Journal of the American Heart Association

ORIGINAL RESEARCH

Vascular Endothelial Growth Factor and the Pathogenesis of Intracranial Aneurysms: A Systematic Review on the Missing Link in a Complex Pathway

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BACKGROUND: Aneurysms are one of the most common and yet devastating cerebrovascular diseases after rupture. Despite several decades of scientific advancements including the expansion of the endovascular capabilities and noninvasive imaging modalities, no medical treatment exists to date. This failure is likely largely attributed to the complex and multifactorial nature of aneurysm pathophysiology. Recent research has increasingly implicated vascular endothelial growth factor (VEGF) in the development and rupture of intracranial aneurysms. Regarded as one of the most potent inducers of angiogenesis, it is a key factor in vascular wall maintenance, inflammation, and regulation of vascular permeability. Whether abnormal VEGF expression is directly related to aneurysm development or acting merely as an acute phase reactant remains uncertain. No review of the current-state-of-evidence on this topic exists yet.

METHODS AND RESULTS: A systematic literature search was performed following PRISMA guidelines May 2024 that queried PubMed (1946–2024), Wiley Cochrane Library: Central Register of Controlled Trials (1898–2024), Thompson Reuters Web of Science: Citation Index (1900–2024), and Google Scholar (1946–2024). Inclusion criteria encompassed human and animal studies that investigated the relationship of intracranial aneurysm and VEGF. Several human and animal models revealed significantly elevated expression of VEGF in intracranial aneurysm tissue, along with greater levels in the cerebrospinal fluid and systemically. Overexpression has been shown to inhibit endothelial cell migration, proliferation, and induce cell apoptosis. Recently, genetic polymorphisms of VEGF have also been shown to significantly correlate with the presence of intracranial aneurysms, establishing the first genetic link between the two.

CONCLUSIONS: Despite lacking definitive evidence of a causal relationship, the wealth of supporting data substantiates VEGF as a promising topic for future investigation into aneurysm pathophysiology and as a potential therapeutic target.

Key Words: brain aneurysm ■ cerebral aneurysm ■ intracranial aneurysm ■ pathophysiology ■ systematic review ■ vascular endothelial growth factor

One of the most common and life-threatening vascular pathologies is intracranial aneurysms (IA). Approximately 500 000 people die worldwide from ruptured IAs and account for 3% to 5% of all strokes.[1] Before rupturing, these lesions are most often clinically silent and only found incidentally or recognized after a hemorrhage, which carries as high as a 65% mortality rate.[2] Despite several decades of scientific advancements including the expansion of the endovascular capabilities and noninvasive

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This manuscript was sent to Neel S. Singhal, MD, PhD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.124.035638

For Sources of Funding and Disclosures, see page 14.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What New Question Does This Study Raise?

- Several human and animal models have revealed significantly elevated vascular endothelial growth factor (VEGF) in intracranial aneurysm tissue, along with greater levels in the cerebrospinal fluid and systemically.
- Overexpression of VEGF has been shown to inhibit endothelial cell migration, proliferation, and induce cell apoptosis. Recently, genetic polymorphisms of VEGF have also been shown to significantly correlate with the presence of intracranial aneurysms, establishing the first genetic link between the two.
- The abundance of corroborating evidence substantiates VEGF as a promising topic for future investigation into aneurysm pathophysiology and as a potential therapeutic target.

What Question Should be Addressed Next?

- Additional research is needed to delineate upon VEGF's involvement with intracranial aneurysms, with an emphasis in establishing both a temporal and dose–response relationship.

Nonstandard Abbreviations and Acronyms

EC	endothelial cell
IA	intracranial aneurysms
MMP	matrix metalloproteinase

imaging modalities, no medical treatment exists to date.

Although it is well-known that IAs possess an abnormal wall structure that differs from the normal cerebral artery, the mechanism behind these changes remains unclear.[3] Since IAs preferentially occur at the site of arterial bifurcation or sharp curves, it is suspected that a key triggering event is endothelial cell (EC) injury caused by focal high wall shear stress and a cascade of inflammatory changes.[4,5] ECs experience malfunction at the site of aneurysms with disruption of tight junctions, reduced EC markers (CD 31 and VE-Cadherin), and lower expression of phosphorylated form of endothelial nitric oxide synthase.[6] Using electron microscopy, a study into EC morphological changes over time revealed the early development of interendothelial gap formations with a loss of tight junction protein occludin and ZO-1, followed by leukocyte adhesion and migration into the paracellular space of IAs.[7] Infiltrated with inflammatory cells, greater vascular remodeling and degradation of the extracellular matrix ensues.[8,9] Among the many enzymes involved, matrix metalloproteinases (MMP) have an integral part in mediating this destructive pathway.[10] Eventually loss of smooth muscle cells occurs and the breakdown of elastic lamina causes vessel dilation and the formation of an aneurysm.[11] Once formed, the wall of the aneurysm may undergo degenerative or adaptive remodeling, leading either to further degradation and thinning of the wall or migration and proliferation of mural cells with subsequent thickening.

While hemodynamic factors likely play an essential role in this process, these flow dynamic phenomena take place on every bifurcation or high sheer-stress-related area without causing indiscriminate aneurysm formation. What triggers inflammatory changes in certain patients but not others and what leads to a maladaptive response to hemodynamic stresses is unclear. An emerging topic of interest has been vascular endothelial growth factor (VEGF) and aneurysms.[12,13] Regarded as one of the most potent inducers of angiogenesis, this cytokine exerts direct effects on ECs and surrounding extracellular matrix. It can alter endothelial cell gene expression of collagenase, tissue factor, GLUT-1, uPAR, tPA, and various integrins and protect against EC senescence and apoptosis.[14]

A member of the cystine-knot superfamily of growth factors, VEGF is located on chromosome 6p21.3.[15] It is a 40-kDa heterodimeric glycoprotein that contains a series of bisulfide bridges. Five proteins within this family exist in the mammalian genome sharing a VEGF homology domain: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor.[16] Using a media exposed to bovine pituitary follicular cells, Ferrara et al. first coined the term VEGF after an isolated protein showed growth-promoting activity only toward vascular ECs.[17] VEGF molecules signal through 3 tyrosine-kinase receptors, known as VEGF-R1, VEGF-R2, and VEGF-R3.[18] The VEGF isomers bind to specific receptors, each with different functions. VEGF-R1 and VEGF-R2 promote angiogenesis while VEGF-R3 lymphangiogenesis.[19] VEGF-A has a leading role in angiogenesis while VEGF-C and VEGF-D primarily regulate lymphogenesis.[20]

Through these receptors, the effect VEGF has on ECs is vast and complex. Some of these include increased gene expression through activity of E2F transcription factors, reorganization of the cytoskeleton, prosurival signaling through the mitogen-activated protein kinase pathway, and even autophagy via AMP-activated protein kinase, to name a few.[21–24] However, one of its most potent and powerful functions is as a vascular permeabilizing agent.[25] In fact, at the time of its initial discovery it was identified as a vascular permeability factor

by Senger et al. in 1983.[26] VEGF causes permeability through the development of capillary fenestrations, vesicular vacuolar organelles, and transendothelial gaps.[25] VEGF exposure has been shown to lead to the disassembly of VE-cadherin and loss of occludins and ZO-1 at tight junctions.[27,28] These structural changes may be induced, in part, through endothelial nitric oxide synthase and activation of protein kinase G.[29] Rho kinase (ROCK) also plays a role in altering cellular permeability through the phosphorylation of RhoGTPases (Rho, RAC, and Cdc42) that regulate the cytoskeleton of the cell.[30] While these are just some of the biological actions VEGF has on ECs, a complete understanding of its pleiotropic effects, particularly in the context of specific disease states, is unknown.

Vascular endothelial growth factor has been more extensively studied in the oncologic literature with treatments directed at blocking neovascularization associated with tumor growth.[31,32] Yet, a growing body of evidence has also implicated its role in IAs. There is concern that VEGF may be acting as an upstream regulator in the aforementioned inflammatory cascade of aneurysm formation. However, no review exists yet on this topic. Additionally, the available literature spans several medical disciplines with potentially limited interspecialty crossover.[33–35] Pertinent findings related to VEGF may also have only been presented as discrete items within the context of other investigational aims and are not well-known.[36–39] As such, a comprehensive review of known findings and the current-state-of-evidence is needed. Herein we summarize all the findings and laboratorial investigations available in the published literature on VEGF and IAs.

