pathology.

VEGF/VPF expression is modulated by hypoxia, glucose deficiency, tumor suppressor genes, oncogenes and other cytokines (Claffey and Robinson, 1996). The CNS tumoral or reparative processes that we have studied are likely to be associated with some degree of hypoxia. Astrocytomas of both low (Giannini and Scheithauer, 1997) and high grade (Barker et al., 1996) display necrotic and/or infarcted areas. Abscesses have necrotic centers and thus have ischemic/hypoxic tissues. Infarcts by their nature have an obvious ischemic component. Besides infarction that can be identified in 90% of fatal head injuries (Graham et al., 1995), vasospasm due to subarachnoid hemorrhage with cerebral contusions can contribute to the developing brain edema by induction of ischemia. Moreover, cerebral contusions are usually associated with full thickness necrosis of the cortex. In both, the signal in elongated cells is consistent with microglial cells (Barleon et al., 1996), where a VEGF/VPF signal was detected. VEGF/VPF is expressed by cells in the deep portion of the subdural membrane. It is interesting that these cells expressing VEGF/VPF also express CD31 (PECAM-1) that has been recently shown to be implicated in angiogenesis (DeLisser et al., 1997). VEGF/VPF may represent the angiogenic factor that has been extracted from SDH (Nakamura and Tsubokawa, 1989). VEGF/VPF upregulation is detected as quickly as 3 hours *in vitro* in astrocytes exposed to hypoxia (Ijichi et al., 1995), and within 30 minutes in polymorphonuclear neutrophils after injury *in vivo* (Nag et al., 1997). Thus, hypoxia is critical for the upregulation of VEGF/VPF. HIF-1a plays an essential role in oxygen homeostasis (Wang and Semenza, 1995; Wang et al., 1995; Iyer et al., 1998). HIF-1 activates a large battery of genes whose protein products include VEGF/VPF (Forsythe et al., 1996; Semenza et al., 1996; Iyer et al., 1998; Semenza, 1998; Tazuke et al., 1998; Feldser et al., 1999). HIF-1α has been demonstrated in many tumors (Zagzag et al, 2000). However, in some

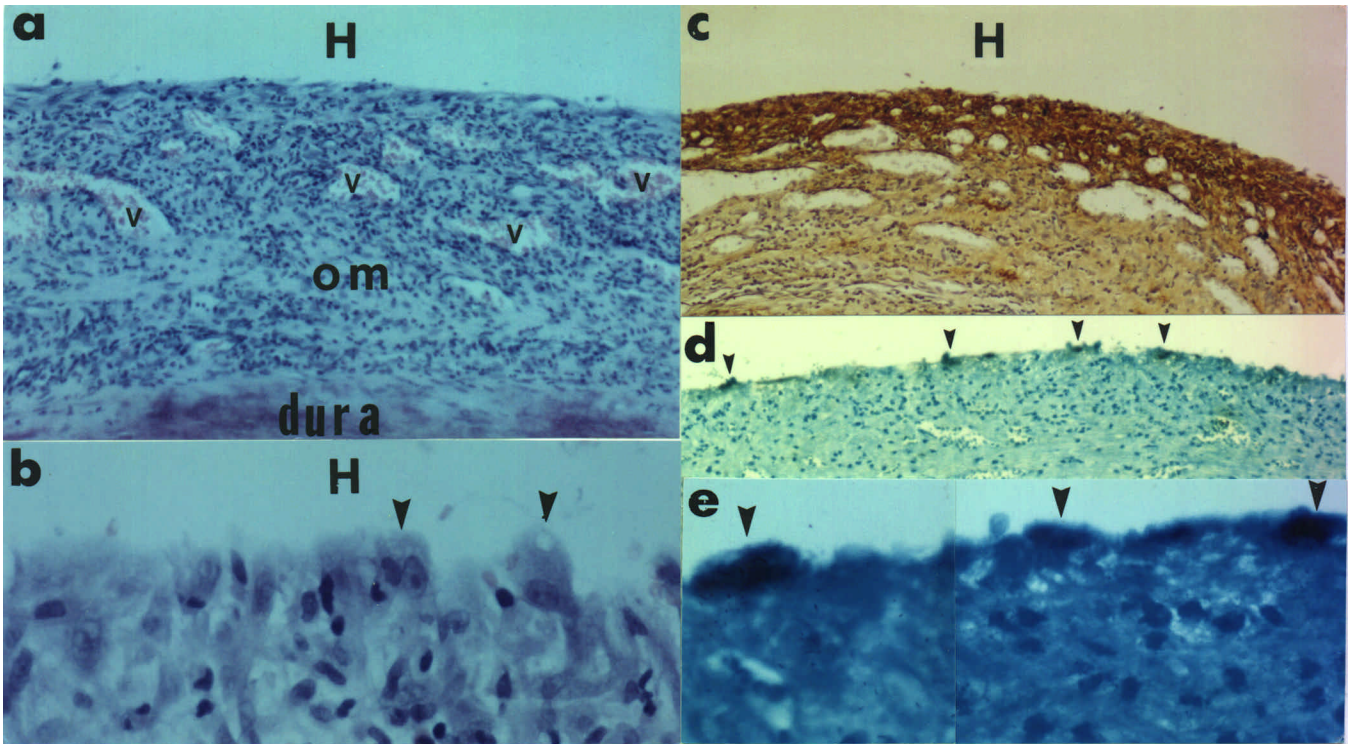


Fig. 9. Chronic SDH. **a.** Outer membrane (OM) adjacent to the dura mater lining the hematoma cavity (H) showing the presence of an inflammatory infiltrate and numerous newly formed vascular channels (V). H&E, x 50. **b.** Higher magnification of the inner portion of the membrane shows large cells (arrowheads) with large nuclei and prominent nucleoli lining the hematoma cavity (H). These cells were also immunopositive for vimentin, smooth muscle actin, CD31 (PECAM-1) and negative for Factor VIII related antigen and CD34 (Q-BEND10) (data not shown). Smaller mononuclear cells are located deeper in the membrane. H&E, x 400. **c.** TN-C is heterogeneously expressed in the outer membrane. TN-C immunostaining is primarily seen in the areas adjacent to the hematoma cavity (H) i.e. in the inner deeper layer of the outer membrane where it is marked around vascular channels. Immunoperoxidase and hematoxylin counterstain, x 50. **d.** ISH for VEGF/VPF mRNA shows upregulation within the large cells (arrowheads) lining the hematoma cavity in the deeper portion of the outer membrane. NBT/BCIP, x 50. **e.** Higher magnification of these cells (arrowheads). NBT/BCIP, x 400

conditions, e.g. hemangioblastomas, other mechanisms (i.e. loss of the Von Hippel-Lindau tumor suppressor gene (Siemeister et al., 1996; Stratmann et al., 1997; Vortmeyer et al., 1997; Lee et al., 1998), are responsible for the upregulation of VEGF/VPF. Other tumor suppressor genes and oncogenes including ras (Rak et al., 1995), src (Mukhopadhyay et al., 1995; Jiang et al., 1997), and p53 (Kieser et al., 1994; Mukhopadhyay et al., 1995) have been shown to modulate VEGF/VPF expression. Both src (Kieser et al., 1994; Mukhopadhyay et al., 1995), which at least in part involve HIF-1 upregulation (Jiang et al., 1997), and ras (Rak et al., 1995) upregulate VEGF/VPF. The exact implication of p53 is unclear. While mutant p53 was reported to potentiate the upregulation of VEGF/VPF by protein kinase C (Keiser et al., 1994), other studies have demonstrated that wild type p53 suppresses the src-induced VEGF/VPF transcription (Mukhopadhyay et al., 1995). Some studies have implicated the p53-MDM2 pathway in the regulation of HIF-1α degradation (Ravi et al., 2000). Epidermal growth factor (EGF) receptor upregulates VEGF/VPF (Petit et al., 1997). Both P53 and EGFR are important in the pathogenesis of high grade gliomas (Louis and Gusella, 1995). The role of src and ras in human brain tumors remains to be clarified (Wasson et al., 1990; Brustle et al., 1992; Patt et al., 1993). Glucose deficiency is also able to induce VEGF/VPF expression (Shweiki et al., 1995).

