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Brain Arteriovenous Malformation Pathogenesis: A Response-to-Injury Paradigm

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Abstract

Brain arteriovenous malformations (AVMs) are a rare but important cause of intracranial hemorrhage (ICH) in young adults. In this paper, we review both human and animal studies of brain AVM, focusing on the: (1) natural history of AVM hemorrhage; (2) genetic and expression studies of AVM susceptibility and hemorrhage; and (3) strategies for development of a brain AVM model in adult mice. These data target various mechanisms which must act in concert to regulate normal angiogenic response to injury. Based on the various lines of evidence reviewed in this paper, we propose a “response-to-injury” model of brain AVM pathogenesis.

Keywords

Brain arteriovenous malformations; Intracranial hemorrhage; Gene expression; Genetics; Angiogenesis; Inflammation; Animal models

Brain arteriovenous malformations (AVM) represent a relatively infrequent but important source of neurological morbidity in relatively young adults [5]. Brain AVMs have a population prevalence of 10–18 per 100,000 adults [4, 7], and a new detection rate (incidence) of ~1.3 per 100,000 person-years [12, 53]. The basic morphology is of a vascular mass, called the nidus, that directly shunts blood between the arterial and venous circulations without a true capillary bed. There is usually high flow through the feeding arteries, nidus and draining veins. The nidus is a complex tangle of abnormal, dilated channels, not clearly artery or vein, with intervening gliosis.

Seizures, mass effect and headache are causes of associated morbidity, but prevention of new or recurrent intracranial hemorrhage (ICH) is the primary rationale to treat AVMs, usually with some combination of surgical resection, embolization and stereotactic radiotherapy. The risk of spontaneous ICH has been estimated in retrospective and prospective observational studies to range from 2–4% per year [26], but approximately 50%

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of patients present initially with a bleed. Other than non-specific control of symptoms, e.g., headache and seizures, primary medical therapy is lacking.

Treatment of unruptured AVMs is controversial and has led to an ongoing randomized clinical trial to test whether best medical therapy has better outcomes than procedural intervention (http://clinicaltrials.gov/ct/show/NCT00389181). Due to the complexity of AVM treatment and a wide range of expert opinions, it is unlikely that a single clinical trial can settle all of the questions related to management strategies. Thus, understanding the pathogenesis of AVM formation and progression to ICH will be important for informing patient management decisions.

In this review, we propose a novel "response-to-injury" paradigm to explain sporadic brain AVM pathogenesis, based on findings from clinical research studies of AVM patients and animal models investigating AVM formation to date. Figure 1 shows a speculative synthesis of pathways involved in AVM pathogenesis. Inciting event(s), while not known, might include sequelae of even modest injury from an otherwise unremarkable episode of trauma, infection, inflammation, irradiation, or a mechanical stimulus such as compression. The normal response to these inciting events would involve angiogenesis, endothelial mitogenesis, and vascular stabilization. However, when superimposed on an underlying structural defect, such as a microscopic developmental venous anomaly or some sort of venous outflow restriction in a microcirculatory bed, or an underlying genetic background, such as mutations in key angiogenic genes, the normal injury response is shifted towards an abnormal dysplastic response. In the next few sections, we will review the available data on factors involved in the abnormal "response-to-injury" in AVMs.

Evidence for Abnormal Angiogenesis and Inflammation in AVM

Studies of surgically-resected AVM tissue suggest an active angiogenic and inflammatory lesion rather than a static congenital anomaly. Several groups [21, 43] have shown that a prominent feature of the AVM phenotype is relative overexpression of vascular endothelial growth factor (VEGF-A), at both the mRNA and protein level. Extrapolating from animal models, VEGF may contribute to the hemorrhagic tendency of AVMs [30]. The vascular phenotype of AVM tissue may be explained, in part, by an inadequate recruitment of peri-endothelial support structures, which is mediated by angiopoietins and TIE-2 signaling. For example, angiopoietin-2 (ANG-2), which allows loosening of cell-to-cell contacts, is overexpressed in the perivascular region in AVM vascular channels [20].