METHODS

The authors declare that all supporting data are available within the article (and its online Supplementary Files).

Eligibility Criteria

Inclusion criteria specified all studies that investigated or reported on the topic of VEGF and IAs. Both animal and human studies were included. Aneurysms in other locations such as the aorta, common carotid artery, renal artery, and arterial retina were excluded.

Literature Search

A literature search per the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines was performed on May 19, 2024 in the following resources: PubMed (1946–2024), Wiley Cochrane Library: Central Register of Controlled Trials (1898–2024), Thompson Reuters Web of Science: Citation Index (1900–2024), and Google Scholar (1946–2024) (Figure 1). The search strategy for these databases included various MeSH and text word combinations. There were no year limits placed on the search; any articles that were not available in English or a translated version were excluded.

Study Screening and Selection

Primary screening was conducted by 2 reviewers (P.L.N. and O.C.). All references were accessible to the coauthors. These reviewers independently screened the titles and abstracts of two-thirds of the total records thus ensuring that 2 reviewers had screened each record to limit reviewer error. The same 2 reviewers then conducted secondary screening by examining full-text articles that were deemed potentially relevant during primary screening. Any discrepancies were resolved by consensus. An example of the search strategy is provided in Data S1.

Data Abstraction and Analysis

Data were collected directly from each full-length article included in the study. The authors, year published, study subject (ie, human versus animal), number of subjects, pertinent groups compared during the study, major study findings related to IA and VEGF, and *P* value were recorded.

RESULTS

A literature search revealed 391 articles of which 23 met inclusion criteria. Studies were either conducted on humans (n=18), animals (n=4), or a hybrid of both (n=1). Animal models most commonly used Sprague–Dawley rats (n=4), or C57BL/6J mice (n=1). Eighty-three percent of studies (n=19) were published 2010 or later (Figure 2). All studies were categorized by the substrates used in the experiment(s). This included the aneurysm wall tissue, serum/plasma, cerebrospinal fluid (CSF), and genome. Aneurysm wall studies were further subcategorized into VEGF receptors, aneurysm wall VEGF expression, and VEGF modulators. A summary of human studies identified are listed in Table 1 and animal studies in Table 2.

VEGF and Aneurysm Wall Tissue

Elevated VEGF Expression at Aneurysm Wall in Intracranial Aneurysms

One of the most consistent findings has been the presence of VEGF at the site of aneurysm tissue. First published by Skigaudas et al. in 1996, the authors collected aneurysm wall tissue at the time of surgery. Direct immunostaining of the tissue for VEGF was positive in 10 out of 10 samples. Intensity of the immunostaining was also evaluated using assigned gradations with all but 1 patient sample staining as “moderate” or “strong.”

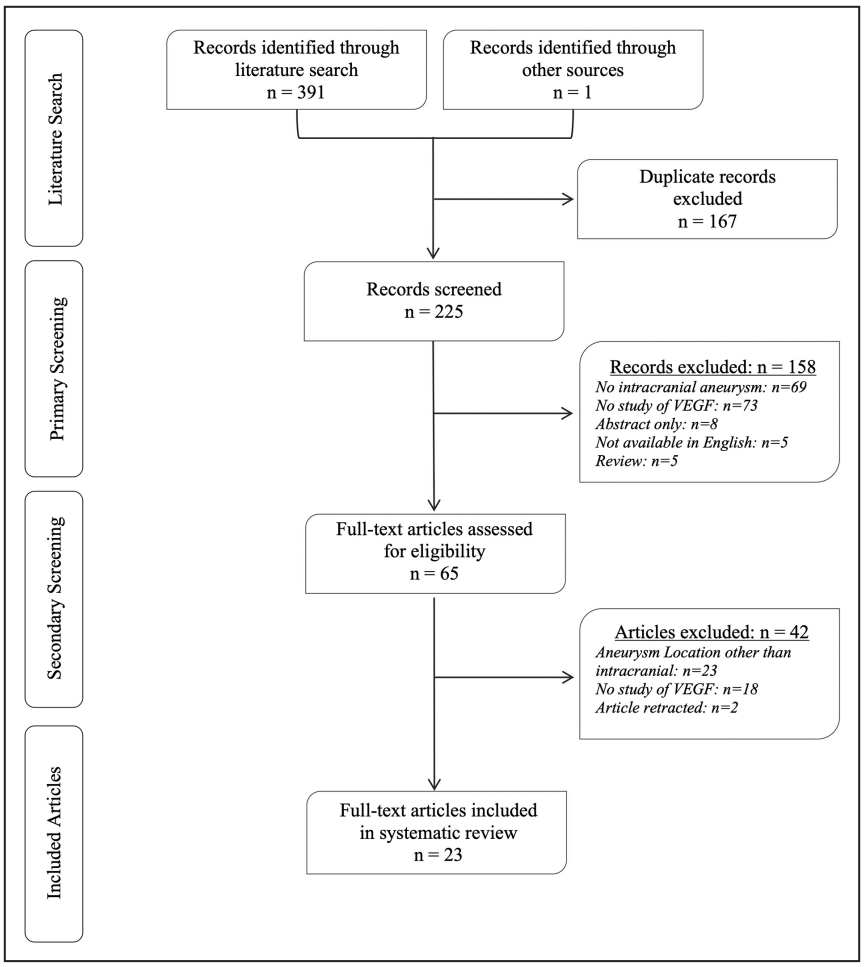

Figure 1. Flow diagram of article selection for the systematic review search.

Conversely, non-aneurysmal vascular tissue located at the Circle of Willis was collected from 3 different autopsies and none stained positive for VEGF except for a few small areas with atherosclerosis.[12] Interestingly, this finding also underscores the relationship between VEGF, atherosclerotic disease, and aneurysms. Positive staining for VEGF has been previously identified at atherosclerotic lesions and shown to effect lipid metabolism, inflammation, and plaque rupture.[55,56] It has been proposed that atherosclerosis may play a role in IA pathogenesis.[57,58] In abdominal aortic aneurysms (AAA), calcifications are recognized as a risk factor for rupture and associated with an increase in mortality.[59] How atherosclerotic changes in the IA wall affect the risk of rupture remains incompletely understood, though lipid accumulation has been associated with wall degeneration.[60] This implies that atherosclerotic degeneration could be 1 pathway leading to IA wall rupture. VEGF may impact this degenerative pathway through affecting endothelial permeability and hence the accumulation of lipids to the IA wall, the presumed initial trigger of atherosclerotic degeneration.

A subsequent investigation also revealed positive VEGF staining in 4 of the 6 unruptured IA tissue samples.[35] Using an animal model of Sprague–Dawley rats (n=3), Ono et al. redemonstrated positive staining pattern of VEGF along the aneurysm wall in addition to all 3 human IA samples, although no normal vascular control tissues were included for comparison.[36] Investigations performing quantitative comparisons of VEGF between IA and non-IA tissue have also been conducted. Fan et al. demonstrated over twice the protein expression of VEGF in human IA tissue compared with normal control arterial wall tissues using Western blot assays ($P<0.01$).[46] This was corroborated in a subsequent study.[37] Others have also demonstrated significantly greater VEGF gene expression in IA tissue compared with controls using polymerase chain reaction (PCR).[37,39,46,53] Another study collected the culture medium (inflammatory smooth muscle medium) from purchased human brain vascular smooth muscle cells that were exposed to mechanical stretching using flexible silicone rubber plates for 48 hours. Human umbilical cord vein endothelial cells were then exposed to this

