Am J Physiol Cell Physiol 316: C252–C263, 2019.
First published November 21, 2018; doi:10.1152/ajpcell.00151.2018.

THEME | *Vascular and Cellular Pathophysiology of Stroke*

Roles of blood-brain barrier integrins and extracellular matrix in stroke

Danielle N. Edwards[1,2] and Gregory J. Bix[1,2,3,4]

[1]*Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky;* [2]*Department of Neuroscience, University of Kentucky, Lexington, Kentucky;* [3]*Department of Neurology, University of Kentucky, Lexington, Kentucky; and* [4]*Department of Neurosurgery, University of Kentucky, Lexington, Kentucky*

Submitted 18 April 2018; accepted in final form 20 November 2018

Edwards DN, Bix GJ. Roles of blood-brain barrier integrins and extracellular matrix in stroke. *Am J Physiol Cell Physiol* 316: C252–C263, 2019. First published November 21, 2018; doi:10.1152/ajpcell.00151.2018.—Ischemicstroke isa leading cause of death and disability in the United States, but recent advances in treatments [i.e., endovascular thrombectomy and tissue plasminogen activator (t-PA)] that target the stroke-causing blood clot, while improving overall stroke mortality rates, have had much less of an impact on overall stroke morbidity. This may in part be attributed to the lack of therapeutics targeting reperfusion-induced injury after the blood clot has been removed, which, if left unchecked, can expand injury from its core into the surrounding at risk tissue (penumbra). This occurs in two phases of increased permeability of the blood-brain barrier, a physical barrier that under physiologic conditions regulates brain influx and efflux of substances and consists of tight junction forming endothelial cells (and transporter proteins), astrocytes, pericytes, extracellular matrix, and their integrin cellular receptors. During, embryonic development, maturity, and following stroke reperfusion, cerebral vasculature undergoes significant changes including changes in expression of integrins and degradation of surrounding extracellular matrix. Integrins, heterodimers with α and β subunits, and their extracellular matrix ligands, a collection of proteoglycans, glycoproteins, and collagens, have been modestly studied in the context of stroke compared with other diseases (e.g., cancer). In this review, we describe the effect that various integrins and extracellular matrix components have in embryonic brain development, and how this changes in both maturity and in the poststroke environment. Particular focus will be on how these changes in integrins and the extracellular matrix affect blood-brain barrier components and their potential as diagnostic and therapeutic targets for ischemic stroke.

blood-brain barrier; extracellular matrix; integrins; permeability; stroke

INTRODUCTION

Stroke is the fifth most common cause of death in the United States (separate from cardiovascular disease), with a person experiencing a stroke every 40 seconds (12). The most common type of stroke, ischemic stroke, is defined as obstruction of blood flow to part of the brain due to a thrombus or blood clot, and results in a one year patient survival rate of 60% (12, 46, 104, 105). While all are potentially at risk for having a stroke, factors such as being male (or a postmenopausal female), African-American, being of advanced age, and the presence of hypertension all increase a person's risk of experiencing a stroke in their lifetime (32, 35, 36, 60, 106, 110, 147). Additionally, functional deficits induced by ischemic stroke are the leading cause of disability in the United States and cause a $36–65 billion economic burden that is expected to increase to $180 billion by 2030 (60). Taken together, ischemic stroke is a significant health issue with limited therapeutic options. The current therapies, exogenously administered clot-busting tissue plasminogen activator (t-PA) and endovascular mechanical thrombectomy (clot removal), are efficient in removing the thrombus, thereby increasing reperfusion rates by 60%, and decreasing mortality (since 2013) (104, 105). However, increased efficacy (i.e., morbidity) due to therapeutic input has largely lagged behind these gains in mortality (31, 153).