Direct [basic fibroblast growth factor (FGF) (Stavri et al., 1995; Tsai et al., 1995; Ryuto et al., 1996), platelet derived growth factor (PDGF) (Finkenzeller et al., 1992; Tsai et al., 1995), EGF (Tsai et al., 1995)] and indirect [tumor necrosis factor (TNF) (Ryuto et al., 1996; Yoshida et al., 1997), transforming growth factor (TGF) (Pertovaara et al., 1994) and interleukin-1 (Li et al., 1995; Ryuto et al., 1996)] angiogenic factors also upregulate VEGF/VPF expression. Some have synergistic effects with VEGF/VPF, e.g. basic FGF (Goto et al., 1993). Moreover, FGF-induced angiogenesis, is at least in part, mediated by VEGF/VPF (Deroanne et al., 1997). Thus, a variety of potentially interrelated pathways can lead to the upregulation of VEGF/VPF. These modulating factors, including hypoxia, glucose deficiency, tumor suppressor genes, oncogenes and growth factors, probably interact *in vivo* in a complex interplay. For example, hypoxia modulates p53 expression (Graeber et al., 1994) and is linked to permeability (Tanno et al., 1992), probably in part through upregulation of VEGF/VPF. Thus, each of these regulating mechanisms alone or in conjunction with others may lead to the upregulation of VEGF/VPF. Further studies are needed to elucidate their interaction. High (Plate et al., 1992; Shweiki et al., 1992) and low (Weindel et al., 1994; Leung et al., 1997) grade human astrocytomas, and hemangioblastomas (Stratmann et al., 1997) and experimental animal models of CNS neoplasms (Plate et al., 1993), cold injury (Nag et al., 1997) and ischemia (Kovacs et al., 1996), are associated with VEGF/VPF upregulation. We have demonstrated that a variety of human hypoxic/ischemic, inflammatory, infectious and traumatic conditions of the CNS associated with angiogenesis are also associated with upregulation of VEGF/VPF, a potent angiogenic and edematogenic cytokine.

TN-C and angiogenesis

In contrast to the low levels of TN-C found in normal adult brain, enhanced expression occurs in human astrocytomas (Zagzag et al., 1995, 1996). For example, by Western blot its expression is elevated up to 4 fold in GBMs as compared to normal control tissue (Zagzag et al., 1995). It is expressed around tumor cells mainly of high-grade tumors as well as in hyperplastic vessels of astrocytomas regardless of grade, and its expression correlates with angiogenesis (Zagzag et al., 1995, 1996). Immunohistochemistry and ISH for TN-C showed enhanced TN-C expression in all high and low-grade astrocytomas (Figs. 1, 2) and hemangioblastomas (Fig. 5), and in a variety of non-neoplastic diseases of the CNS which are associated with neovascularization. These included infectious, inflammatory and ischemic diseases of the CNS, e.g. cerebral abscesses (Fig. 6) and infarcts (Fig. 7), as well as traumatic conditions such as cerebral contusions (Fig. 8) and SDHs (Fig. 9). TN-C was observed around hyperplastic blood vessels of tumors regardless of their grade or type as well as around newly formed vascular channels of non-neoplastic processes. Thus, TN-C expression correlates spacially and temporally with angiogenesis in both neoplastic and non-neoplastic human diseases of the brain. Moreover, because TN-C mRNA and protein were not upregulated in vessels of normal brain, it is possible that TN-C expression might be important for vascular cell activation. Vascular cells able to synthesize TN-C include endothelial cells (Webersinke et al., 1992; Hahn et al., 1995; Zagzag et al., 1996), and pericytes/smooth muscle cells (Schor et al., 1991; Mackie et al., 1992; Zagzag et al., 1996). Other cell types capable of expressing TN-C include astrocytes (Grumet et al., 1985; Dorries et al., 1993; Brodkey et al., 1995; Zagzag et al., 1996; Ness and David, 1997), fibroblasts (Copertino et al., 1997), and neurons (Ferhat et al., 1996).

Evidence linking TN-C and angiogenesis includes: 1) TN-C, which has both adhesive and counteradhesive domains for a variety of cell types (Prieto et al., 1992) modulates endothelial cell adhesion (Murphy-Ullrich et al., 1991; Joshi et al., 1993; Sriramarao et al., 1993; Chung and Erickson, 1994). This is mediated in part by $\alpha_v\beta_3$ integrin (Joshi et al., 1993; Sriramarao et al., 1993) that is required for angiogenesis (Brooks et al., 1994). 2) TN-C modulates microvascular migration (Kaplony et al., 1991; Canfield and Schor, 1995; Hahn et al., 1995; Chung et al., 1996). For example, TN-C-rich matrices are permissive for endothelial cell migration, by contrast to inhibitory thrombospondin-rich matrices (Canfield and Schor, 1995). TN-C is specifically expressed at the

site of migration of developing embryonic vasculature (Spence and Poole, 1994). Moreover, during cornea development, cells derived from the neural crest and destined to become endothelia migrate exactly along the line of the TN-C-rich stroma (Kaplony et al., 1991). Furthermore, antibodies against TN-C inhibit endothelial cell sprouting *in vitro* (Canfield and Schor, 1995; Hahn et al., 1995; Chung et al., 1996). TN-C also modulates the migration of glial (Wehrle-Haller and Chiquet, 1993) and glioma cells (Deryugina and Bourdon, 1996), cerebellar granular layer cells (Husmann et al., 1992) and supports lymphocyte rolling (Clark et al., 1997). TN-C also plays a similar role during embryogenesis (Erickson and Bourdon, 1989). For example, TN-C is expressed at the site of migration of neural crest cells (Mackie et al., 1988), which could be inhibited by anti-TN-C antibodies (Bronner-Fraser, 1988). 3) TN-C modulates migration and proliferation of vascular cells, both crucial steps of the angiogenic cascade (Ausprunk and Folkman, 1977). TN-C modulates the proliferation of a variety of cell types (Chiquet-Ehrismann et al., 1986; Crossin, 1991). However, the mitogenic effect of TN-C on endothelial cells is seen only if TN-C is added before or simultaneously when bFGF is added to endothelial cell cultures (Chung et al., 1996). TN-C enhances cell migration both by its anti-adhesive effects (Murphy-Ullrich et al., 1991; Joshi et al., 1993; Sriramarao et al., 1993; Chung and Erickson, 1994) and by stimulation of the expression of genes encoding matrix metalloproteinases (Tremble et al., 1994). One additional important mechanism is the loss of focal adhesion in endothelial cells induced by the alternatively spliced region of TN-C that is a step associated with cell migration and proliferation (Murphy-Ullrich et al., 1991; Chung and Erickson, 1994). This effect can be blocked by antibodies against annexin II, a 35 kD non-integrin receptor for TN-C on endothelial cells (Murphy-Ullrich et al., 1991; Chung and Erickson, 1994). Interestingly, overexpression of an immediate early gene, e.g. c-jun which is upregulated in brain infarcts (Liu, 1995) and injuries (Nag et al., 1997) and associated with angiogenesis (Michel et al., 1994; Liu, 1995; Nag et al., 1997) stimulate TN-C (Mettouchi et al., 1997). Recently TN-C has been shown to be a survival factor for vascular smooth muscle cells (Jones et al., 1997), which have been implicated in the neovascular proliferative phenomena associated with GBMs (Haddad et al., 1992); 4) TN-C is up-regulated spatially and temporally in newly formed vessels of granulation tissue in experimentally induced skin wounds (Mackie et al., 1988; Chuong and Chen, 1991) and is not detectable or markedly reduced in the scar when wound contraction is complete (Chuong and Chen, 1991; Fassler et al., 1996). 5) TN-C is expressed in vascular tumors and reactive vascular proliferations e.g. bacillary angiomatosis (Kostinanovsky et al., 1997). 6) TN-C binds to heparin, (Weber et al., 1995) an important modulator of angiogenesis (Folkman and Shing, 1992). 7) vascular cells including endothelial cells and smooth muscle cells/pericytes contribute to the deposition of TN-C (Webersinke et al., 1992; Hahn et al., 1995; Zagzag et al., 1996) present at sites of vascular hyperplasia. 8) several factors known to stimulate angiogenesis in cerebral embryogenesis and neoplasia (Zagzag, 1995), including basic FGF (Rettig and Garin-Chera, 1989; Meiners et al., 1993; Tucker et al., 1993; Rettig et al., 1994), TGF (Rettig and Garin-Chesa, 1989; Adams Pearson et al., 1988; Mackie et al., 1992; Hahn et al., 1995), PDGF (Adams Pearson et al., 1988; Mackie et al., 1992), EGF (Sakai et al., 1995), interleukin-1 (Rettig et al., 1994), and TNF-alpha (Rettig and Garin-Chesa, 1989; Rettig et al., 1994) can upregulate TN-C expression.