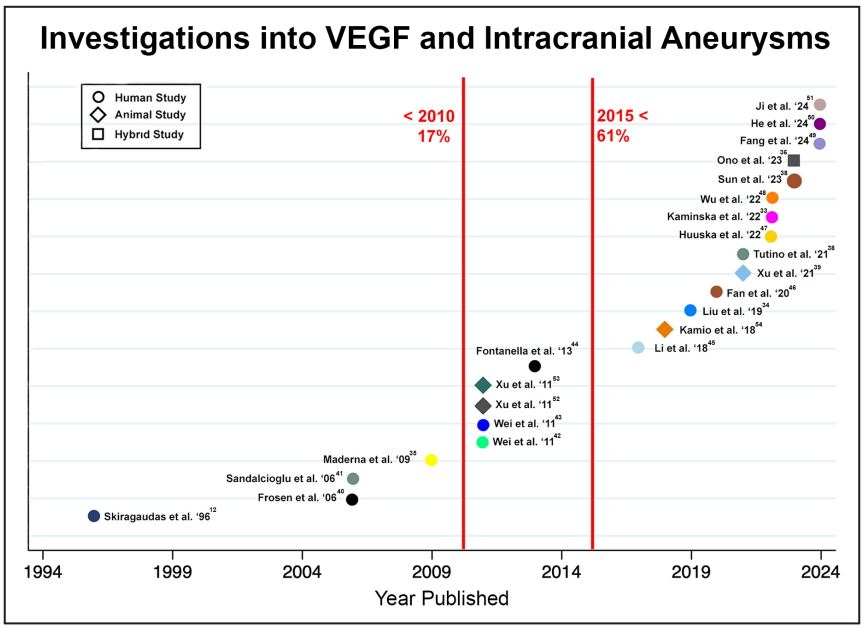

Figure 2. Plot illustrating the studies published on VEGF and intracranial aneurysms over time.
The first investigation was conducted by Skirgauda et al in 1996.[12] Over the following years, there has been a significant expansion of research published on this topic, most of which within the past 5 to 10 years. Since 2015 (depicted by the second red line from left to right) 10 studies were published, comprising 53% of all those available in the literature. Before 2010 (depicted by the first red vertical line from left to right) there were only a total of 4 studies published. Studies that used human subjects are represented by a "circle" while those with a "diamond" shape indicate those that used animals. The single hybrid study with both human and animal subjects is represented by a "square" symbol. VEGF indicates vascular endothelial growth factor.

inflammatory smooth muscle medium, demonstrating reduced VEGF-A expression compared with human umbilical cord vein endothelial cells exposed to other control cell culture mediums. These cells also had reduced tube formation, migration, and reduced viability. Results of this study indicated a unique, proinflammatory phenotype of smooth muscle cells contributed to EC dysfunction through decreased VEGF-A expression, along with integrin protein.[34]

VEGF Receptors and Unruptured IA Versus Ruptured IA

Frosen et al. (2006) analyzed VEGF receptors (VEGF-R1 and VEGF-R2) between ruptured and unruptured IA tissue collected at the time of surgery with immunofluorescence. A total of 56 specimens were scored as having either negative staining, moderate, or strong. The walls of ruptured IAs had a significantly greater proportion of strong or moderate staining for VEGF-R1 compared with unruptured lesions (33% versus 5%, P=0.024) while VEGF-R2 showed no difference (83% versus 74%, P=0.424). VEGF-R1 was associated with wall remodeling (P=0.027), t-cell, macrophage, and organization of luminal thrombosis (P=0.019). Alternatively, VEGF-R2 was associated with the presence of myointimal hyperplasia (P=0.017) and cellular proliferation (P<0.001).[40] A smaller sized study (Maderna et al., 2010) found VEGF-R1 expression loss associated with aneurysm formation. Positive staining for VEGF-R2 was present in all unruptured IA tissue samples (n=6) and 1 healthy control while VEGF-R1 was positive for only 2 of the IA patients and in the 1 healthy control.[35] Lastly, in a recent study positive staining for VEGF-R3 was found in 100% of aneurysms with a thrombus (n=17).[47] While the significance of these findings remains uncertain, it may suggest that not only is VEGF abnormally expressed at these IA locations, but so are its receptors.

Modulators of VEGF

Multiple investigations identified protein interactions or the administration of a particular drug that influenced VEGF expression. Kamio et al. discovered nicotine administration led to an increase in both VEGF expression (P<0.01) and IA rupture rate (P<0.01) using a C57BL/6J mice model.[54] mRNA expression of VEGF was calculated by harvesting the Circle of Willis in the mice with IAs and compared with those without

Table 1. Literature Review Summary of Human Studies on Vascular-Endothelial-Growth-Factor and Intracranial Aneurysms

Author	Year	Tissue location	No.	Comparison groups	Results/conclusions	*P* value
Skirgaudas et al.[12]	1996	IA wall	13	IA vs Control	VEGF expression 10/10 on IA tissue	NA
Frosen et al.[40]	2006	IA wall	56	IA vs Control	VEGF-R1 associated with ruptured aneurysm	0.024
					VEGF-R2 no difference between rupture and unruptured group	0.424
					VEGF-R1 associated with wall remodeling	0.027
					VEGF-R1 associated with T-cell and macrophage infiltration	0.019
					VEGF-R2 associated with myointimal hyperplasia	0.017
Sandalcioglu et al.[41]	2006	Serum	84	IA vs Control	IA had elevated VEGF serum level	0.05 (not sig)
					Elevated serum VEGF levels in females compared with males	0.04
					Elevated serum VEGF in IA males vs non-IA males	0.04
					Serum VEGF levels in women age-dependent	0.04
Maderna et al.[35]	2009	IA wall	7	IA vs Control	Abnormal distribution of VEGF, VEGFR1, VEGFR2 in IA tissue	NA
					Less VEGFR1 expression in IA tissue versus control patient	NA
Wei et al.[42]	2011	IA wall	96	Ruptured vs Unruptured IA	No difference in plasma VEGF level between ruptured and unruptured IA patients	0.298
				IA vs Control	Greater plasma VEGF level in IA than non-IA patients	<0.01
Wei et al.[43]	2011	IA wall	32	Ruptured IA vs Controls (without IA)	Positive correlation of plasma VEGF levels and epithelial progenitor cells	<0.001
					VEGF levels rise after coiling and peak on day 14	<0.01
Fontanella et al.[44]	2013	IA wall	400	Ruptured IA vs Controls (without IA)	No difference in VEGF 18 bp deletion between Ruptured IA and controls without IA	0.157
					No difference in VEGF +936C>T polymorphism between Ruptured IA and controls	0.328
Li et al.[45]	2017	Genome	242	IA vs Control	VEGFA rs3025039 TT genotype higher in IA patients (OR 3.09)	0.025
					VEGFA rs3025039 T allele higher in IA patients (OR 1.81)	0.006
					VEGFA rs3025039 TT genotype significantly related to the number and size of IAs	<0.05
Liu et al.[34]	2019	IA wall	10	IA vs Control	iSMC medium had decreased VEGF-A expression compared with other cells	NA
Fan et al.[46]	2020	IA wall	100	IA vs Control	Greater expression of VEGF in IA tissue	<0.01
					VEGF expression positively correlates with miR-566 expression	<0.01
Tutino et al.[38]	2021	Genome	58	Ruptured IA vs Control	VEGFA identified as one of the leading immune signaling molecules involved in aneurysm rupture	NA
Huuska et al.[47]	2022	IA wall	36	Ruptured vs Unruptured IA	VEGFR-3 widely expressed on saccular aneurysm walls	NA
					VEGFR-3 found in aneurysm lymphatic vessel	NA
Kaminska et al.[33]	2022	CSF	55	IA vs Control	VEGF significantly greater in CSF of patients with IAs	0.0497

(*Continued*)

