



# Reduced Vascular Endothelial Growth Factor A Levels are Correlated with the Incidence of Brain Edema in Acute Ischemic Stroke Patients

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## Authors' contributions

This work was carried out in collaboration between all authors. Author IS designed the study, wrote the protocol, conducted the study and wrote the first draft of the manuscript. Authors SW, AHS and RL helped in designing the study and in manuscript preparation. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** To study the correlation between the vascular endothelial growth factor A/VEGF-A level and the incidence of brain edema in acute ischemic stroke patients.

**Study Design:** A prospective observational analytic case-control study.

**Place and Duration of Study:** Stroke Unit at the Dr. Sardjito General Hospital, Yogyakarta, Indonesia, between December 2010 and August 2011.

**Methodology:** Seventy-one hospitalized acute ischemic stroke patients were recruited, consisting of 37 subjects in the brain edema group and 34 subjects in the non-brain edema group. Comparative analysis of the VEGF-A levels in blood was performed between the brain edema and non-brain edema groups.

**Results:** The average level of VEGF-A in the brain edema group was 436 pg/mL and the one in

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the non-brain edema group was 746 pg/mL. This difference was statistically significant (95%CI: 5.5-615;  $P=0.046$ ). The proportion of VEGF-A levels less than the calculated cut-off point (638.3 pg/mL) in the brain edema group were significantly greater than the ones in the non-brain edema (83.78% and 58.82%, respectively; OR=3.6; 95%CI=1.06-13.26;  $P=0.020$ ).

**Conclusions:** The decreased levels of VEGF-A in blood were correlated with the incidence of brain edema in acute ischemic stroke patients.

*Keywords: Acute ischemic stroke; VEGF-A; blood; brain edema.*

## 1. INTRODUCTION

Stroke with a characteristic of arterial blockage causes brain ischemia due to the focal interruption of blood flow to the brain [1]. The brain ischemia induces tissue hypoxia that can result in brain edema [2]. This type of edema is known as the vasogenic edema, in which the disruption in the blood-brain barrier increases the cellular permeability, causing displacement of protein and intravascular ions into the extracellular compartment [3]. It is worthy to note that another type of brain edema can also occur during ischemic stroke, i.e., cytotoxic edema. Cytotoxic edema occurs in the core ischemic zone within a few minutes due to the loss of energy supply and anoxic membrane depolarization, resulting in the intracellular accumulation of  $\text{Na}^+$  that further attracts water and causes cell swelling [3]. Progressive cerebral edema subsequently incites severe neurologic deficits due to the displacement of the brain structure, resulting in trans-tentorial and uncal herniation [4]. The severity of brain edema is correlated with the brain infarct and high mortality rates [5]. Unfortunately, majority of patients with a space-occupying lesion due to the infarct hemisphere have a bad outcome with the mortality rate as high as 80% [6,7].

The tissue hypoxia during acute ischemic stroke also activates factors responsible for angiogenesis, including vascular endothelial growth factors (VEGFs) [8]. As a vascular endothelial cell-specific mitogen, VEGFs and their receptors are the cardinal factor during angiogenesis [9,10]. VEGF isoforms belong to a family of growth factors that comprise of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor, in which each of them displays distinct physical as well as biological characteristics and functions through the binding to the tyrosine kinase receptors, i.e., VEGFR-1, VEGFR-2 and VEGFR-3 [9]. During ischemic stroke, VEGF-A (often simply referred as VEGF) serves a much larger role than other VEGF isoforms because VEGF-A can influence

the cell migration, organize the blood vessels, differentiate the endothelial cells and remodel the primary capillary vasculature [11]. Taken together, the VEGF-A-induced angiogenesis may support neuro-restorative processes after ischemic stroke [12]. However, VEGF-A can also increase the vascular permeability [13]. It potentially can further disrupt the blood-brain barrier, thus may deteriorate the clinical condition of ischemic stroke patients [14].