A key downstream consequence of VEGF and ANG-2 signaling, contributing to the angiogenic phenotype, is matrix metalloproteinase (MMP) expression. MMP-9 expression, in particular, appears to be at least an order of magnitude higher in AVM than in control tissue [8, 22] with levels of naturally occurring MMP inhibitors, TIMP-1 and TIMP-3, also increased, but to a lesser degree. Additional inflammatory markers that are overexpressed include myeloperoxidase (MPO) and interleukin 6 (IL-6), both of which are highly correlated with MMP-9 [8, 9]. MMP-9 expression is correlated with the lipocalin-MMP-9 complex, suggesting neutrophils as a major source. In a subset of unruptured, non-embolized AVMs, neutrophils (MPO) and macrophages/microglia (CD68) were all prominent in the vascular wall and intervening stroma of AVM tissue, whereas T and B lymphocytes were present but rarely observed [10]. Higher immunoglobulin levels have been reported in AVM tissue than in control brain [48].

Exactly how the dysplastic response propagates is not known, but recruitment of progenitor cell populations may be one source influencing AVM growth and development and is an area in need of further exploration. For example, endothelial progenitor cells (EPCs) are present in the nidus of brain and spinal cord AVMs, and may mediate pathological vascular

remodeling and impact the clinical course of AVMs. Gao et al. demonstrated that both brain and spinal AVM tissues displayed more CD133, SDF-1, and CD68-positive signals than epilepsy and basilar artery control tissues [14]. EPCs, identified as CD133 and KDR double stained-positive cells, were increased in the brain and spinal cord AVM nidus, mainly at the edge of the vessel wall. The expression of SDF-1 was co-localized with CD31-positive and α-smooth muscle cell expression, and was predominantly found within the vessel wall. More generally, circulating bone-marrow derived cells have a major role in both microcirculatory angiogenesis [16, 17] and conductance vessel remodeling [35, 36]. If AVM pathogenesis involves these two processes, it is reasonable to infer that bone-marrow derived cells may have an underappreciated role in lesion formation and growth. An unresolved issue with all stem cell interactions is the extent to which progenitor cells actually integrate into existing tissue compartments, or whether they provide a nursing function by supplying critical components of the repair response such as cytokines, growth factors and enzymes to the tissue, i.e., do progenitor cells supply “troops” or merely “ordinance”.

Evidence for Genetic Influences in AVMs

Candidate genes and pathways for brain AVM pathogenesis have been suggested by Mendelian disorders, which exhibit AVMs as part of their clinical phenotype, and gene expression studies. AVMs in various organs, including the brain, are highly prevalent in patients with hereditary hemorrhagic telangiectasia (HHT, OMIM#187300), an autosomal dominant disorder of mucocutaneous fragility. Compared to sporadic lesions, brain AVMs in HHT tend to be smaller and are more likely to have single draining veins, be located superficially, and be multiple. However, they are generally similar to the sporadic lesions and cannot be distinguished individually on the basis of their angioarchitecture.

The two main subtypes of HHT (HHT1 and 2) are caused by loss-of-function mutations in two genes [33] originally implicated in TGF-β signaling pathways (Figure 1). The first is endoglin (*ENG*), which encodes an accessory protein of TGF-β receptor complexes. The second is activin-like kinase 1 (*ALK1*, or *ACVLR1*), which codes for a transmembrane kinase also thought to participate in TGF-β signaling. There are hundreds of reported mutations in *ALK1* and *ENG* [1], but the functional effect appears to be haploinsufficiency rather than a mutation-specific set of dysfunctions. A third candidate gene for AVM pathogenesis is *SMAD4*, encoding a downstream participant in TGF-β and bone morphogenic protein (BMP) signaling. *SMAD4* is mutated in a combined syndrome of juvenile polyposis and HHT [13]. These HHT mutations can be viewed as risk factors for brain AVM since the prevalence in HHT1 (*ENG*) is 1000-fold higher and HHT2 (*ALK1*) is 100-fold higher compared to the prevalence of brain AVMs in the general population (10/100,000) [24].