Table 1. Continued

Author	Year	Tissue location	No.	Comparison groups	Results/conclusions	*P* value
Wu et al.[48]	2022	Genome	79	Ruptured vs Unruptured IA	Using databases of differentially expressed genes, predictive software identified VEGFR inhibitor (Tivozanib) as a potential treatment target for ruptured IAs	NA
Ono et al.[36]	2023	IA wall	3	None	VEGF present at aneurysm wall	NA
					VEGF location aligns with macrophage location	NA
Sun et al.[37]	2023	IA wall	150	IA vs Control	VEGFA increased in IA tissue compared with controls	<0.01
					miR-34a-5p binds VEGFA	<0.01
					miR-34a-5p silencing prevents HDAC9 inhibition of VEGFA	<0.05
					In aneurysms, VEGFA negatively correlated with miR-34a-5p and positively correlated with HDAC9	<0.01
					VEGFA expression increased by overexpression of HDAC9	<0.05
					VEGFA expression inhibited by downregulation of HDAC9	<0.05
					Overexpression of VEGFA associated with cell apoptosis, inhibition of vascular endothelial cells migration and proliferation	<0.05
Fang et al.[49]	2024	Genome	348015	Ruptured vs Unruptured IA	Mendellian randomization estimate for VEGF was associated with ruptured IA but not present for external population group	NA
He et al.[50]	2024	Genome	79429	IA vs Control	Mendellian randomization showed level of VEGF increased with the development of IA	NA
Ji et al.[51]	2024	IA wall	4	IA	Single-cell RNA sequencing revealed enriched expression for genes in the signaling pathways of VEGF	NA
					VEGF-C highly expressed	NA

CSF indicates cerebrospinal fluid; IA, intracranial aneurysm; iSMC, inflammatory smooth muscle culture; NA, not available; and VEGF, vascular endothelial growth factor.

in this study. Cigarette smoking is associated with a 3- to 4-times risk for IA rupture.[61] Elevated plasma VEGF levels has been discovered in smokers compared with non-smokers and an in vitro study revealed endothelial cells produced VEGF after exposure to nicotine and cotinine.[62,63]

A non-coding micro ribonucleic acid (miR-566) implicated in various pathophysiological processes has also been investigated. Aneurysmal wall tissue was compared in 50 IA patients to arterial wall in 50 healthy volunteers. Western blot and quantitative real time-PCR revealed VEGF levels positively correlated with miR-566 (r=0.77, $P<0.01$). miR-566 was significantly higher in aneurysm tissue than controls ($P<0.01$).[46] In a second study, micro-RNA called miR-34a-5p was compared between human IA tissue (n=75) and normal intracranial arterioles as controls from trauma patients undergoing surgery (n=75). It was hypothesized that histone deacetylase 9 (HDAC9) regulated miR-34a-5p which in turn was involved in immunomodulation and VEGF-A regulation in IAs. Vascular endothelial cells were harvested from each group. Some were transfected with VEGF-A and HDAC9 sequences using pcDNA3.1 vectors (Invitrogen, Carlsbad, CA) to induce the overexpression of the respective genes. Others were treated with an miR-34a-5p inhibitor vector. Using this model, miR-34a-5p was found to specifically bind to VEGF-A using Dual Luciferase assay and was negatively correlated with VEGF-A expression (r=−0.37, $P<0.01$). Conversely, VEGF-A expression positively correlated with HDAC9 (r=0.56, $P<0.05$). Cells with overexpression of VEGF-A were associated with apoptosis along with inhibition of vascular EC migration and proliferation ($P<0.05$).[37] These findings add to the complexity of VEGF-A function, which has traditionally been viewed as a prosurvival factor and yet can act as an apoptotic signal as well. This corroborates a prior study where VEGF in combination of transforming growth factor beta 1 (TGF-B1) acted as a proapoptotic signal through a VEGF/flk-1 activated $p38^{MAPK}$ pathway.[64] Of note, concomitant expression of TGF-B1 receptors and VEGF receptors has been reported in the walls of some human IAs.[40]

Table 2. Literature Review Summary of Animal Studies on Vascular Endothelial Growth Factor and Intracranial Aneurysms

Author	Year	Animal Model	No.	Comparison Groups	Results/Conclusions	*P* value
Xu et al.[52]	2011	Sprague–Dawley rats	49	(±Erythropoietin: IA vs Controls)	Erythropoietin treated mice have higher serum VEGF levels after aneurysm induction surgery	<0.01
Xu et al.[53]	2011	Sprague–Dawley rats	100	(±Hyperhomocysteinemia: IA vs Controls)	Increased RNA VEGF expression at IA wall site in +hyperhomocysteinemia IA group	<0.01
Kamio et al.[54]	2018	C57BL/6J mice	NA	(IA: Nicotine vs a7*-nAChR Antagonist vs Control)	Nicotine causes increased levels of mRNA VEGF and nicotine associated with IA rupture rate	<0.01
Xu et al.[39]	2021	Sprague–Dawley rats	50	(±Hyperhomocysteinemia: IA+Simvastatin vs Controls)	Increased Serum VEGF level in hyperhomocysteinemia IA group compared with controls	<0.01
					Serum VEGF levels suppressed in aneurysm group treated with simvastatin than IA group without simvastatin	<0.01
					RNA VEGF expression at aneurysm wall site elevated in IA group	<0.01
					RNA VEGF expression at aneurysm wall site suppressed in IA group treated with simvastatin	<0.01
Ono et al.[36]	2023	Sprague–Dawley rats	3	None	VEGF present at IA wall	NA
					VEGF location aligns with macrophage location	NA

a7*-nAChR indicates nicotine acetylcholine receptor containing 7 alpha subunits; IA, intracranial aneurysm; and VEGF, vascular endothelial growth factor.

Elevated VEGF Serum/Plasma in Patients With IA

The first study to report elevated serum VEGF levels in patients with IA was Sandalcioglu et al. (2006). Though IA serum VEGF levels did not reach significance (*P*=0.05) when compared between those with and without IAs (n=84), a significant difference was found in a subanalysis looking specifically at IAs in males (*P*=0.04). Significantly greater VEGF levels were found in women compared with men, (*P*=0.04) along with a female age-dependence increase in VEGF (*P*=0.04).[41]

This particular finding is corroborated by Byrne et al. who reported significantly greater levels of circulating VEGF in postmenopausal women than premenopausal.[65] Females have approximately a 3 times greater prevalence of IAs compared with males.[2] Additionally, they experience double the rate of subarachnoid hemorrhage and higher rates of aneurysm multiplicity (11% versus 6%) compared with males.[66–68] Curiously, the discrepancy in IA prevalence between sexes is near equal until after the age of 50 years, at which point it substantially rises for women. This happens to correlate with the mean age of menopause which occurs between 50 and 52 years in the United States.[69]

In a follow-up study (n=96), Wei et al. (2011) found a significantly greater plasma VEGF levels in patients with IA than healthy controls (99.2 versus 35.75 pg/mL, *P*<0.01). However, there was also a significantly greater proportion of current smokers versus non-smoker in the unruptured IA group compared with controls (33% versus 3%, *P*=0.03), which may have also affected their results given the known association of elevated VEGF with smoking.[42,62,63] Within the IA group, no difference in VEGF levels was found between the ruptured (n=35) and unruptured patients (n=21) (*P*=0.298).[42] Another study (Wei et al., 2011) measured serum VEGF levels in 14 patients with ruptured IAs. Blood was collected at the time of admission, and post-procedure days 1, 4, 14, and 21. The serum levels demonstrated a stepwise increase of plasma VEGF after treatment with a peak at day 14. A positive correlation of endothelial progenitor cells in the serum and VEGF was identified as well (r=0.636, *P*<0.001).[43] Two other studies measured serum VEGF levels in male Sprague–Dawley rat IA models. These studies showed groups in the IA surgery group that received hyperhomocysteinemia had the greatest VEGF levels (*P*<0.01) along with those with the largest internal carotid aneurysm sizes (*P*<0.01).[39,52]

However, the interpretation of VEGF levels may be limited because of differences in the collection of VEGF-A from blood. Since VEGF-A is secreted by alpha granules within platelets, serum is considered the least reliable because of ex vivo platelet activation.[70] Currently, no standardized protocol exists for measuring VEGF from plasma. Some studies recommend for the use of Citrate-Theophylline-Adenosine-Dipyidamole (CTAD) collection tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey) as they have been shown to have the least platelet activation compared with EDTA and sodium citrate tubes.[71,72] Other protocols call for a double centrifuge technique to obtain "platelet depleted plasma" or "platelet poor plasma" to allow for the maximal reduction of platelets.[65] Type of centrifuge, time-before-centrifuge, placement on ice after collection, anticoagulant use, and tube filling amount may also impact VEGF-A levels.[73] A