It is known that majority of acute ischemic stroke patients displayed elevation of VEGF-A levels in their blood sera as compared to the ones in healthy controls [15]. However, among acute ischemic stroke patients, it is elusive whether the elevated levels of VEGF-A mediate a protective or destructive effect. In particular, it is unknown whether there is any correlation between the levels of sera VEGF-A and the incidence of brain edema. We therefore performed a prospective observational analytic case-control study on hospitalized acute ischemic stroke patients in Yogyakarta, Indonesia, in order to correlate the levels of sera VEGF-A to the incidence of brain edema in acute ischemic stroke patients.

## 2. MATERIALS AND METHODS

This study was approved by the ethical committee of the Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. This was a prospective observational analytic study using the case-control design. The inclusion criteria for the case were: 1) both male and female with first-ever acute ischemic stroke between the age of 50 and 70 years old who started to exhibit symptoms maximum for 6 days before the admission into the Dr. Sardjito General Hospital; 2) patients suffered from neurologic deficits affecting either the cortical-subcortical area (including aphasia, hemianopia, reduced consciousness, hemiparesis, or hemi-anesthesia) or the brainstem region (including decreased strength and drooping of the face, dysarthria and dysphagia, reduced consciousness, hemiparesis, or hemi-

anesthesia); and 3) patients exhibited brain edema and ischemic stroke verified by the cranial CT scan. The control group itself comprised of acute ischemic stroke patients without brain edema verified by the cranial CT scan who were hospitalized in the Stroke Unit at the Dr. Sardjito General Hospital within the same period as the ones in the case group. The cranial CT scan was performed immediately when patient with suspected stroke was admitted into the emergency department of the Dr. Sardjito General Hospital, as a part of the standard operating procedure for management of stroke. The exclusion criteria for both the case ('brain edema') and control ('non-brain edema') groups were: 1) patients suffered from hemorrhagic stroke, including transformation hemorrhagic or space-occupying lesion due to other causes; and 2) patients who did not complete the research procedure, such as discharged against medical advice.

By heeding the VEGF-A proportion, the odds ratio and the confidence level of 90%, the Schlesselman sample size calculation [16] for each group (with and without brain edema) was 32, thus the minimum total samples were 64 subjects. The independent variable was the VEGF-A level and the dependent variable was brain edema in acute ischemic stroke patient that was verified by the cranial CT scan.

Subjects were recruited via the consecutive sampling method until the targeted sample size was obtained. Identities and clinical characteristics of consented subjects were recorded in a designated case form and subsequently were monitored based on their medical records in the Stroke Unit. The duration of this study was approximately 12 months.

Following 24-hour hospitalization of the subjects, venous blood samples of 20 mL were collected after a minimum 12-hour fasting period. The VEGF-A levels were measured in the collected blood samples by using the quantitative technique of sandwich enzyme linked-immunosorbent assay (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA). Data analysis was done on the VEGF-A levels in acute ischemic stroke subjects with and without brain edema. Chi-square test was performed with the significant level of 0.05 or the confidence level of 90% ( $P < 0.05$ ) by using STATA software version 9 (StataCorp. 2011. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP).

### 3. RESULTS AND DISCUSSION

This study was conducted from December 2010 until August 2011 with total subjects were 74, comprising 37 and 37 patients in the brain edema and non-brain edema group, respectively. However, there were 3 subjects in the latter group who did not have complete data, hence were excluded (hence 34 subjects only in the non-brain edema group). Thus, the subsequent comparison was performed on total 71 subjects. The baseline characteristics of subjects within both groups were depicted in Table 1. Of note, several co-morbidities of these stroke patients were also assessed, including hypertension, hypercholesterolemia, diabetes mellitus and cardiac diseases. As shown in Table 1, the distribution of these co-morbidities were approximately equal between the edema and non-edema groups.

Fig. 1 illustrates the distribution of VEGF-A concentrations within both groups. Interestingly, we observed that the median of VEGF-A levels within the non-brain edema group was greater than the one within the brain edema group (496.65 and 375.40 pg/mL, respectively). A larger deviation of VEGF-A levels within the non-brain edema group was also observed than the ones in the brain edema group.