At the earliest stages of vascular development, mice lacking *Alk-1* (Acvrl1) form systemic A-V fistulae from fusion of major arteries and veins [57]. Endothelial cell-specific ablation of the murine *Alk-1* gene causes vascular malformations to form during development, whereas mice harboring an EC-specific knockout of *Alk-5* (the type I TGF-β receptor) or *Tgfbr2* show neither vascular malformation formation nor any other perturbation in vascular morphogenesis [37]. The exact signaling pathways for ALK-1 and ENG are complex, interrelated and their relative importance and cellular specificity are controversial [55]. ENG interacts with multiple TGF-β related signaling pathways and interacts with TGFBR2 (the type II TGF-β receptor) as well as with type I TGF-β receptors, ALK-1 and ALK-5 [31]. ENG can also bind ligands besides TGF-β, including activins and BMP family members [6, 45]. Regardless of the exact signaling mechanism leading to vascular malformation, it is clear that mutations and likely genetic variation in TGF-beta signaling genes are important players in the “response-to-injury” paradigm of AVM pathogenesis.

Candidate Gene Studies in non-HHT AVM Patients

The mechanism of AVM initiation is as yet unknown. Even if it involves a structural aberration, or mechanical insult—per se not a heritable trait—the subsequent growth and behavior of the lesion may still be influenced by genetic variation. For example, multiple genetic loci influence VEGF-induced angiogenesis [42, 46]. Therefore, a pathogenesis that involves a "response-to-injury" at any level may be at least partially influenced by heritable aspects of such a response.

Candidate gene studies of sporadic AVM cases have identified single nucleotide polymorphisms (SNPs) in several genes associated with risk of AVM susceptibility and/or progression to ICH. Previously, SNPs in *ALK1* (IVS3–35A>G) and *ENG* (207G>A) were found to be associated with an increased risk of AVM [40]. The *ALK1* finding was later replicated in an independent cohort of AVM patients from Germany [49, 50]. Additionally, common SNPs in interleukin (IL) genes have been associated with increased risk of AVM among certain race-ethnic groups. Among Hispanics, a promoter SNP in *IL-6* (−174G>C) was associated with a 2-fold increased risk of AVM after adjusting for age, sex, and genetic ancestry. Among self-reported Caucasians, common SNPs in *IL-1*β, 2 promoter (−31T>C and −511C>T) and 1 exonic (+3953C>T), were also associated with increased risk of AVM susceptibility [23]. The *IL-1*β promoter polymorphisms have also been reported to have functional effects on *IL-1*β transcription. Thus, genetic variation in these cytokines may contribute to AVM pathogenesis by enhancing or maintaining a pro-inflammatory state necessary for lesion formation.

Evidence for genetic influences on clinical course of AVM rupture resulting in intracranial hemorrhage (ICH) have also been reported in three different settings: presentation with ICH [23, 41, 60], new ICH after diagnosis [3, 39], and ICH after treatment [2]. The same *IL-6* promoter polymorphism (−174G>C) was associated with clinical presentation of ICH [41], and the high-risk G allele correlated with increasing IL-6 mRNA and protein levels in AVM tissue [9]. More recently, SNPs in the *EPHB4* gene, encoding a tyrosine kinase receptor involved in embryogenic arterial-venous determination, were also reported to be associated with increased risk of ICH presentation [60]. Loss of function mutations in *EphB4* (receptor) and *Efnb2* (ligand) cause vascular defects and AVM formation in mice similar to that observed in *Notch1* gain of function mutants, but these results suggest that different mechanisms can lead to the same phenotype [28].

Not surprisingly, SNPs in inflammatory genes also appear to influence risk of ICH in the natural course of AVMs, including promoter SNPs in *TNF*-α (−238G>A) [3] and *IL1B* (−31T>C and −511C>T) [23]. In addition to their association with spontaneous ICH in the natural, untreated course, both *APOE*ε2 [39] and *TNF*-α −238 A [3] alleles appear to confer greater risk for post-radiosurgical and post-surgical hemorrhage [2].

Genome-wide SNP and Expression Studies in AVM Patients

A drawback of candidate gene studies is that, while they are hypothesis driven, they represent at best an educated guess as to which genes are involved. An alternative approach is to conduct a genome-wide association (GWAS) or expression profiling study. The GWAS approach relies upon scanning all common variation in the genome utilizing microarrays that feature hundreds of thousands to millions of SNPs or probes covering known genes. GWAS can identify associated genes if the causal variants are common in the general population and have shown moderate success for several common complex diseases. An advantage of the GWAS approach is the ability to uncover completely novel biological mechanisms. For example, inflammation was not previously known to be causally involved

in age-related macular degeneration (AMD), but a series of studies published in 2005, including the first successful example of GWAS [27], implicated the Y204H polymorphism in the complement factor H gene with risk of AMD [11]. These genetic findings were subsequently replicated in several independent cohorts and have paved the way for development of new therapeutic interventions [11]. Preliminary results from the first GWAS study in Caucasian brain AVM patients have recently been reported [25].