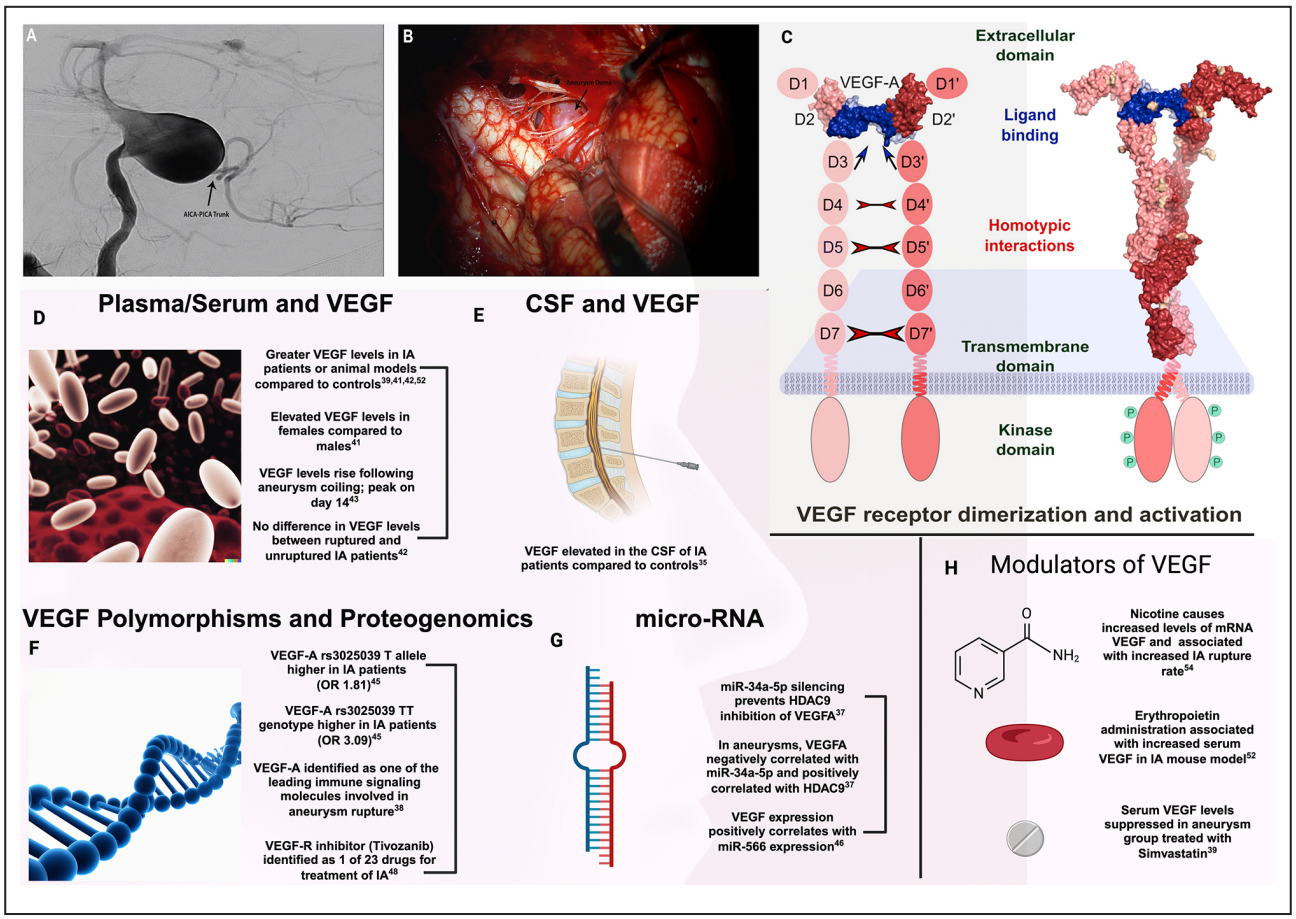

Figure 3. Summary of major investigative findings related to VEGF and other anatomical locations in the setting of intracranial aneurysms is provided.
A, An example of a patient with a giant, complex saccular aneurysm located at the junction of the vertebral artery and a posterior inferior cerebellar artery-anterior inferior cerebellar artery trunk. The patient was treated with microsurgical aneurysm clipping for trapping of the aneurysm and followed by end-to-end occipital artery-anterior inferior cerebellar artery-posterior inferior cerebellar artery bypass for revascularization. Reprinted from Nisson et al[75] with permission. Copyright ©2020, Wolters Kluwer Health, Inc. **B,** The intraoperative findings of the large aneurysm. **C,** The crystal structure of VEGF-A in complex with VEGF-R1 domains D1-6 and dimerization. Reprinted from Markovic-Mueller et al[76] with permission. Copyright ©2017, Elsevier. **D–H,** Investigative findings by anatomical location with associated references provided as a numerical superscript. AICA indicates anterior inferior cerebellar artery; CSF, cerebrospinal fluid; PICA, posterior inferior cerebellar artery; and VEGF, vascular endothelial growth factor.

detailed protocol of blood collection and or measures taken to avoid contamination by platelet activation is missing from the articles in this review.[39,41–43,52] This limitation does not necessarily invalidate the findings as both groups being compared would theoretically be exposed to the same confounders. Since platelets are a known acute phase reactant, groups with ruptured IAs may have had reactive thrombocytosis which could affect the results, but this would not be expected for those with unruptured IAs or controls.[74]

Elevated VEGF in CSF for Patients With Unruptured IA

Only one study has compared the level of VEGF in the CSF between patients with and without IAs. Forty subjects that underwent microsurgical clipping of an unruptured IA had CSF collected at the time of surgery from the subarachnoid space. A control group of 15 patients who underwent microvascular decompression for trigeminal neuralgia also had CSF collected in a similar fashion. Using an ELISA assay, VEGF was nearly 6x higher in the IA group (63.80 versus 10.86 pg/mL, P=0.0497).[33]

Genetic VEGF Polymorphisms, Differentially Expressed Genes, and IA

Several studies have focused on VEGF genetics and IAs. Fontanella et al. was the first to do so in 2013.[44] Two functional polymorphisms were compared between patients with ruptured IA and healthy controls