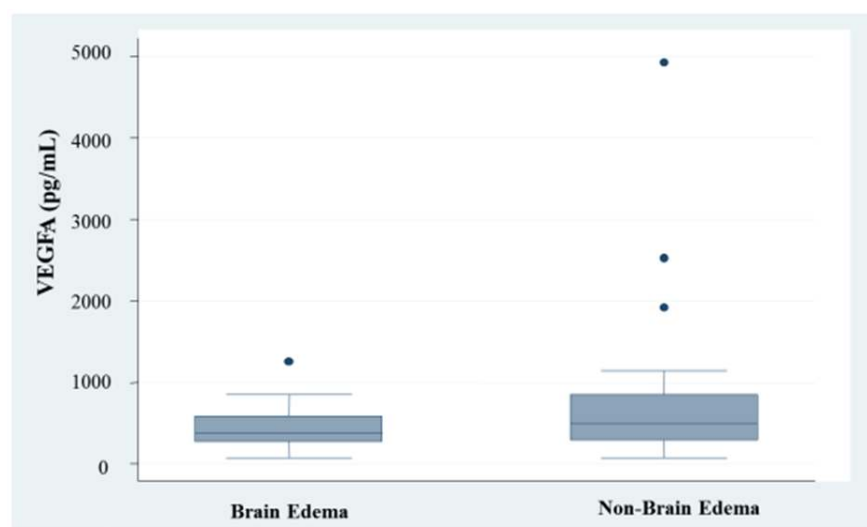
Next, the overall comparison of VEGF-A mean values between the brain edema and the non-brain edema groups is shown in Table 2. The mean value of VEGF-A in the brain edema group was lower than the one in the non-brain edema group (436 and 746 pg/mL, respectively). This difference is statistically significant (95% confidence interval=5.5-615;  $P=.046$ ), suggesting that the reduced levels of blood VEGF-A was correlated with the incidence of brain edema in acute ischemic stroke patients. Furthermore, by using the ROC curve, we obtained the cut-off point for VEGF-A as  $\leq 638.3$  pg/mL (data not shown). While the mean value of VEGF-A within the brain edema group (436 pg/mL) was lower than the cut-off point, the mean value of VEGF-A within the non-brain edema group (746 pg/mL) was higher than the cut-off point.

The influence of VEGF-A levels to the incidence of brain edema based on the cut-off point obtained from the ROC curve is depicted in Table 3. Within the brain edema group, the proportion of patients with VEGF-A levels  $\leq 638.3$  pg/mL was 83.78%. This was greater than the proportion within the non-brain edema group

(58.82%). Indeed, the difference is statistically significant based on the estimated risk rate (OR=3.6; 95%CI=1.06-13.26; P=.020). In addition, this result also shows the higher probability of patients within the brain edema group to have VEGF-A levels  $\leq 638.3$  pg/mL.