Although a variety of ECM molecules including laminin (Kubota et al., 1988), fibronectin (Nicosia et al., 1993), collagen (Montesano et al., 1983), thrombospondin (Iruela-Arispe et al., 1991), SPARC (Lane et al., 1994), and vitronectin (Davis et al., 1993) have been implicated in the regulation of angiogenesis, it appears that the interaction of endothelial cells with TN-C is different from that of the other ECM molecules. For example, endothelial cells *in vitro* attach to TN-C substrata where they elongate and extend and have interconnecting processes (Sriramarao et al., 1993). These features are lacking when endothelial cells are grown on fibronectin, collagen, vitronectin or laminin substrata (Sriramarao et al., 1993). Because of its particular implication in brain pathology, and its potential role in each of the crucial steps of the angiogenic cascade, TN-C may prove to be the most important ECM molecule in CNS pathological angiogenesis.

Conclusion

The strong association of increased VEGF/VPF and TN-C expression in angiogenic conditions of the CNS suggests a link between their expression. Whether VEGF/VPF upregulates TN-C expression or how TN-C precisely modulates angiogenesis is unknown. The effect of VEGF/VPF, a hypoxia-inducible angiogenic factor, on TN expression is unknown. Since TN-C lacks a hypoxia response element, the upregulation of TN in a hypoxic environment, could be mediated by VEGF/VPF. Thus, it is of interest to investigate if VEGF/VPF upregulates TN expression. TN-C may be an angiogenic cofactor by presenting VEGF/VPF to the cell surface as it was described for proteoglycans and FGF (Schlessinger et al., 1995). Besides VEGF/VPF and TN-C, there are other molecules which are also upregulated in a variety of neoplastic and non-neoplastic conditions of the CNS associated with angiogenesis. For example, $\alpha_v ß_3$ integrin required for angiogenesis (Brooks et al., 1994), is upregulated in embryogenesis (Sutherland et al., 1993), in brain neoplasms (Gladson, 1996) and also in cerebral ischemia (Okada et al., 1996). VEGF/VPF and TN-C follow the same paradigm of upregulation in embryogenesis, become almost undetectable in adult

quiescency, and are re-upregulated in tissue injury and activated state. It is therefore likely that embryological, neoplastic and non-neoplastic angiogenesis in the brain is mediated by similar biological compounds and molecules. The accurate regulation of the well-controlled angiogenesis occurring in embryogenesis as opposed to the uncontrolled neovascularization of tumors remains unclear. Nevertheless, VEGF/VPF and TN-C are upregulated in several human pathological neoplastic and non-neoplastic processes of the CNS associated with angiogenesis.

Acknowledgments. The authors wish to thank Dr. Kevin Claffey for generous gift of the VEGF/VPF clone, Dr. Ramona Polvere for preparation of the probe and Wai Chan for excellent technical help. This work was supported by a grant from the American Cancer Society RPG-00-060-01-CCE to DZ.

References

Adams Pearson C., Pearson D., Shibahara S., Hofsteenge J. and Chiquet-Ehrismann R. (1988). Tenascin: cDNA cloning and induction by TGF. EMBO J. 7, 2977-2982.

Ausprunk D.H. and Folkman J. (1977). Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc. Res. 14, 53-65.

Barker F.G., Davis R.L., Chang S.M. and Prados M.D. (1996). Necrosis as a prognostic factor in glioblastoma multiforme. Cancer 77, 1161-1166.

Barleon B., Sozzani S., Zhou D., Weich H.A., Mantovani A. and Marme D. (1996). Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood 87, 3336-3343.

Berse B., Brown L.F., Van De Water L., Dvorak H.F. and Senger D.R. (1992). Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol. Biol. Cell. 3, 211-220.

Binetruy B. (1997). The c-jun-induced transformation process involves complex regulation of Tenascin-C expression. Mol. Cell Biol. 17, 3202-3209.

Bourdon M.A., Wikstrand C.J., Furthmayr H., Matthews T.J. and Bigner D.D. (1983). Human glioma-mesenchymal extracellular matrix antigen defined by monoclonal antibody. Cancer Res. 43, 2796-2805.

Breier G., Clauss M. and Risau W. (1995). Coordinate expression of VEGF receptor-1 (flt-1) and its ligand suggests a paracrine regulation of murine vascular development. Dev. Dynam. 204, 228-239.

Brem S., Cotran R. and Folkman J. (1972). Tumor angiogenesis: a quantitative method for histologic grading. J. Natl. Canc. Inst. 48, 347-356.

Britt R.H. and Enzmann D.R. (1983). Clinical stages of human brain abscesses on serial CT scans after contrast infusion. Computerized tomographic, neuropathological, and clinical correlations. J. Neurosurg. 59, 972-989.

Brodkey J.A., Laywell E.D., O'Brien T.F., Faissner A., Stefansson K., Dorries H., Schachner M. and Steindler D.A. (1995). Focal brain injury and upregulation of a developmentally regulated extracellular matrix protein. J. Neurosurg. 82, l06-112.

Bronner-Fraser M. (1988). Distribution and function of tenascin during cranial neural crest development in the chick. J. Neurosci. Res. 21, 135-147.

Brooks P.C., Clark R.A.F. and Cheresh D.A. (1994). Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. Science 264, 569-571.

Brown L.F., Detmar M., Claffey K., Nagy J.A., Feng D., Dvorak A.M. and Dvorak H.F. (1997). Vascular Permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. EXS. 79, 233-269.

Brown L.F., Detmar M., Tognazzi K., Abu-Jawdeh G. and Iruela-Arispe M.L. (1997). Uterine smooth muscle cells express functional receptors (flt-1 and KDR) for vascular permeability factor/vascular endothelial growth factor. Lab. Invest. 245, 245-255.

Bruce D.A., Alavi A., Bilaniuk L., Dolinskas C., Obrist W. and Uzzell B. (1981). Diffuse cerebral swelling following head injuries in children: the syndrome of “malignant brain edema”. J. Neurosurg. 54, 170-178.