Genome-wide expression profiling can also be used to identify genes that are likely to have a functional role in the disease process. The basic premise is that different patient groups (diseases) can be distinguished by their gene expression "signature", defined as the unique and consistent pattern of up- and down-regulation of genes. Two small genome-wide expression studies of brain AVM tissue have identified overexpression of inflammatory and angiogenesis-related genes, including *VEGFA*, *ENG*, *ANGPT2*, *ITGAV*, *VEGFR1 (FLT1)*, and *MMP9* [21, 47]. Decreased expression was observed for *TIE1*, *TEK (TIE2)*, and *ANGPT1* [21, 47].

Increasingly, there is interest in performing genome-wide expression profiling of peripheral blood to identify vascular disease-specific gene expression signatures that may serve as clinically useful molecular biomarkers [15, 51, 59, 63]. Identifying blood biomarkers for ICH may have clinical utility in identifying high-risk AVM patients, especially those who come to clinical attention without ICH. The first such study in brain AVM patients has recently been published in abstract form [61], demonstrating differential blood expression profiles in ruptured compared to unruptured brain AVM patients. Pathway analysis of differentially expressed genes implicated inflammatory pathways and VEGF, MAPK, and Wnt signaling, which has relevance for AVM model development as discussed below. Integration of data from multiple genome-wide approaches, including both SNP genotype and gene expression data, may offer additional insight into disease mechanisms.

Experimental AVM Models

Model systems for studying AVM are needed to test mechanistic hypotheses and develop novel therapies. We have previously discussed development of cerebral microvascular dysplasia, a surrogate model for brain AVM [54]. There has been considerable progress in AVM model development during the past year.

A logical approach to animal models is to focus on genes that are clearly related to the human disease phenotype, which for AVM are those genes described above leading to HHT. It is known that both *Eng*+/− [56] and *Alk1*+/− [52] adult mice develop vascular lesions in various organs, but spontaneous lesions in the brain are quite modest, and only seen in older *Eng*+/−mice using scanning electron microscopy [44]. Our group showed that more pronounced forms of cerebral microvascular dysplasia can be induced using VEGF stimulation in *Eng*+/− or *Alk1*+/−mice [18, 19, 62], which can be enhanced by local increases in tissue perfusion rates in the *Alk1*+/− background [18]. Recently, we found that, for a given degree of virally-mediated VEGF overexpression, *Eng*+/− mice have more severe cerebrovascular dysplasia than *Alk1*+/− mice, which simulates the relative penetrance of brain AVM in HHT patients (HHT1>HHT2) (Figure 2C) [19]. These experiments result in enlarged, dysmorphic vascular structures at the capillary level, not the large vessels seen in the human disease.

Oh and colleagues have developed several innovative inducible knockout systems using a novel endothelial Cre transgenic line [37, 38]. Antenatal conditional deletion of *Alk1* causes severe cerebrovascular dysplasia and apparent fistula formation (Figure 2A). Interestingly, conditional *Alk1* deletion in adult mice induced AV fistulas and hemorrhage in the lung and GI tracks, but not in skin or brain. Importantly, upon induction of skin wounding, *Alk1*

deleted mice developed vascular dysplasia and direct A–V connections, suggesting an abnormal response to injury (Figure 2B). Direct A–V connections have also been detected in the retina of Eng-deficient neonatal mice [32]. The combination of local angiogenic stimulation (Matrigel + VEGF/FGF) and Eng loss led to gross venous enlargement [32]. These results suggest that physiological or environmental factors, in addition to genetic variation, are required for Alk1 and Eng-deficient vessels to develop vascular malformations in adult mice. In support of this notion, Walker et al recently described cerebrovascular dysplasia and apparent A–V shunting after focal VEGF stimulation in mice subjected to regional conditional *Alk1* deletion [58].