A proposed hypothesis to explain the lack of correlation between improved mortality rates and patient outcomes involves the mechanisms following reperfusion, so-called reperfusion-induced injury. When reperfusion injury occurs, it often expands the initial brain injury caused by the occlusion (referred to as the core) to at risk brain tissue (referred to as the penumbra or peri-infarct region (17, 30, 113). This occurs, in part, as the result of cerebral edema (brain swelling). The first phase of edema occurring at 0–24 h after injury is cytotoxic [ionic and metabolic dysfunction (113)] in nature, followed by vasogenic [new blood vessel growth (angiogenesis) and reas-

Address for reprint requests and other correspondence: G. J. Bix, University of Kentucky 800 S. Limestone 430 Sanders-Brown Center on Aging Lexington, KY 40536 (e-mail: gregorybix@uky.edu).

sembly of endothelial cell tight junctions (TJs) (56, 113, 115)] causes.

Edema greatly contributes to breakdown of the blood-brain barrier (BBB), a three layer defense system around the vasculature preventing unwanted molecules from entering the brain parenchyma, and composed of nonfenestrated endothelial cells with intercellular tight junctions and various influx and efflux cellular transporters, extracellular matrix (ECM) and its cellular integrin receptors, pericytes, and astrocytic endfeet (Fig. 1) (113). BBB dysfunction in transient ischemic stroke models, in which a cerebral blood vessel is closed for a predetermined period of time and then reopened, occurs in two phases (biphasic permeability); at 30 min after reopening of the blood vessel, and then again at 2–5 days after occlusion, lasting for up to 5 wk (1, 81, 123, 144). The loss of organization leading to BBB dysfunction can be observed by the loss of sharp distinction in the basement membrane 12–24 h after ischemia, as shown by transmission electron microscopy (72). The importance of BBB dysfunction has come under high scrutiny over recent years, as studies have shown that BBB dysfunction can predict the probability of having an ischemic stroke as well as its outcome (34, 38, 39, 134, 149). This review focuses on the modulation of integrins and ECM components (existing in and around all cell types in the cerebrum) following reperfusion, and how this can lead to BBB dysfunction following ischemic stroke (Fig. 2).

PROTEINS OF THE BLOOD-BRAIN BARRIER

Integrins

Integrins as a whole are located on every cell type in the body. They are heterodimeric transmembrane proteins composed of non-covalently bound α and β subunits forming 24 known combinations (128). The different subunits have varying roles; the α subunit is responsible for binding the respective ligand, while both the α and β subunits are responsible for intracellular signaling (28, 138, 148). Integrins exist in three states—active, at rest, and inactive—represented by different conformations (21, 113). The conformational states differentially expose the binding domain, typically arginine-glycine-aspartate (RGD) and determine the relative (none, intermediate, or high) affinity an integrin will have for ECM components (26, 111). Once bound to their corresponding ECM protein, the integrin-ECM complex functions to promote cellular signaling, proliferation, migration, differentiation, and survival (58, 59, 63, 150). We will first discuss several key integrins that have been implicated in BBB function after stroke, and, where known, their respective therapeutic target potential. Here, we will discuss integrins associated with the extracellular matrix, though blood-borne integrins (α2β1 and α11bβ3) are also affected following ischemic stroke.

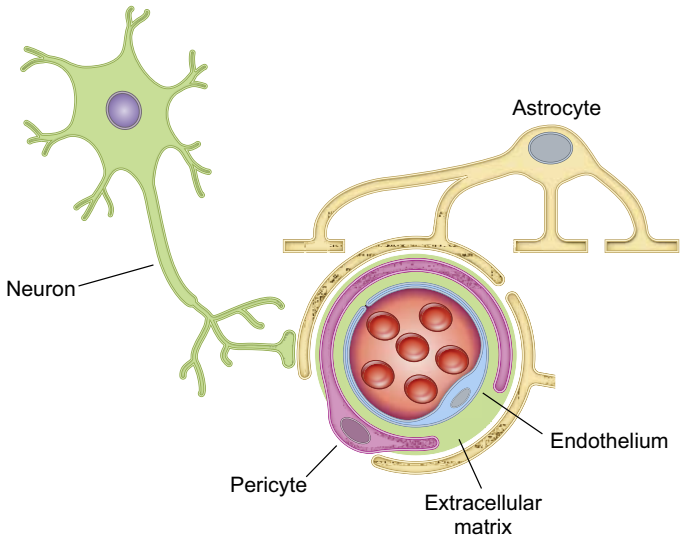

Fig. 1. Representation of the blood-brain barrier during normal conditions.