Brustle O., Ohgaki H., Schmitt P., Walter F., Ostertag H. and Kleihues P. (1992). Primitive neuroectodermal tumors after prophylactic central nervous system irradiation in children. Associated with an activated K-ras gene. Cancer 69, 2385-2392.

Burger P.C., Vogel F.S., Green S.B. and Strike T.A. (1985). Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. Cancer 56, 1106-1111.

Cancilla P.A., Frommes S.P., Kahn L.E. and Debault L.E. (1979). Regeneration of cerebral microvessels. A morphologic and histochemical study after local freeze-injury. Lab. Invest. 40, 74-82.

Canfield A.E. and Schor A.M. (1995). Evidence that tenascin and thrombospondin-1 modulate sprouting of endothelial cells. J. Cell Sci. 108, 797-809.

Castaigne P., David M., Pertuiset B., Escourolle R. and Poirier J. (1968). L’ultrastructure des hemangioblastomes du systeme nerveux central. Rev Neurol (Paris) 118, 6-26.

Cheng S.Y., Huang H.J.S., Nagane M., Ji X.D., Wang D., Shih C.C., Arap W., Huang C.M. and Cavenee W.K. (1996). Suppression of glioblastoma angiogenicity and tumorigenicity by inhibition of endogenous expression of vascular endothelial growth factor. Proc. Natl. Acad. Sci. USA 93, 8502-8507.

Chiquet-Ehrismann R., Mackie E.J., Pearson C.A. and Sakakura T. (1986). Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47, 131-139.

Chung C.Y. and Erickson H.P. (1994). Cell surface annexin II is a high affinity receptor for the alternatively spliced segment of tenascin-C. J. Cell Biol. 126, 539-548.

Chung C.Y., Murphy-Ullrich J.E. and Erickson H.P. (1996). Mitogenesis, Cell migration, and loss of focal adhesions induced by tenascin-C interacting with its cell surface receptor, annexin II. Mol. Biol. Cell 7, 883-892.

Chuong C.M. and Chen H.M. (1991). Enhanced expression of neural cell adhesion molecules and tenascin (cytotactin) during wound healing. Am. J. Pathol. 138, 427-440.

Claffey K.P. and Robinson G.S. (1996). Regulation of VEGF/VPF expression in tumor cells: consequences for tumor growth and metastasis. Cancer Met. Rev. 15, 165-176.

Clark R.A., Erickson H.P. and Springer T.A. (1997). Tenascin supports lymphocyte rolling. J. Cell Biol. 137, 755-765.

Copertino D.W., Edelman G.M. and Jones F.S. (1997). Multiple

promoter elements differentially regulate the expression of the mouse tenascin gene. Proc. Natl. Acad. Sci. USA 93, 1846-1851.

Criscuolo G.R., Merrill M.J. and Oldfield E.H. (1988). Further characterization of malignant glioma-derived vascular permeability factor. J. Neurosurg. 69, 254-262.

Crossin K.L. (1991). Cytotactin binding: inhibition of stimulated proliferation and intracellular alkalinization in fibroblasts. Proc. Natl. Acad. Sci. USA 88, 11403-11407.

Daumas-Duport C., Scheithauer B.W., O'Fallon J. and Kelly P.J. (1988). Grading of astrocytomas: a simple and reproducible method. Cancer 62, 2152-2165.

Davis C.M., Danehower S.C., Laurenza A. and Molony J.L. (1993). Identification of a role for the vitronectin receptor and protein kinase C in the induction of endothelial cell vascular formation. J. Cell Biochem. 51, 206-218.

De Vries C., Escobedo J.A., Ueno H., Houck K., Ferrara N. and Williams L.T. (1992). The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 255, 989-991.

Del Maestro R.F., Megyesi J.F. and Farrell C.L. (1990). Mechanisms of tumor-associated edema: a review. Can. J. Neurol. Sci. 17, 177-183.

DeLisser H.M., Christofidou-Solomidou M., Strieter R.M., Burdick M.D., Robinson C.S., Wexler R.S., Kerr J.S., Garlanda C., Merwin J.R., Madri J.A. and Albelda S.M. (1997). Involvement of endothelial PECAM-1/CD31 in angiogenesis. Am. J. Pathol. 151, 671-677.

Deroanne C.F., Hajitou A., Calberg-Bacq C.M., Vusgens B.V. and Lapiere C.M. (1997). Angiogenesis by fibroblast growth factor 4 is mediated through an autocrine up-regulation of vascular endothelial growth factor expression. Cancer Res. 57, 5590-5597.

Deryugina E.I. and Bourdon M.A. (1996). Tenascin mediates human glioma cell migration and modulates cell migration on fibronectin. J. Cell Sci. 109, 643-652.

Detmar M., Brown L.F., Berse B., Jackman R.W., Elicker B.M., Dvorak H.F. and Claffey K.P. (1997). Hypoxia regulates the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptors in human skin. J. Invest. Dermatol. 108, 263-268.

Dorries U., Bartsch U., Nolte C., Roth J. and Schachner M. (1993). Adaptation of a non-radioactive in situ hybridization method electron microscopy: detection of tenascin mRNAs in mouse cerebellum with digoxigenin-labelled probes and gold-labelled antibodies. Histochemistry 99, 251-262.

Dvorak H.F., Detmar M., Claffey K.P., Nagy J.A., van de Water L. and Senger D.R. (1995). Vascular permeability factor/vascular endothelial growth factor: An important mediator of angiogenesis in malignancy and inflammation. Int. Arch. Allergy Immunol. 107, 233-235.

Erickson H.P. (1993). Tenascin-C, tenascin-R, tenascin-X: a family of talented proteins in search of functions. Curr. Op. Cell Biol. 5, 869-876.

Erickson H.P. and Bourdon M.A. (1989). Tenascin: an extracellular matrix protein prominent in specialized embryonic tissues and tumors. Annu. Rev. Cell Biol. 5, 71-92.

Fassler R., Sasaki T., Timpl R., Chu M.L. and Werner S. (1996). Differential regulation of fibulin, tenascin-C, and nidogen expression during wound healing of normal and glucocorticoid-treated mice. Exp. Cell. Res. 222, 111-116.

Feldser D., Agani F., Iyer N.V., Pak B., Ferreira G. and Semenza G.L. (1999). Reciprocal positive regulation of hypoxia-inducible factor 1α and insulin-like growth factor 2. Cancer Res. 59, 3915-3918.

Feng D., Nagy J.A., Hipp J., Dvorak H.F. and Dvorak A.M. (1996). Vesiculo-vacuolar organelles and the regulation of venule permeability of macromolecules by vascular permeability factor, histamine, and serotonin. J. Exp. Med. 183, 1981-1986.

Ferhat L., Chevassus-Au-Louis N., Khrestchatisky M., Ben-Ari Y. and Represa A. (1996). Seizures induce tenascin-C mRNA expression in neurons. J. Neurocytol. 25, 535-546.

Ferrara N., Houck K.A., Jakeman L.B., Winer J. and Leung D.W. (1991). The vascular endothelial growth factor family of polypeptides. J. Cell. Biochem. 47, 211-218.

Finkenzeller G., Marme D., Weich H.A. and Hug H. (1992). Platelet-derived growth factor-induced transcription of the vascular endothelial growth factor gene is mediated by protein kinase C1. Cancer Res. 52, 4821-4823.

Fischer S., Sharma H.S., Karliczek G.F. and Schaper W. (1995). Expression of vascular permeability factor/vascular endothelial growth factor in pig cerebral microvascular endothelial cells and its upregulation by adenosine. Mol. Brain. Res. 28, 141-148.