**Table 1. The baseline characteristics of the subjects belong to brain edema and non-brain edema groups**

Variables	Brain edema		Non-brain edema		Total		t/ $\chi^2$	P
	Mean	SD	Mean	SD	Mean	SD		
<b>Demography:</b>								
- Age (years)	58.84	10.39	60.0	10.81	71.0	10.53	0.127	.721
- Male (n; %)	20	54.05	21	61.76	41	57.75	0.65	.518
- Female (n; %)	17	5.95	13	38.24	30	42.25		
Therapeutic window (hours)	27.97	30.57	25.74	21.40	26.9	26.42	0.354	.724
<b>History of risk factor:</b>								
- Hypertension (n; %)	26	70.3	21	61.8	47	66.2	1.95	.377
- Diabetes mellitus (n; %)	8	21.6	7	20.6	15	21.1	0.19	.909
- Cardiac diseases (n; %)	3	8.1	3	8.8	6	8.5	0.29	.867
- Hypercholesterolemia (n; %)	4	10.8	9	26.5	13	18.3	2.91	.234
- Smoking habits (n; %)	14	37.8	9	26.5	23	32.4	1.05	.307
<b>Blood pressure (mmHg):</b>								
- Systolic	151	29.3	149	19.4	150	24.9	0.41	.684
- Diastolic	86	14.5	89	10.9	87	12.9	-0.69	.490
<b>Blood chemistry:</b>								
- BUN (mg/dL)	18.9	11.4	17.2	12.7	18.1	12.0	0.58	.561
- Creatinine (mg/dL)	1.07	0.51	1.01	0.50	1.04	0.50	0.55	.585
- Total cholesterol (mmol/L)	195.5	48.2	197	48.2	196.3	47.9	-0.14	.891
- HDL (mmol/L)	40.6	19.0	38.5	12.0	39.6	16.0	0.56	.579
- LDL (mmol/L)	122.9	47.7	127	40.8	124.8	44.3	-0.39	.700
- Triglyceride (mmol/L)	159.7	67.6	145.7	62.9	153.0	65.3	0.90	.372
- Glucose (mmol/L)	159.9	69.6	134.6	48.3	147.8	61.2	1.76	.083
- Albumin (g/dL)	3.10	0.60	3.18	0.65	3.14	0.62	-0.54	.593



**Fig. 1. The distribution of VEGF-A levels in blood within the brain edema and the non-brain edema groups**

The median is indicated as a horizontal line within the box. The bottom and top of the box indicate the first and third quartiles, while whisker indicated the range between third quartile +1.5 inter quartile range (IQR) and first quartile -1.5 IQR. The outlier data are indicated as circles

**Table 2. The comparison of mean levels of VEGF-A between the brain edema and the non-brain edema groups**

	Brain edema		Non-brain edema		P	95% CI	
	Mean	SD	Mean	SD		Lower limit	Upper limit
VEGF-A (pg/mL)	436.0	247.1	746.0	893.2	.046	5.5	615.0

**Table 3. Bi-variate analysis of VEGF-A levels within the brain edema and non-brain edema groups**

VEGF-A	Brain edema		Non-brain edema		$\chi^2$	P	OR	95%CI	
	n	%	N	%				Lower limit	Upper limit
≤638.3	31	83.78	20	58.82	5.5	.020	3.6	1.06	13.26
>638.3	6	16.22	14	41.18					

Our findings indicated an inverse relationship between VEGF-A levels and brain edema among acute ischemic stroke patients. We indeed observed that the reduced levels of sera VEGF-A were linearly correlated with the incidence of brain edema. We therefore speculate that high levels of VEGF-A might actually be beneficial in acute ischemic stroke patients.

The levels of VEGF-A were reported to decrease during the period of 2 to 12 hours post-stroke, but were increased after 24 hours in acute ischemic stroke patients [17]. Its levels peaked after 7 days and it was maintained up to 14 days [15]. Importantly, VEGF-A levels were increased in the penumbra as compared to the infarct region and the healthy contralateral hemisphere [18], suggesting that VEGF-A might help to attenuate the disturbed perfusion by preventing endothelial cell dysfunction and/or inducing angiogenesis [12]. This VEGF-A-dependent rescue mechanism appears to be at least time-dependent. It has been shown in a study using a rat model of focal cerebral embolic ischemia that early post-ischemic (1 hour) administration of recombinant human VEGF<sub>165</sub> worsen the stroke by increasing the blood-brain barrier leakage, enlarging the ischemic lesions and eventually causing hemorrhagic transformation [19]. Nonetheless, the late post-ischemic (48 hours) administration of recombinant human VEGF<sub>165</sub> actually enhanced angiogenesis in the ischemic penumbra and significantly improved neurological deficits [19]. This finding is supported by other studies demonstrating that the intraventricular administration of VEGF-A on 24-72 hours after the middle cerebral artery occlusion in rats indeed resulted in improvement of neurology deficits [20,21]. Taken together, these findings suggest that the late elevation (after 24 hours) of endogenous VEGF-A levels in ischemic stroke can actually mediate the neuro-restorative processes, while avoiding the

potentially deleterious effect of VEGF-A on the blood-brain barrier. Of note, we measured sera VEGF-A levels in our patients starting from 24 hours post-stroke. Therefore, this can partly explain the potential protective effect of VEGF-A observed in this study due to its inverse correlation with the incidence of brain edema.