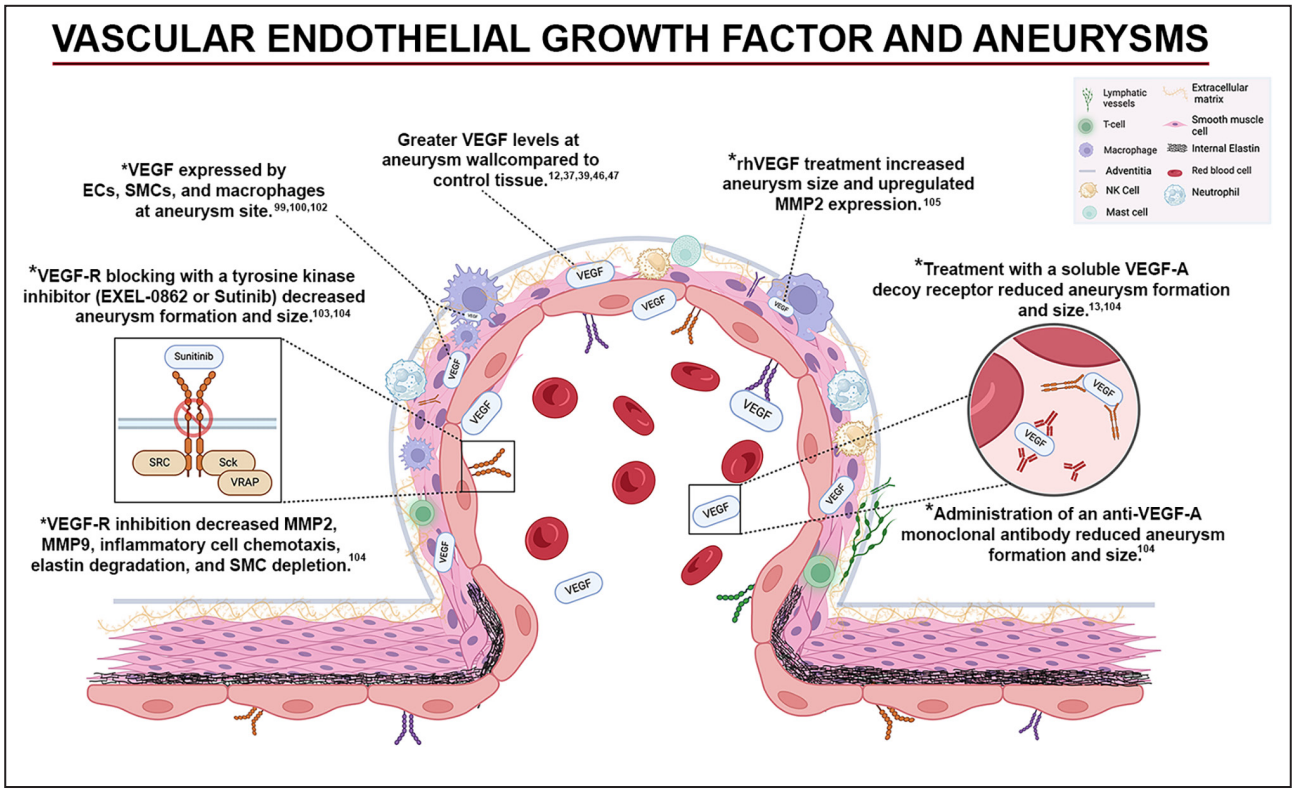

Figure 4. Summary of major investigative findings related to VEGF and aneurysm walls.
The complex inflammatory response with recruitment of t-lymphocytes, mast cells, natural killer cells, neutrophils, and macrophages is shown with loss of the internal elastic lamina. There is dysfunctional remodeling of the extracellular matrix and thinning of the smooth muscle cell layer and disorganization. VEGF is widely distributed throughout the aneurysmal wall (ie, endothelium, tunica media, and adventia) along with its receptors including VEGF-R1, VEGF-R2, and VEGF-R3. The illustration is not drawn to scale. References to the studies where the findings were obtained are provided as a numerical superscript value. The symbol * designates study findings specific to abdominal aortic aneurysms. EC indicates endothelial cells; MMP2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; NK cell, natural killer cell; rhVEGF, recombinant VEGF; SMCs, smooth muscle cells; VEGF, vascular endothelial growth factor; VEGF-R, VEGF-receptor.

(n=400). The polymorphisms were located on the promoter region of the VEGF gene (+396 C>T and 18 bp microdeletion). No difference was found for either one between groups. A follow-up case–control study by Li et al. (2017) compared 2 single nucleotide polymorphisms (SNPs) for VEGF-A (rs3025039 and rs201096) between patients with IAs and healthy controls (n=242). rs3025039 allele TT (11% versus 5%) and T (29% versus 18%) were both significantly greater in the IA group than the control (P<0.05). They also found the number and size of IAs correlated with rs30205039 genotypes (P<0.05).[45] Ji et al. (2024) performed single-cell RNA sequencing (ssRNA) on ruptured and unruptured human IA domes. They found ECs had enriched expression for genes in the signaling pathways of VEGF and transforming growth factor-B. VEGF-C was also highly expressed.[51]

Two investigations with similar designs performed Mendelian randomization using summary statistics obtained from a genome-wide association study. One identified VEGF levels as a risk factor for aneurysmal subarachnoid hemorrhage in the primary analysis. Although, a subsequent external analysis failed to show any correlation of VEGF molecular subtypes and subarachnoid hemorrhage.[49] Conversely, the other study found levels of VEGF increased in unruptured IAs compared with controls but not for subarachnoid hemorrhage.[50]

Other studies have compared genetic data from the Gene Expression Omnibus database between ruptured IAs, unruptured IAs, and controls. VEGF-A (along with IL-1, tumor necrosis factor, and PPARG) was identified as one of the leading immune signaling molecules responsible for functions such as recruitment and activation of leukocytes, adhesion to immune cells, and degranulation in ruptured aneurysms.[38] Additionally, using the Connectivity Map database for small drug molecules, VEGF-R inhibitor (Tivozanib) was revealed as a potential treatment target.[48] A summary of these unique findings including an aneurysm illustrative case and the molecular structure of VEGF-A is provided in Figure 3.[75,76]

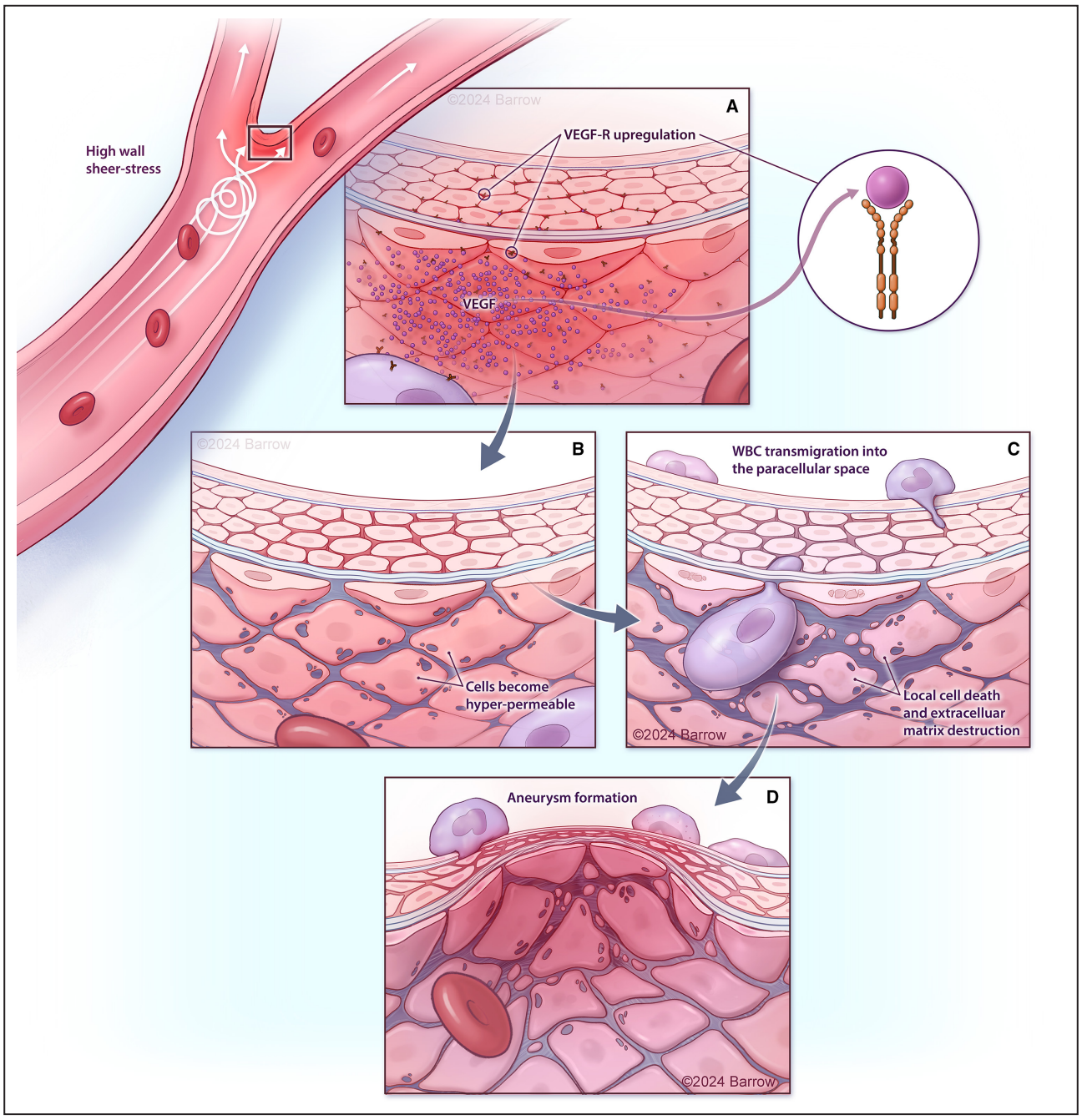

Figure 5. Illustration depicting one of the major proposed mechanisms by which VEGF contributes toward intracranial aneurysms progression.
A, A zoomed-in image at the site of persistent high wall sheer stress shows local inflammation with VEGF release and binding to its receptors, initiating a positive feedback loop. **B,** Overactivation of VEGF-Rs induces changes in the EC barrier, including loss of tight junctions, interendothelial gaps, cellular contraction, and increased permeability. **C,** White blood cells are recruited into the paracellular space, leading to local destruction of the extracellular matrix and cell death. **D,** Eventually, degradation and dilation of the vessel wall result in IA formation. VEGF indicates vascular endothelial growth factor; and VEGF-R, vascular endothelial growth factor receptor.