Folkman J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med. 1, 27-31.

Folkman J. and Klagsburn M. (1987). Angiogenic factors. Science 235, 442-447.

Folkman J. and Shing Y. (1992). Control of angiogenesis by heparin and other sulfated polysaccharides. Adv. Exp. Med. Biol. 313, 355-364.

Forsberg E., Hirsch E., Frohlich L., Meyer M., Ekblom P., Aszodi A., Werner S. and Fassler R. (1996). Skin wounds and severed nerves heal normally in mice lacking tenascin- C. Proc. Natl. Acad. Sci. USA 93, 6594-6599.

Forsythe J.A., Jiang B-H., Iyer N.V., Agani F., Leung S.W., Koos R.D. and Semenza G.L. (1996). Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol. Cell Biol. 16, 4604-4613.

Freeman M.R., Schneck F.X., Gagnon M.L., Corless C., Soker S., Niknejad K., Peoples G.E. and Klagsbrun M. (1995). Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. Cancer Res. 55, 4140-4145.

Garcia J.H. (1992). The evolution of brain infarcts: A review. J. Neuropathol. Exp. Neurol. 51, 387-393.

Giannini C. and Scheithauer B.W. (1997). Classification and grading of low-grade astrocytic tumors in children. Brain Pathol. 7, 785-798.

Gladson C.L. (1996). Expression of integrin αvß3 in small blood vessels of glioblastoma tumors. J. Neuropath. Exp. Neurol. 55, 1143-1149.

Goto F., Goto K., Weindel K. and Folkman J. (1993). Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. Lab Invest. 69, 508-517.

Graeber T.G., Peterson J.F., Tsai M., Monica K., Fornace A.J. Jr and Giaccia A.J. (1994). Hypoxia induces accumulation of p53 protein, but activation of a Gl-phase checkpoint by low-oxygen conditions is independent of p53 status. Mol. Cell Biol. 14, 6264-6277.

Graham D.I., Hume Adams J., Nicoll J.A.R., Maxwell W.L. and Gennarelli T.A. (1995). The nature, distribution and causes of traumatic brain injury. Brain Pathol. 5, 397-406.

Grumet M., Hoffman S., Crossin K.L. and Edelman G.M. (1985). Cytotactin, an extracellular matrix protein of neural and non-neural tissues that mediates glia-neuron interaction. Proc. Natl. Acad. Sci. USA 82, 8075-8079.

Haddad S.F., Moore S.A., Schelper R.L. and Goeken J.A. (1992).

Vascular smooth muscle hyperplasia underlies the formation of glomeruloid vascular structures in glioblastoma multiforme. J. Neuropathol. Exp. Neurol. 51, 488-492.

Hahn A.W.A., Kern F., Jonas U., John M., Buhler F.R. and Resink T.J. (1995). Functional aspects of vascular tenascin-C expression. J. Vasc. Res. 32, 162-174.

Hardman J.M. (1979). The pathology of traumatic brain injuries. Adv. Neurol. 22, 15-50.

Hariri R.J. (1994). Cerebral edema. Neurosurg Clin. N. Am. 5, 687-706.

Heffner R.R. Jr (1993). Inflammatory myopathies. A review. J. Neuropathol. Exp. Neurol. 52, 339-350.

Hoffman E.C., Reyes H., Chu F.F., Sander F., Conley L.H., Brooks B.A. and Hankinson O. (1991). Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 52, 954-958.

Huang L.E., Arany Z., Livingston D.M. and Bunn H.F. (1996). Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J. Biol. Chem. 271, 32253-32259.

Husmann K., Faissner A. and Schachner M. (1992). Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III repeats. J. Cell Biol. 116, 1475-1486.

Ijichi A., Sakuma S. and Tofilon P-J. (1995). Hypoxia-induced vascular endothelial growth factor expression in normal rat astrocyte cultures. Glia 14, 87-93.

Iruela-Arispe M.L., Bornstein P. and Sage H. (1991). Thrombospondin exerts an antiagiogenic effect on cord formation by endothelial cells in vitro. Proc. Natl. Acad. Sci. U.S.A. 88, 5026-5030.

Ivan M., Kondo K., Yang H., Kim W., Valiando J., Ohh M., Salic A., Asara J.M., Lane W.S. and Kaelin W.G. Jr (2001). HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 292: 464-468.

Iyer N.V., Kotch L.E., Agani F., Leung S.W., Laughner E., Wenger R.H., Gassmann M., Gearhart J.D., Lawler A.M., Yu A.Y. and Semenza G.L. (1998). Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1a. Genes Dev. 12, 149-162.

Jaakkola P., Mole D.R., Tian Y.M., Wilson M.I., Gielbert J., Gaskell S.J., Kriegsheim A.V., Hebestreit H.F., Mukherji M., Schofield C.J., Maxwell P.H., Pugh C.W. and Ratcliffe P.J. (2001). Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2 regulated prolyl hydroxylation. Science 292, 468-472.

Jakeman L.B., Armanini M., Phillips H.S. and Ferrara N. (1993). Developmental expression of binding sites and messenger ribonucleic acid for vascular endothelial growth factor suggests a role for this protein in vasculogenesis and angiogenesis. Endocrinology 133, 848-859.

Jallo G.I., Friedlander D.R., Kelly P.J., Wisoff J.H., Grumet M. and Zagzag D. (1997). Tenascin-C expression in the cyst wall and fluid of human brain tumors correlates with angiogenesis. Neurosurgery 41, 1052-1059.

Jelsma R. and Bucy P.C. (1967). The treatment of glioblastoma multiforme of the brain. J. Neurosurg. 27, 388-400.

Jeltsch M., Kaipainen A., Joukov V., Meng X., Lakso M., Rauvala H., Swartz M., Fukumura D., Jain R.K. and Alitalo K. (1997). Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 276, 1423-1425.

Jiang B.H., Semenza G.L., Bauer C. and Marti H.H. (1996). Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O_2 tension. Am. J. Physiol. 271, 1172-1180.

Jiang B-H., Agani F., Passaniti A. and Semenza G.L. (1997). V-SRC induces expression of hypoxia-inducible factor 1 (HIF-1) and transcription of genes encoding vascular endothelial growth factor and enolase 1: Involvement of HIF-1 in tumor progression. Cancer Res. 57, 5328-5335.

Jones P.L., Crack J. and Rabinovitch M. (1997). Regulation of Tenascin-C, a vascular smooth muscle cell survival factor that interacts with the αvß3 integrin to promote epidermal growth factor receptor phosphorylation and growth. J. Cell Biol. 139, 279-293.

Joshi P., Chung C.Y., Aukhil I. and Erickson H.P. (1993). Endothelial cells adhere to the RGD domain and the fibrinogen-like terminal knob of tenascin. J. Cell. Sci. 106, 389-400.

Kaplony A., Zimmerman D.R., Fischer R.W., Imhof B.A., Odermatt B.F., Winterhalter K.H. and Vaughan L. (1991). Tenascin M_r 220 000 isoform expression correlates with corneal cell migration. Development 112, 605-614.

Kieser A., Weich H., Brandner G., Marme D. and Kolch W. (1994). Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. Oncogene 9, 963-969.

Kim K.J., Li B., Winer J., Armanini M., Gillett N., Phillips H.S. and Ferrara N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo. Nature 362, 841-844.

Klatzo I. (1967). Neuropathological aspects of brain edema. J. Neuropathol. Exp. Neurol. 26, 1-14.

Kleihues P., Burger P.C. and Scheithauer B.W. (1993). The new WHO classification of brain tumors. Brain Pathol. 3, 255-268.