As priorly mentioned, VEGF-A is not the only pro-angiogenic molecules [17,22]. In addition, the overall angiogenic activity might depend on the balance of concentration and activity between angiogenic promoters and inhibitors. Interestingly, it was reported that acute ischemic stroke patients with a higher endogenous pro-angiogenic balance did not suffer from brain bleeding [17]. This finding indeed supports our argument that the elevated VEGF-A levels might be beneficial in acute ischemic stroke patients.

It is worthy to mention that recent studies also suggest that VEGF-A has direct protective effects on the nervous system [12,23]. It was initially reported that exogenous VEGF-A exerted positive neurotrophic effects in cultured superior cervical and dorsal route ganglion neurons [24] as well in cultured ventral mesencephalon *in vitro* [25]. Subsequent *in vivo* studies using a rat model of middle cerebral artery occlusion demonstrated that exogenous VEGF-A also could induce neuroprotection and neurogenesis. It was indeed reported that delayed administration of VEGF-A (24-72 hours after stroke) reduced the infarct size, enhanced the survival of new neurons, stimulated angiogenesis in the penumbra region, as well as improved recovery of sensorimotor and cognitive deficits [20,21]. To sum, these findings support the potential protective effects of VEGF-A in acute ischemic stroke, i.e., to incite neuroprotection, neurogenesis and angiogenesis.

We acknowledge that are several limitations in this study. First, we did not stratify the patients

based on the size and the location of infarct lesion. In addition, in cases of middle cerebral artery infarction, we did not distinguish patients with malignant middle cerebral artery infarction [26]. It is unknown whether these extreme patients would also display an inverse relationship between VEGF-A and the incidence of brain edema. Intriguingly, several published studies suggested that longer duration of ischemia actually did not enhance the induction of VEGF-A expression [27-29]. In addition, as compared to longer ischemia, reoxygenation of ischemic tissue via reperfusion was more important to rapidly induce VEGF-A expression [27]. These findings suggest that tissue infarct due to extended ischemia probably is not a major factor contributing to the differential expression of VEGF-A levels in our study. However, because we did not stratify the patients based on the size and location of the infarct, we could not verify this. Second, we did not stratify the patients into different subtypes of ischemic stroke, e.g., large-artery atherosclerosis or cardiac embolism. It is still not clear whether various subtypes of ischemic stroke patients would display an inverse relationship between VEGF-A and the incidence of brain edema. Third, we did not obtain detailed information about the medications the subjects used before enrolling in this study. We confirmed that none of our patients used anti-VEGF treatment, including bevacizumab, sorafenib or sunitinib [30], before and/or during the study. However we did not obtain information about the use of other medications affecting VEGF. We therefore recommend performing future studies on acute ischemic stroke patients with more number of subjects, more analysis on size and location of the infarct, and more stringent inclusion criteria in order to elucidate the above mentioned concerns.

#### 4. CONCLUSION

We observed an inverse correlation between the levels of VEGF-A in blood with the incidence of brain edema in acute ischemic stroke patients. We calculated that the probability of a patient to have level of sera VEGF-A smaller or equal to the cut-off point based on the ROC curve would be encountered more within the brain edema group. We therefore suggest that the elevated levels of sera VEGF-A might be protective in acute ischemic stroke patients.