αvβ3 Integrin. Embryonically, αvβ3 integrin is highly expressed on endothelial cells, astrocytes, and microglia, binding to a number of ECM components including fibronectin, vitronectin, osteopontin, laminin, etc. (7, 33, 95, 141). Functionally, αvβ3 is essential to angiogenesis, as 80% of αv knockout mice are embryonically lethal by E10–12 with placental and heart defects, though this is not completely attributed to the β3 subunit as αv can also associate with β3, β5, β6, and β8 subunits with varying viability (29). The remaining 20% of mutants survive through gestation, but they end up succumbing to intestinal and cerebral hemorrhages at birth (29). Interestingly, conditional αv knockout mice also result in nonsurvivability due to intracerebral hemorrhages, but not by loss of integrin αv on endothelial cells. Instead, integrin αv absence on glial cells and astrocytes facilitates detachment of astrocytes from the ECM, an increase in permeability of the BBB, and intracerebral hemorrhage (83). Completion of development results in total loss of αvβ3 expression under physiologic conditions (95).

After experimental ischemic stroke [middle cerebral artery occlusion (MCAO)] in adult rodents, expression of αvβ3 integrin is significantly increased by 2 h in the ischemic core and continues to increase in the ischemic penumbra until expression peaks at 7 days poststroke (2, 57). This correlates with increases in the ECM proteins fibronectin and vitronectin as well as increasing vascular density (as determined through brain endothelial proliferation) through poststroke day 14 in the ischemic penumbra (2, 57). Furthermore, vascular endothelial growth factor (25), a known inducer of αvβ3 integrin expression, activity, and ligand affinity, has a similar pattern of upregulation after MCAO, increasing around 1 h postreperfusion and remaining for up to 7 days (2, 103). The increase in αvβ3 poststroke induces occludin and zonula occludens (ZO-1) (tight junctions of brain endothelial cells) internalization, disrupts VE-cadherin localization (a tight junction regulating protein), induces stress fiber formation, and increases expression of ECM degradation proteins, matrix metalloproteinase (MMP) -2 and -9 (4, 37, 43, 95, 97, 116, 134, 139). These outcomes initiate angiogenesis, a process that was previously thought to be beneficial by increasing blood supply to a previously hypoperfused area, but is now known as a main contributor to the chronic (2–5 days) increase in BBB permeability after reperfusion (113). As αvβ3 is a known promoter of the chronic (2–5 days) increase in permeability observed after reperfusion, it may be a good target for ischemic stroke intervention (113).

Over the last few years, different modes of targeting integrin αvβ3 therapeutically have been attempted with contrasting results. The first and most promising combined a 1 h pre-MCAO and 3 h post-MCAO therapeutic treatment with an αvβ3 integrin