DISCUSSION

A complex web of interconnected genetic, environmental, and anatomical factors continue to obscure a full understanding for the pathophysiology of IAs. Since the first article by Skirgaudas et al. in 1996 revealing the presence of VEGF at IA wall tissue, a growing body of literature has implicated VEGF in the pathogenesis of aneurysms.[12,33–37,41,42,44–47,49,51,77] The most frequently reported finding has been greater

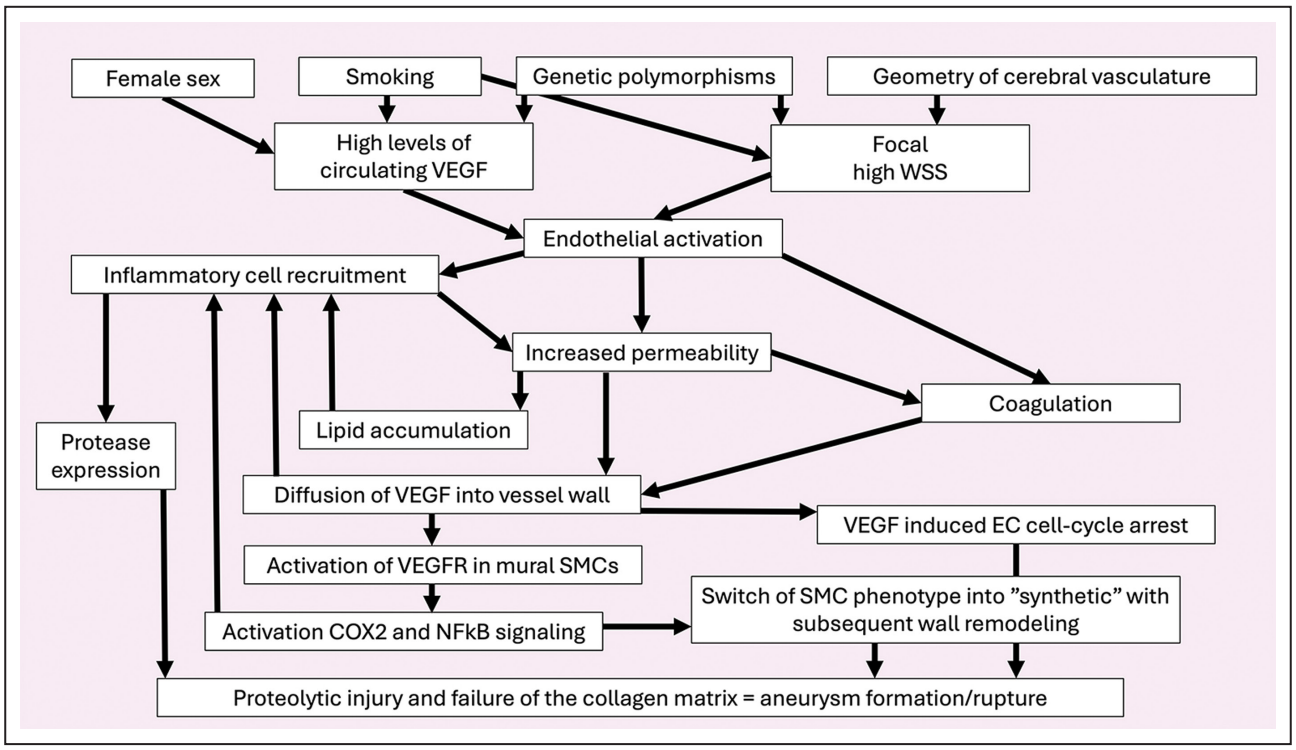

Figure 6. Flow chart demonstrating the web of interconnected factors between VEGF and aneurysms.
COX2 indicates cyclooxygenase 2; NF-kB, nuclear factor-kappa B; SMC, smooth muscle cell; VEGF, vascular endothelial growth factor; WSS, wall shear stress.

VEGF at the aneurysm wall compared with controls. This has been evaluated qualitatively with immunohistochemical staining, semi-quantitatively using assigned gradations, and quantitatively using PCR, and Western blot assay. Moreover, this has been demonstrated in different patient populations of different sizes and reproduced in animal models.[12,36,37,39,46,53] A distinct profile of VEGF receptors at IA tissue has also been identified.[35,40] With the exception of only one study that tittered a decimal away from reaching significance (P=0.05), all investigations have reported elevated VEGF systemically and in the CSF in those with IAs compared with controls.[33,37,41,43,46] However, it remains unclear if VEGF is simply an acute phase reactant at a site of injury or if it has a larger role in aneurysm genesis.

VEGF; Causation Versus Association

Vascular endothelial growth factor is a potent cytokine produced by various cell types including macrophages, monocytes, neutrophils, and enothelium.[78–80] Inflammation is a known stimulus for VEGF production, with increased plasma/serum levels detected in patients with cancer, rheumatoid arthritis, and other chronic inflammatory diseases.[81] Accordingly, macrophages have been identified as a major source of VEGF production at IAs.[36] It is important to make note that elevated cytokine levels in patients with IA were not always exclusive to VEGF. Several other molecules in aneurysm tissue were also elevated in these studies. For instance, greater arachidonate 5-lipoxygenase levels in IA patients was also confirmed with both Western blot and PCR analysis.[46] Multiple interleukin factors were also elevated in the CSF of IA patients.[33]

Yet, unlike the other acute phase reactants elevated in IA patients, VEGF possesses functions that garner suspicion for a greater role in aneurysm formation. Unlike many of the other cytokines that regulate an immune response, VEGF acts as a vital mitogen for vascular ECs along with the migration and recruitment of epithelial progenitor cells.[82,83] This is particularly relevant to aneurysms which are marked by inflammation and a lack ECs.[4,84] It can also act as an inducer of angiotensin II mediated vascular inflammation and remodeling.[85] Consistent with these plausible mechanistic-pathways, VEGF was shown to be elevated IA tissue and associated with significant impairment of epithelial progenitor cell migration in patients with IAs.[12,37,42] The strongest finding that supports a role beyond merely colocalization of VEGF and IAs was the difference in single nucleotide polymorphisms. Li et al. demonstrated a 3.09 (P=0.025) odds ratio for

IAs for those with rs3025039 TT and 1.8 odds ratio for rs3025039 T. The rs3025039 polymorphism also correlated with number of aneurysms and aneurysm size.[45] Further information related to how these genotypes influence VEGF expression and or ligand-receptor affinity is needed.