Kostinanovsky M., Greco M.A., Cangiarella J. and Zagzag D. (1997). Tenascin-C expression in ultrastructurally defined angiogenic and vasculogenic lesions. Ultrastruct. Pathol. 21, 537-544.

Kovacs Z., Ikezaki K., Samoto K., Inamura T. and Fukui M. (1996). VEGF and flt. Expression time kinetics in rat brain infarct. Stroke 27, 1865-1873.

Kubota Y., Kleinman H.K., Martin G.R. and Lawley T.L.J. (1988). Role of laminin and basement membrane in the morphological differentiation of human endothelial cells into capillary structures. J. Cell Biol. 107, 1589-98.

Lane T.F., Iruela-Arispe M.L., Johnson R.S. and Sage E.H. (1994). SPARC is a source of copper-binding peptides that stimulate angiogenesis. J. Cell Biol. 125, 929-943.

Lee J.Y., Dong S.M., Park W.S., Yoo N.J., Kim C.S., Jang J.J., Chi J.G., Zbar B., Lubensky I.A., Linehan W.M., Vortmeyer A.O. and Zhuang Z. (1998). Loss of heterozygosity and somatic mutations of the VHL tumor suppressor gene in sporadic cerebellar hemangioblastomas. Cancer Res. 59, 504-508.

Leung S.Y., Chan Y.S.A., Wong P.M., Yuen T.S. and Chung P.L. (1997). Expression of vascular endothelial growth factor and its receptors in pilocytic astrocytoma. Am. J. Surg. Pathol. 21, 941-950.

Li J., Perrella M.A., Tsai J.C., Yet S.F., Hsieh C.M., Yoshizumi M., Patterson C., Endege W.O., Zhou F. and Lee M.E. (1995). Induction of vascular endothelial growth factor gene expression by interleukin-1 in rat aortic smooth muscle cells. J. Biol. Chem. 270, 308-312.

Liu H.M. (1988). Neovasculature and blood-brain barrier in ischemic brain infarct. Acta Neuropathol. 75, 422-426.

Liu H.M. (1995). Correlation between proto-oncogene, fibroblast growth factor and adaptive response in brain infarct. Prog. Brain Res. 105, 239-244.

Liu Y., Cox S.R., Morita T. and Kourembanas S. (1995). Hypoxia regulates vascular endothelial growth factor gene expression in

endothelial cells. Identification of a 5' enhancer. Circ. Res. 77, 638-643.

Liwnicz B.H., Wu S.Z. and Tew J.M. (1987). The relationship between the capillary structure and hemorrhage in gliomas. J. Neurosurg. 66, 536-541.

Long D.M. (1970). Capillary ultrastructure and the blood-brain barrier in human malignant brain tumors. J. Neurosurg. 32, 127-144.

Lossinsky A.S., Burdo K., Marko M., Pluta R., Mossakowski M.J. and Wisniewski H.M. (1996). Immunoultrastructural and high-voltage electron microscopic studies of endothelial cell tubules and vesicular-vacuolar organelles in human brain tumors. Soc. Neurosci. Abstract 22, 774.

Louis D.N. and Gusella J.F. (1995). A tiger behind many doors: multiple pathways to malignant glioma. Trends Genet. 11, 412-415.

Mackie E.J., Halfter W. and Liverani D. (1988). Induction of tenascin in healing wounds. J. Cell Biol. 107, 2757-2767.

Mackie E.J., Scott-Burden T., Hahn A.W., Kern F., Bernhardt J., Regenass S., Weller A. and Buhler F.R. (1992). Expression of tenascin by vascular smooth muscle cells. Alterations in hypertensive rats and stimulation by angiotensin II. Am. J. Pathol. 141, 377-388.

Mackie E.J., Tucker R.P., Halfter W., Chiquet-Ehrismann R. and Epperlein H.H. (1988). The distribution of tenascin coincides with pathways of neural crest cell migration. Development 102, 237-250.

Markwalder T-W. (1981). Chronic subdural hematomas: a review. J. Neurosurg. 54, 637-645.

Meiners S., Marone M., Rittenhouse J.L. and Geller H.M. (1993). Regulation of astrocytic tenascin by basic fibroblast growth factor. Dev. Biol. 160, 480-493.

Mettouchi A., Cabon F., Montreau N., Dejong V., Vernier P., Gherzi R., Mercier G. and Binetruy B. (1997). The c-Jun-induced transformation process involves complex regulation of tenascin-C expression. Mol. Cell Biol. 17, 3202-3209.

Michel J.B., Ordway G.A., Richardson J.A. and Williams R.S. (1994). Biphasic induction of immediate early gene expression accompanies activity-dependent angiogenesis and myofiber remodeling of rabbit skeletal muscle. J. Clin. Invest. 94, 277-285.

Millauer B., Wizigmann-Voos S., Schnurch H., Martinez R., Moller N.P., Risau W. and Ullrich A. (1993). High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 72, 835-846.

Millauer B., Shawver L.K., Plate K.H., Risau W. and Ullrich A. (1994). Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1 mutant. Nature 367, 576-579.

Mitchell J., Weller R.O. and Evans H. (1978). Capillary regenereation following thermal lesions in the mouse cerebral cortex: an ultrastructural study. Acta Neuropathol. 44, 167-171.

Mohle R., Green D., Moore M.A.S., Nachman R.L. and Rafii S. (1997). Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. Proc. Natl. Acad. Sci. USA 94, 663-668.

Monacci W.T., Merrill M.J. and Oldfield E.H. (1993). Expression of vascular permeability factor/vascular endothelial growth factor in normal rat tissues. Am. J. Physiol. 264, 995-1002.

Montesano R., Orci L. and Vassalli P. (1983). In vitro rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. J. Cell Biol. 97, 1648-1652.

Mukhopadhyay D., Tsiokas L. and Sukhatme V.P. (1995). Wild-type p53 and v-Src exert opposing influences on human vascular endothelial growth factor gene expression. Cancer Res. 55, 6161-6165.

Murata T., Nakagawa K., Khalil A., Ishibashi T., Inomata H. and Sueishi K. (1996). The relation between expression of vascular endothelial growth factor and breakdown of the blood-retinal barrier in diabetic rat retinas. Lab. Invest. 74, 819-825.

Murphy-Ullrich J.E., Lightner V.A., Aukhil I., Yan Y.Z., Erickson H.P. and Hook M. (1991). Focal adhesion integrity is downregulated by the alternatively spliced domain of human tenascin. J. Cell Biol. 115, 1127-1136.

Nag S., Takahashi J.L. and Kilty D.W. (1997). Role of vascular endothelial growth factor in blood-brain barrier breakdown and angiogenesis in brain trauma. J. Neuropathol. Exp. Neurol. 56, 912-921.

Nakagawa Y., Shinno K., Okajima K. and Matsumoto K. (1990). Perifocal brain edema in experimental brain abscess in rats. Acta Neurochir. Suppl. (Wien) 51, 381-382.

Nakamura S. and Tsubokawa T. (1989). Extraction of angiogenesis factor from chronic subdural haematomas. Significance in capsule formation and haematoma growth. Brain Injury 3, 129-136.

Namiki A., Brogi E., Kearney M., Kim E.A., Wu T., Couffinhal T., Varticovski L. and Isner J.M. (1995). Hypoxia induces vascular endothelial growth factor in cultured human endothelial cells. J. Biol. Chem. 270, 31189-31195.

Ness R. and David S. (1997). Leptomeningeal cells modulate the neurite growth promoting properties of astrocytes in vitro. Glia 19, 47-57.

Nicosia RF. (1998). What is the role of vascular endothelial growth factor-related molecules in tumor angiogenesis. Am. J. Pathol. 153, 11-16.