An additional mechanism of potential interest—especially to the phenomenon of AVM rupture—was suggested by a recent study by Lebrin et al [29]. Thalidomide reduced epistaxis and enhanced blood vessel stabilization in nasal mucosa of HHT patients. In *Eng*+/− mice, thalidomide treatment stimulated mural cell coverage and thus rescued vessel wall defects partially through upregulation of platelet-derived growth factor-B (PDGF-B) expression in endothelial cells and stimulated mural cell activation.

Notch signaling appears important for the determination of arterial and venous fate, a process which seems to depend on local levels of VEGF [64]. There is empirical evidence that proteins involved in Notch signaling— including the receptor, its ligands, and downstream signals—are expressed in excised surgical specimens [34, 65]. Animal experiments support a potential link with the human disease. Using conditional endothelial expression, Murphy and colleagues used a tetracycline responsive-promoter to suppress overexpression during development and then by withdrawal of doxycycline, overexpressed the intracellular signaling portion of Notch-4 (int3) in early post-natal mice. They observed a rapidly lethal phenotype, which mimicked aspects of human AVMs, including dysplastic posterior fossa vasculature with apparent A–V shunting.

Taken together, both genetic manipulation and angiogenic stimulation appear to be important aspects of AVM model development. The angiogenic stimulus can be varied, for example via injury, exogenous growth factor delivery, or the use of young, perinatal animals that have high inherent angiogenic activity in the brain. An ideal AVM model should strive to contain the following components: (1) **Anatomic,** nidus of abnormal vessels of varying sizes at micro and macro-circulatory levels; (2) **Physiologic,** A–V shunting, hemodynamically significant, i.e., sufficient to decrease feeding artery or increase draining venous pressures; (3) **Biological,** alterations in angiogenic and inflammatory protein expression, involvement of or intersection with known genetic pathways; and (4) **Clinical,** relative quiescence, spontaneous hemorrhage into the parenchyma or CSF spaces. Currently, such an ideal animal model has not been developed in adults, which would more closely mimic the human phenotype. However, insights from the current AVM models suggest that regional conditional gene deletion plus angiogenic stimulation may promote the ideal AVM development in adult mouse brain.

Summary and synthesis of data regarding etiology and pathogenesis of AVM

Elucidating the mechanisms and factors influencing AVM lesion formation and progression to ICH offers promise for developing innovative treatments and better risk stratification for clinical management or clinical trial design. Further, study of brain AVM may be a powerful platform from which to gain insights into general vascular biologic mechanisms relevant to a wide variety of diseases affecting the vascular system.

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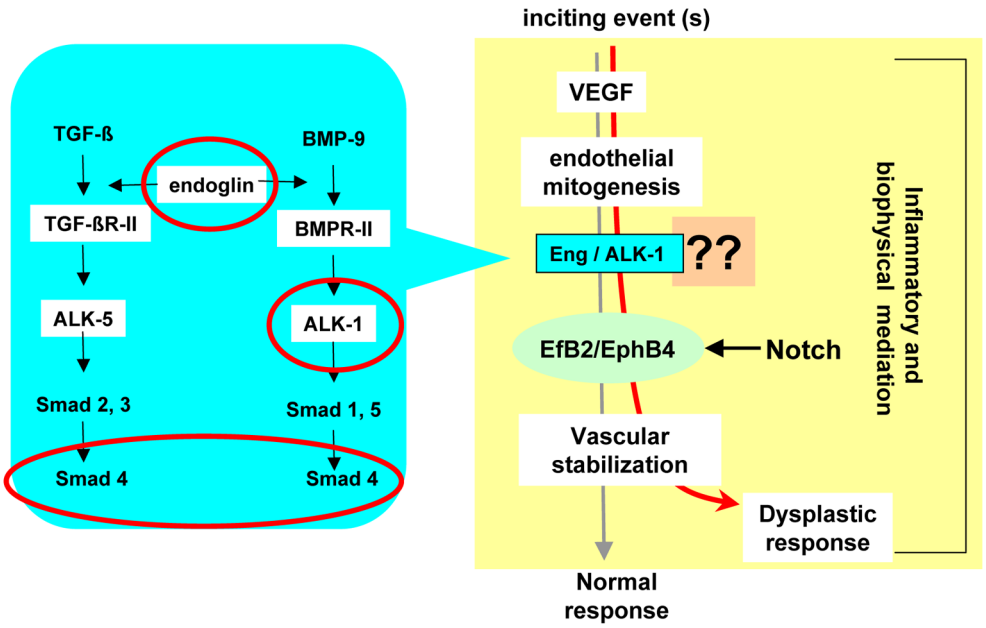