#### CONSENT

All authors declare that written informed consents were obtained from the patients (or

other approved parties) for inclusion in this study. No images of patients or their families are used in this publication.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Taylor JM, Crack PJ. Impact of oxidative stress on neuronal survival. *Clin Exp Pharmacol Physiol.* 2004;31(7):397-406.
2. Hayashi K, et al. Effects of hypoxia on endothelial/pericytic co-culture model of the blood-brain barrier. *Regul Pept.* 2004;123(1-3):77-83.
3. Simard JM, et al. Brain oedema in focal ischaemia: Molecular pathophysiology and theoretical implications. *Lancet Neurol.* 2007;6(3):258-68.
4. Qureshi AI, et al. Timing of neurologic deterioration in massive middle cerebral artery infarction: A multicenter review. *Crit Care Med.* 2003;31(1):272-7.
5. Aiyagari V, Diringner MN. Management of large hemispheric strokes in the neurological intensive care unit. *Neurologist.* 2002;8(3):152-62.
6. Hacke W, et al. 'Malignant' middle cerebral artery territory infarction: Clinical course and prognostic signs. *Arch Neurol.* 1996;53(4):309-15.
7. Berrouschot J, et al. Mortality of space-occupying ('malignant') middle cerebral artery infarction under conservative intensive care. *Intensive Care Med.* 1998;24(6):620-3.
8. Boegehold MA, Kotchen TA. Effect of dietary salt on the skeletal muscle microvasculature in Dahl rats. *Hypertension.* 1990;15(4):420-6.
9. Ogawa S, et al. A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain. *J Biol Chem.* 1998;273(47):31273-82.

10. Leung DW, et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*. 1989;246(4935):1306-9.
11. Felmeden DC, Blann AD, Lip GY. Angiogenesis: Basic pathophysiology and implications for disease. *Eur Heart J*. 2003;24(7):586-603.
12. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: Implications for neurological disorders. *Semin Cell Dev Biol*. 2002;13(1):39-53.
13. Keck PJ, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*. 1989;246(4935):1309-12.
14. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. *Nature*. 2005;437(7058):497-504.
15. Slevin M, et al. Serial measurement of vascular endothelial growth factor and transforming growth factor-beta1 in serum of patients with acute ischemic stroke. *Stroke*. 2000;31(8):1863-70.
16. Schlesselman JJ. Case-control studies: Design, conduct, analysis 1ed. Monographs in Epidemiology and Biostatistics, Oxford: Oxford University Press. 1982;368.
17. Navarro-Sobrino M, et al. A large screening of angiogenesis biomarkers and their association with neurological outcome after ischemic stroke. *Atherosclerosis*. 2011;216(1):205-11.
18. Issa R, et al. Vascular endothelial growth factor and its receptor, KDR, in human brain tissue after ischemic stroke. *Lab Invest*. 1999;79(4):417-25.
19. Zhang ZG, et al. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J Clin Invest*. 2000;106(7):829-38.
20. Sun Y, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest*. 2003;111(12):1843-51.
21. Wang Y, et al. Vascular endothelial growth factor improves recovery of sensorimotor and cognitive deficits after focal cerebral ischemia in the rat. *Brain Res*. 2006;1115(1):186-93.
22. Hermann DM, Zechariah A. Implications of vascular endothelial growth factor for postischemic neurovascular remodeling. *J Cereb Blood Flow Metab*. 2009;29(10):1620-43.
23. Greenberg DA, Jin K. From angiogenesis to neuropathology. *Nature*. 2005;438(7070):954-9.
24. Sondell M, Lundborg G, Kanje M. Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. *J Neurosci*. 1999;19(14):5731-40.
25. Silverman WF, et al. Vascular, glial and neuronal effects of vascular endothelial growth factor in mesencephalic explant cultures. *Neuroscience*. 1999;90(4):1529-41.
26. Arboix A, et al. Malignant middle cerebral artery infarction: A clinical study of 32 patients. *Rev Invest Clin*. 2015;67(1):64-70.
27. Banai S, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation*. 1994;89(5):2183-9.
28. Hashimoto E, et al. Rapid induction of vascular endothelial growth factor expression by transient ischemia in rat heart. *Am J Physiol*. 1994;267(5pt2):H1948-54.
29. Hayashi T, et al. Rapid induction of vascular endothelial growth factor gene expression after transient middle cerebral artery occlusion in rats. *Stroke*. 1997;28(10):2039-44.
30. Ellis LM, Hicklin DJ. VEGF-targeted therapy: Mechanisms of anti-tumour activity. *Nat Rev Cancer*. 2008;8(8):579-91.

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