VEGF; Protective or Pathologic

Conflicting functions of VEGF have likely further complicated understanding its role in aneurysm development. Previous studies have shown under- and over-expression are both lethal during embryonic development.[86] Arterial aneurysms and dissection have also been cited as a possible side effect from the use of VEGF inhibitors in cancer patients.[87] Human umbilical cord vein endothelial cells exposed to inflammatory cultured medium from IA tissue had reduced expression of VEGF-A and cell viability.[42] Studies that investigated recombinant VEGF with carotid aneurysm coiling showed improved endothelial formation at the orifice of the aneurysm and greater occlusion, suggesting a protective rather than pathologic role.[88,89]

Conversely, Sun et al. showed ECs transfected with VEGF-A sequences leading to overexpression was associated with cell apoptosis along with inhibition of EC migration and proliferation.[37] These findings allude to the duality of VEGF with a potential role in aneurysm formation at aberrant levels-either high or low. Given VEGF's known role as a chemoattractant, with the recruitment of progenitor epithelial cells and stem cells, it is possible at abnormally high levels its therapeutic function loses directionality and specificity.[83,90] Similarly, at abnormally low levels there may be an absence of stimulatory signal. This balanced relationship is consistent with the proposed bell-curve dose–response curve by Pontes-Quero et al.[91] Mitogenic activity of ECs was shown to be regulated by Notch, VEGF, Extracellular signal-regulated kinase (ERK), and cell-cycle inhibitor p21. At high levels of VEGF, increased p21 expression leads to cell-cycle arrest. Alternatively, at low of VEGF levels, ECs remain quiescent because of high-NOTCH signaling which suppresses ERK activity. Adding yet another dimension, the unique microenvironment whereby VEGF is exposed to can also influence its function, switching from a pro-growth to an apoptotic signal for ECs.[64]

Intracranial Aneurysm Pathogenesis and VEGF Isomers

Understanding VEGF's role may also lie in delineating the different VEGF isomers at the site of aneurysms. While VEGF-A is considered the most physiologically potent angiographic factor with isomer 165 the most abundant and frequently studied, others likely play a role.[92,93] A more recently discovered variant (VEGF-165b) was found to possess a very unique set of anti-angiogenic properties. It inhibits proliferation of ECs along with migration and tube formation.[94] Ganta et al. demonstrated VEGF165b impaired revascularization through inhibiting VEGF-R1 and induced an anti-angiogenic M1-like phenotype of macrophages, which may have a role in aneurysm formation.[95,96] Among the articles identified in this systematic review, none have investigated the ratio of VEGF165b or other isomers at the site of aneurysms. Rather, immunohistochemical staining, ELISA assays and PCR were most commonly used to identify homologous domains of VEGF or VEGF-A although most studies (n=20) did not specify the VEGF isoform being measured. A technique of growing popularity and accessibility has been ssRNA. Allowing for the comparison of transcriptomes between individual cells, multiple studies have already began applying this technique to investigate IAs.[51,97] Ji et al. were able to identify enriched expression of the VEGF signaling pathway of the human IA dome tissue.[51] Among its expanding applications, ssRNA may also be useful in studying the expression of specific isomers of VEGF and by which cell types in IAs.[98]

Extracranial Aneurysms and VEGF

Though beyond the scope of this review, VEGF has also been implicated in the aneurysm development in other locations of the body. Most notably, the cardiothoracic literature where increased expression of VEGF at AAA has been discovered.[99–102] Tedesco et al. revealed VEGF receptor expression increased in a diameter dependent manner and angiogenesis inhibitors attenuated aneurysm progression.[103] Mice treated with a soluble decoy VEGF-A receptor through intraperitoneal injections had reduced AAA aneurysm size, restored wavy structure of the elastic lamellae and attenuated MMP-9 and MMP-2 activity.[13] This was subsequently corroborated in another study that sequestered VEGF-A with an adenovirus vector that produced a soluble VEGF-R2 extracellular ligand-binding domain. Other cohorts in this study received anti-VEGF-A monoclonal antibody or a multiple RTK inhibitor sunitinib. All groups showed reduced AAA formation and size.[104] Additionally, administration of recombinant VEGF in an AAA mice model increased aneurysm size and MMP2 expression.[105] A summary of major VEGF and aneurysm wall findings is provided in Figure 4.

Proposed Mechanistic Pathway

Although evidence suggests that VEGF direct or indirect involvement with IAs, a clear mechanistic pathway remains elusive. VEGF pleiotropic effects complicate understanding its role in the intricate pathophysiology of IAs. The combination of both a harmful stimulus (such as abnormally high wall shear stress) that fails to abate

and a predisposition for excessive VEGF activation are likely prerequisites for aneurysm progression. Here, we propose plausible mechanism through which VEGF contributes to IA pathogenesis.

At the site of injury and inflammation, dysregulated VEGF release leads to upregulation its own receptors, initiating a positive feedback loop of VEGF/tyrosine-kinase activation.[106] Additionally, tumor necrosis factor-alpha upregulates the expression and function of VEGF-R2 and its co-receptor neurophilin-2.[107] This cascade induces EC changes, including loss of tight junctions, cell contraction, and increased permeability.[25] As a consequence, the important barrier function of the normal EC lining is compromised, allowing cytotoxic and proinflammatory substances such as lipids, antibodies, and components of the complement system to leak into the vessel wall from the circulation.[77,108] Acting as a chemoattractant, VEGF simultaneously recruits and facilitates the transmigration of white blood cells into the paracellular space, resulting in local cell death and destruction of the extracellular matrix via MMPs, cathepsins, and other proteolytic enzymes.[7,10,109]

With continued EC loss and cell contraction, the subendothelium eventually becomes exposed, triggering activation of the clotting cascade and complement system. This results in platelet degranulation and thrombus formation occurs.[108,110] Increased VEGF release by platelets and surrounding cells paradoxically induces EC cycle arrest through p21 activation, hindering the replenishment of healthy ECs needed for repair.[91] Furthermore, circulating VEGF may diffuse into the aneurysm wall from plasma because of loss of endothelial barrier function, also activating mural SMCs that express VEGF receptors.[40] This activation leads to cyclooxygenase 2 expression and subsequent activation and amplification of the NFkB pathway known to mediate the formation and growth of IAs.[111,112] Altogether, this cycle culminates in arterial wall degradation, aneurysm formation, and possible rupture (Figure 5).

Patients may possess a deficiency in a VEGF-R/tyrosine kinase inhibitor (such as anti-angiogenic VEGF165$_b$) or counter-regulatory mechanisms (such as the Delta-like ligand 4-Notch pathway).[94,113,114] This might be otherwise phenotypically silent until exposure to a sustained oculus occurs later in life. Inflammatory processes (such as the buildup of senescence-associated secretory phenotypes with aging and cigarette smoking) and the weakening of vessel wall integrity (because of factors like decreased circulating estrogen or connective tissue diseases) likely promote the initiation or exacerbation of steps in this dysregulated pathway.[54,115–117] A summary of the various, contributing factors is provided in Figure 6. Future research is needed to better elucidate upon the VEGF role in IAs, particularly by establishing both a temporal and dose–response relationship. Next, it is essential to identify the specific abnormality unique to aneurysm patients that causes this deficient counter-VEGF regulatory mechanism or over-expression/activation of the VEGF pathway.

CONCLUSIONS

While the precise mechanism of aneurysm development remains unknown, evidence supporting VEGF's role in this complex pathway is growing. Among the literature on this topic, the most consistently reported finding is elevated VEGF expression in IA patients, observed either locally at the aneurysm tissue site, systemically, or the CSF. Studies on VEGF receptors are fewer but suggest a link between VEGF-R1 expression and ruptured IAs. Additionally, the correlation of specific VEGF genetic polymorphisms with aneurysms adds yet a layer of evidence beyond that of colocalization with inflammation, further implicating the VEGF role in aneurysm formation. Despite lacking definitive evidence of a causal relationship, the wealth of corroborating evidence substantiates VEGF as a promising topic for future investigation into aneurysm pathophysiology and as a potential therapeutic target.

ARTICLE INFORMATION

Received April 4, 2024; accepted August 1, 2024.

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Acknowledgments

We thank the staff of Neuroscience Publications at Barrow Neurological Institute for assistance with manuscript preparation. The authors thank medical illustrator, Ms. Miranda Stano, for her contributions. We also thank Ms. Surya Patil for her editing contributions to the manuscript. Figures 3 and 4 were made using Biorender.com.

Sources of Funding

Funding to conduct this study was provided by the Neurosurgery Education Research Foundation and Bagan Family Foundation Research Grant (PLN), the State funding for university-level health research by Tampere University Hospital, Wellbeing services, county of Pirkanmaa, Finland (T62624) (JF), Brain Aneurysm Foundation (TM), Barrow Neurological Foundation (TM, TH), and the National Institutes of Health R01NS109584, R01AG07780, and R01NS109382 (TH).

Disclosures

None.

Supplemental Material

Data S1

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