Fig. 1. Speculative synthesis of pathways involved in AVM pathogenesis

The blue shaded area is a simplified summary of presumed ALK-1 and ENG signaling via TGF-β and BMP-9 in endothelial cells (EC); the genes mutated in HHT are circled. Main components of the scheme are (a) inciting event(s) upregulate the expression of angiogenic factors, such as VEGF, which induce EC mitogenesis; newly formed vessels will develop into a stable neovasculature; (b) this process leads to a vascular dysplastic response when signaling through aberrant ALK-1 and/or ENG, or in a closely related pathway (question marks); (c) ephrinB2 and EPHB4 imbalance, possibly through involvement of Notch signaling; and (d) modifier influences, potentially genetic and/or hemodynamic. Inflammation and involvement of circulating precursor cells may be relevant.

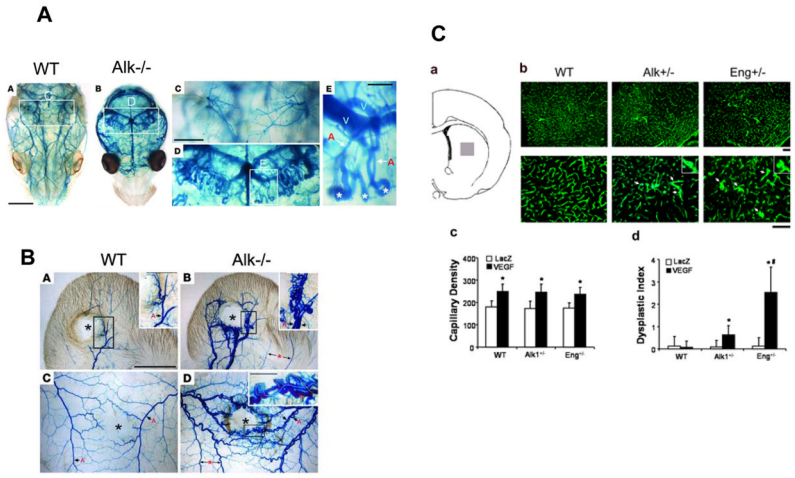

Fig. 2. Brain AVM in *Alk1 or Eng* deficient mice

A. Endothelial *Alk1* deletion results in AVMs in the brain [38]. A–E. Dissection microscopic views of vascular images of control (WT, A, C) and mutant (*Alk1*−/−; B, D, E) in postnatal day 3 mouse brains by latex dye injected into the left ventricle of the heart. Magnified views of blood vessels in the hipocampal area (D, E). Asterisks indicate peculiar looping of vessels at the distal tips of arteries shunting to veins (E). A, artery; V, vein. **B.** Wounding can induce *de novo* AVM formation in *Alk1*-deleted adult mice [38]. Vascular patterns shown by latex dye injected into the left heart of control (WT, A, C) and mutant (*Alk1*−/−, B, D) mice bearing wounds in the ear (A, B) or dorsal skin (C, D), 8 days after induction of *Alk1* gene deletion. The images were taken after clearing in organic solvents. Center of the wound is indicated by asterisks. Note that only mutant mice developed AV shunts shown by the presence of latex dye in both arteries and veins. AV shunting and abnormal vascular morphologies were apparent only in the wound areas. Blood vessels away from the wound indicated by arrows with asterisks (B and D) showed normal appearance. Inset in D shows a magnified view of AV fistulas formed in the rim area of the mutant wound. **C.** Overexpression of VEGF in the striatum of *Alk1* and *Eng* haploinsufficient mice resulted in vascular dysplasia [19]. a. Injection site (grey square). b. Angiogenic foci and dysplastic capillaries (arrows). Inserts are enlarged images of dysplastic capillaries. Scale bars: 100 μm (top panel) and 50 μm (bottom panel). c and d. Capillary density and dysplasia index. * = $p<0.05$, vs. AAV-LacZ group. # = $p<0.05$, vs. AAV-VEGF-transduced WT or *Alk1*+/− mice. VEGF: AAV-VEGF-injected mice; LacZ: AAV-LacZ-injected mice.