Nicosia R.F., Bonanno E. and Smith M. (1993). Fibronectin promotes the elongation of microvessels during angiogenesis in vitro. J. Cell Physiol. 154, 654-661.

Nies D.E., Hemesath T.J., Kim J-H., Gulcher J.R. and Stefansson K. (1991). The complete cDNA sequence of human hexabrachion (tenascin). A multidomain protein containing unique epidermal growth factor repeats. J. Biol. Chem. 266, 2818-2823.

Okada Y., Copeland B.R., Hamann G.F., Koziol J.A., Cheresh D.A. and del Zoppo G.J. (1996). Integrin αvß3 is expressed in selected microvessels after focal cerebral ischemia. Am. J. Pathol. 149, 37-44.

Patt S., Thiel G., Maas S., Lozanova T., Prosenc N., Cervos-Navarro J., Witkowski R. and Blumenstock M. (1993). Chromosomal changes and correspondingly altered proto-oncogene expression in human glioma. Value of combined cytogenetic and molecular genetic analysis. Anticancer Res. 13, 113-118.

Peiffer J. (1988). Encephalomyelitis and demyelinating diseases in patients with extracerebral malignant tumors. J. Neuroimmunol. 20, 263-267.

Pertovaara L., Kaipainen A., Mustonen T., Orpana A., Ferrara N., Saksela O. and Alitalo K. (1994). Vascular endothelial growth factor is induced in response to transforming growth factor-ß in fibroblastic and epithelial cells. J. Biol. Chem. 269, 6271-6274.

Petit A.M., Rak J., Hung M.C., Rockwell P., Goldstein N., Fendly B. and Kerbel R.S. (1997). Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo angiogenic implications for signal transduction therapy of solid tumors. Am. J. Pathol. 151, 1523-1530.

Plate K.H., Breier G., Weich H.A. and Risau W. (1992). Vascular

endothelial growth factor is a potential tumor angiogenesis factor in human gliomas in vivo. Nature 359, 845-848.

Plate K.H., Breier G., Millauer B., Ullrich A. and Risau W. (1993). Up-regulation of vascular endothelial growth factor and its cognate receptor in a rat glioma model of tumor angiogenesis. Cancer Res. 53, 5822-5827.

Plate K.H., Beck H., Danner S., Allegrini P.R. and Wiessner C. (1999). Cell type specific upregulation of vascular endothelial growth factor in an MCA-occlusion model of cerebral infarct. J. Neuropathol. Exp. Neurol. 58, 654-66.

Prieto A.L., Andersson-Fisone C. and Crossin K.L. (1992). Characterization of multiple adhesive and counteradhesive domains in the extracellular matrix protein cytotactin. J. Cell Biol. 119, 663-678.

Prineas J.W. and McDonald I.W. (1997). Demyelinating diseases. In: Greenfield neuropathology. 1st Ed. Graham D.I. and Lantos P.L. (eds). Oxford University Press. New York. pp. 813-899.

Putnam T. and Cushing H. (1925). Chronic subdural hematoma. Arch. Surg. 11, 329-393.

Rak J., Mitsuhashi Y., Bayko L., Filmus J., Shirasawa S., Sasazuki T. and Kerbel R.S. (1995). Mutant ras oncogenes upregulated VEGF/VPF expression: Implications for induction and inhibition of tumor angiogenesis. Cancer Res. 55, 4575-4580.

Ravi R., Mookerjee B., Bhujwalla Z.M., Sutter C.H., Artemov D., Zeng Q., Dillehay L.E., Madan A., Semenza G.L. and Bedi A. (2000). Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev. 14, 34-44.

Redick S.D. and Schwarzbauer J.E. (1995). Rapid intracellular assembly of tenascin hexabrachions suggests a novel cotranslational process. J. Cell Sci. 108, 1761-1769.

Rettig W.J. and Garin-Chesa P. (1989). Cell type-specific control of human neuronectin secretion by polypeptide mediators and phorbol ester. J. Histochem. Cytochem. 37, 1777-1786 .

Rettig W.J., Erickson H.P., Albino A.P. and Garin-Chesa P. (1994). Induction of human tenascin (neuronectin) by growth factors and cytokines: cell type-specific signals and signalling pathways. J. Cell Sci. 107, 487-497.

Roberts W.G. and Palade G.E. (1995). Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J. Cell Sci. 108, 2369-2379.

Ropper A.H. and Shafran B. (1984). Brain edema after stroke. Clinical syndrome and intracranial pressure. Arch. Neurol. 41, 26-29.

Ryuto M., Ono M., Izumi H., Yoshida S., Weich H.A., Kohno K. and Kuwano M. (1996). Induction of vascular endothelial growth factor by tumor necrosis factor in human glioma cells. J. Biol. Chem. 271, 28220-28228.

Saga Y., Yagi T., Ikawa Y., Sakakura T. and Aizawa S. (1992). Mice develop normally without tenascin. Genes Dev. 6, 1821-1831.

Sakai T., Ohta M., Furukawa Y., Saga Y., Aizawa S., Kawakatsu H. and Saito M. (1995). Tenascin-C induction by a diffusible factor epidermal growth factor in stromal-epithelial interactions. J. Cell Physiol. 165, 18-29.

Scherer H.J. (1940). The forms of growth in gliomas and their practical significance. Brain 63, 1-53.

Schlessinger J., Lax I. and Lemmon M. (1995). Regulation of growth factor activation by proteoglycans: What is the role of the low affinity receptors? Cell 83, 357-360.

Schor A.M., Canfield A.E., Sloan P. and Schor S.L. (1991). Differentiation of pericytes in culture is accompanied by changes in the extracellular matrix. In Vitro Cell. Dev. Biol. 27, 651-659.

Schweiki D., Itin A., Sofer D. and Keshet E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359, 843-845.

Seghezzi G., Patel S., Ren C.J., Gualandris A., Pintucci G., Robbins E.S., Shapiro R.L., Galloway A.C., Rifkin D.B. and Mignatti P. (1998). Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. J. Cell Biol. 141, 1659-1673.

Semenza G.L., Jiang B.H., Leung S.W., Passantino R., Concordet J-P, Maire P. and Giallongo A. (1996). Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. J. Biol. Chem. 271, 32529-32537.

Semenza G.L. (1998). Hypoxia-inducible factor 1: master regulator of O_2 homeostasis. Curr. Opin. Genet. Dev. 8, 588-594.

Senger D.R., Galli S.J., Dvorak A.M., Perruzzi C.A., Harvey V.S. and Dvorak H.F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219, 983-985.

Shweiki D., Itin A., Soffer D. and Keshet E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359, 843-845.

Shweiki D., Neeman M., Itin A. and Keshet E. (1995). Induction of vascular endothelial growth factor expression by hypoxia and by glucose deficiency in multicell spheroids: Implications for tumor angiogenesis. Proc. Natl. Acad. Sci. USA 92, 768-772.

Siemeister G., Weindel K., Mohrs K., Barleon B., Martiny-Baron G. and Marme D. (1996). Reversion of deregulated expression of vascular endothelial growth factor in human renal carcinoma cells by von Hippel-Lindau tumor suppressor protein. Cancer Res. 56, 2299-2301.

Soker S., Takashima S., Miao H.Q., Neufeld G. and Klagsbrun M. (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 92, 735-745.

Spence S.G. and Poole T.J. (1994). Developing blood vessels and associated extracellular matrix as substrates for neural crest migration in Japanese quail, *Coturnix coturnix japonica*. Int. J. Dev. Biol. 38, 85-98.

Sriramarao P., Mendler M. and Bourdon M.A. (1993). Endothelial cell attachment and spreading on human tenascin is mediated by α2ß1 and αvß3 integrins. J. Cell Sci. 105, 1001-1012.

Stavri G.T., Zachary I.C., Baskerville P.A., Martin J.F. and Erusalimsky JD. (1995). Basic fibroblast growth factor upregulates the expression of vascular endothelial growth factor in vascular smooth muscle cells: synergistic interaction with hypoxia. Circulation 92, 11-14.

Stratmann R., Krieg M., Haas R. and Plate K.H. (1997). Putative control of angiogenesis in hemangioblastomas by the von Hippel-Lindau tumor suppressor gene. J. Neuropathol. Exp. Neurol. 56, 1242-1252.

Sutherland A.E., Calarco P.G. and Damsky C.H. (1993). Developmental regulation of integrin expression at the time of implantation in the mouse embryo. Development 119, 1175-1186.

Tanno H., Nockels R.P., Pitts L.H. and Noble L.J. (1992). Breakdown of the blood-brain barrier after fluid percussive brain injury in the rat: part 2: Effects of hypoxia on permeability to plasma proteins. J. Neurotrauma 9, 335-347.

Tazuke S.I., Mazure N.M., Sugawara J., Carland G., Faessen G.H., Suen L.F., Irwin J.C., Powell D.R., Giaccia A.J. and Giudice L.C. (1998). Hypoxia stimulates insulin-lke growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: a possible model for IGFBP-1 expression in fetal hypoxia. Proc. Natl. Acad. Sci. USA 95, 10188-10193.

Terman B.I., Dougher-Vermazen M., Carrion M.E., Dimitrov D., Armellino D.C., Gospodarowicz D. and Bohlen P. (1992). Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem. Biophys. Res. Commun. 187, 1579-1586.

Tremble P., Chiquet-Ehrismann R. and Werb Z. (1994). The extracellular matrix ligands fibronectin and tenascin collaborate in regulating collagenase gene expression in fibroblasts. Mol. Biol. Cell 5, 439-453.

Tsai J.C., Goldman C.K. and Gillespie G.Y. (1995). Vascular endothelial growth factor in human glioma cell lines: Induced secretion by EGF, PDGF-BB and BFGF. J. Neurosurg. 82, 864-873.

Tucker R.P., Hammarback J.A., Jenrath D.A., Mackie E.J. and Xu Y. (1993). Tenascin expression in the mouse: in situ localization and induction in vitro by bFGF. J. Cell Sci. 104, 69-76.

Uchida K., Uchida S., Nitta K., Yumura W., Marumo F. and Nihei H. (1994). Glomerular endothelial cells in culture express and secrete vascular endothelial growth factor. Am. J. Physiol. 266, 81-88.

Volpert O.V., Dameron K.M. and Bouck N. (1997). Sequential development of an angiogenic phenotype by human fibroblasts progressing to tumorigenicity. Oncogene 14, 1495-1502.

Vortmeyer A.O., Gnarra J.R., Emmert-Buck M.R., Katz D., Linehan W.M., Oldfield E.H. and Zhuang Z. (1997). von Hippel-Lindau gene deletion detected in the stromal cell component of a cerebellar hemangioblastoma associated with von Hippel-Lindau disease. Hum. Pathol. 28, 540-543.

Wang G.L. and Semenza G.L. (1995). Purification and characterization of hypoxia-inducible factor 1. J. Biol. Chem. 270, 1230-1237.

Wang G.L., Jiang B.H., Rue E.A. and Semenza G.L. (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc. Natl. Acad. Sci. USA 2, 5510-5514.

Wang W., Merrill M.J. and Borchardt R.T. (1996). Vascular endothelial growth factor affects permeability of brain microvessel endothelial cells in vitro. Am. J. Physiol. 271, 1973-1980.

Wasson J.C., Saylors R.L. 3d, Zeltzer P., Friedman H.S., Bigner S.H., Burger P.C., Bigner D.D., Look A.T., Douglass E.C. and Brodeur G.M. (1990). Oncogene amplification in pediatric brain tumors. Cancer Res. 50, 2987-2990.

Weber P., Zimmerman D.R., Winterhalter K.H. and Vaughan L. (1995). Tenascin-C binds heparin by its fibronectin type III domain five. J. Biol. Chem. 270, 4619-4623.

Webersinke G., Bauer H., Amberger A., Zach O. and Bauer H.C. (1992). Comparison of gene expression of extracellular matrix molecules in brain microvascular endothelial cells and astrocytes. Biochem. Biophys. Res. Commun. 189, 877-884.

Wehrle-Haller B. and Chiquet M. (1993). Dual function of tenascin: simultaneous promotion of neurite growth and inhibition of glial migration. J. Cell Sci. 106, 597-610.

Weindel K., Moringlane J.R., Marme D. and Weich H.A. (1994). Detection and quantification of vascular endothelial growth factor/vascular permeability factor in brain tumor and cyst fluid: the key to angiogenesis? Neurosurgery 35, 439-449.

White O.B., Norris J.W., Hachinski V.C. and Lewis A. (1979). Death in early stroke, causes and mechanisms. Stroke 10, 743.

Yamashima T. and Yamamoto S. (1984). How do vessels proliferate in the capsule of a chronic subdural hematoma? Neurosurgery 15, 672-678.

Yamashima T., Yamamoto S. and Friede R.L. (1983). The role of endothelial gap junctions in the enlargement of chronic subdural hematomas. J. Neurosurg. 59, 298-303.

Yancopoulos G.D., Klagsbrun M. and Folkman J. (1998). Vasculogenesis, angiogenesis, and growth factors: Ephrins enter the fray at the border. Cell 93, 661-664.

Yoshida S., Ono M., Shono T., Izumi H., Ishibashi T., Suzuki H. and Kuwano M. (1997). Involvement of interleukin-8 vascular endothelial growth factor and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. Mol. Cell Biol. 17, 4015-4023.

Zagzag D. (1995). Angiogenic growth factors in neural embryogenesis and neoplasia. Am. J. Pathol. 146, 293-309.

Zagzag D., Brem S. and Robert F. (1988). Neovascularization and tumor growth in the rabbit brain: A model for experimental studies of angiogenesis and the blood-brain barrier. Am. J. Pathol. 131, 361-372.

Zagzag D., Goldenberg M. and Brem S. (1989). Angiogenesis and blood-brain barrier breakdown modulate CT contrast enhancement: An experimental study in a rabbit brain-tumor model. AJNR 10, 529-534.

Zagzag D., Friedlander D.R., Miller D.C., Dosik J., Cangiarella J., Kostianovsky M., Cohen H., Grumet M. and Greco M.A. (1995). Tenascin expression in astrocytomas correlates with angiogenesis. Cancer Res. 55, 907-914.

Zagzag D., Friedlander D.R., Dosik J., Chikramane S., Chan W., Greco M.A., Allen J.C., Dorovini-Zis K. and Grumet M. (1996). Tenascin-C expression in angiogenic vessels in human astrocytomas and by human brain endothelial cells in vitro. Cancer Res. 56, 182-189.

Zagzag D., Zhong H., Scalzitti J.M., Laughner E., Simons J.W. and Semenza G.L. (2000). Expression of hypoxia-inducible factor 1α in brain tumors: association with angiogenesis, invasion, and progression. Cancer 88, 2606-2618.

Zagzag D., Amirnovin R., Greco M.A., Yee H., Holash J., Wiegand S.J., Zabski S., Yancopoulos G.D. and Grumet M. (2000). Vascular apoptosis and involution in gliomas precede neovascularization: a novel concept for glioma growth and angiogenesis. Lab. Invest. 80, 837-849.

Accepted October 10, 2001