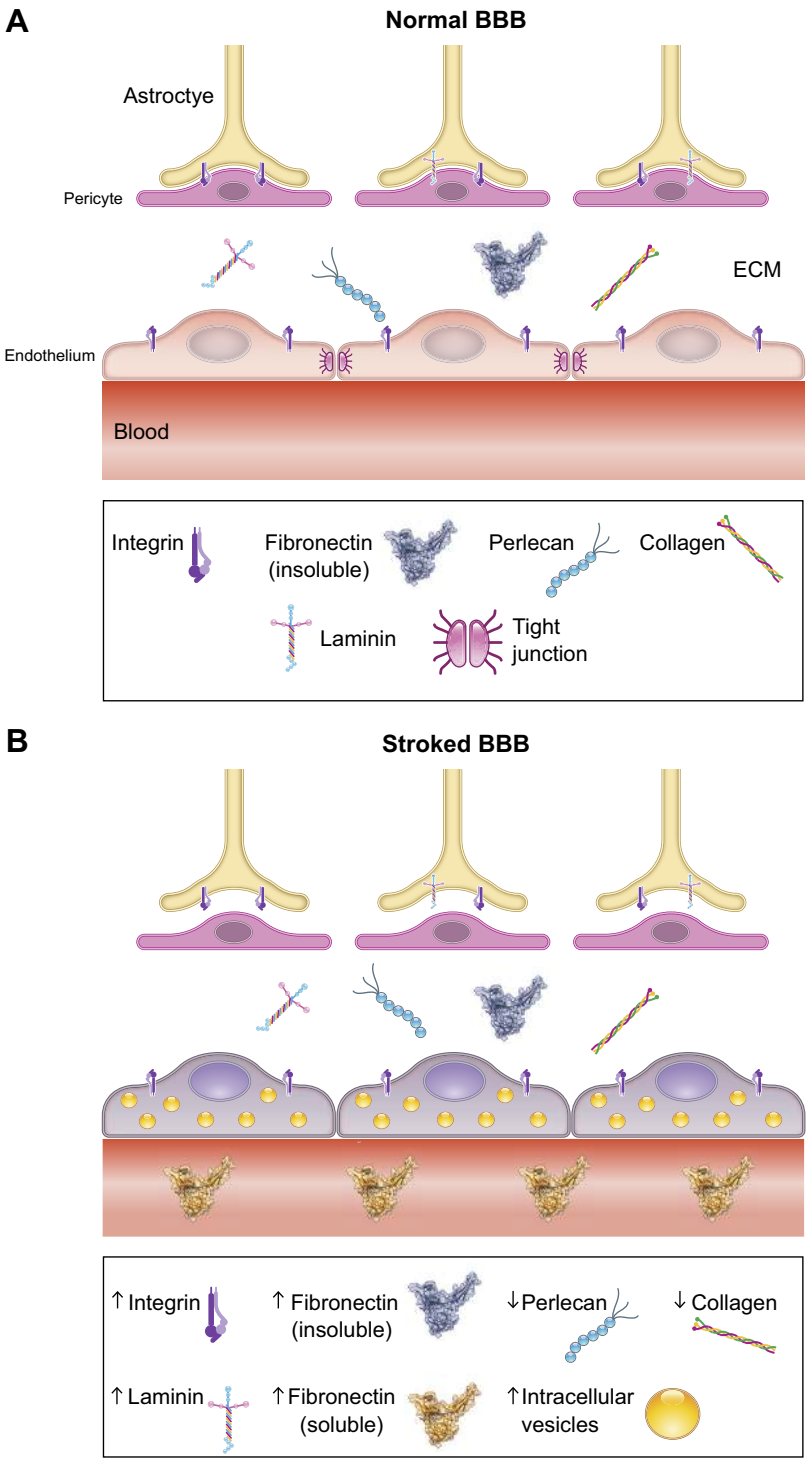

Fig. 2. Representation of integrin and extracellular matrix (ECM) effects of the cerebral neurovascular unit during maturity (*A*) and following reperfusion after ischemic stroke (*B*). BBB, blood-brain barrier.

selective inhibitor, cyclo-RGDfV, which showed reduced infarct volumes and BBB permeability (118). However, prophylactic treatment for ischemic stroke is not a viable clinical option as we cannot predict the moment a stroke will occur. Because of this, the same group attempted to inhibit αvβ3 3 h post-MCAO. A decrease in BBB permeability was still observed, but the resulting decrease in infarct volume was not significant in this dosing paradigm (117). Additional results with the cyclo-RGDfV inhibitor demonstrated a decrease in phosphorylated Flk-1, a specific VEGF receptor, that when phosphorylated, increases VEGFa production, consistent in both dosing windows (117, 118). This suggests that the decreases in BBB permeability are due to changes in VEGF- αvβ3-mediated angiogenesis, but inhibition of this mechanism alone is insufficient to modulate infarct volumes. This may be due to inhibitor dosing schedule (first dose is too late or repetitive dosing is necessary) or that additional mechanisms are required for effects on infarct volume.

α5β1 Integrin. The α5β1 integrin's main function is to promote embryonic angiogenesis, but it has also been implicated in cell migration, cell adhesion, and cell survival (114). It is also present on many different cell types, most notably on endothelial cells and astrocytes in the cerebrum, with a preference for interacting with the ECM proteins fibronectin, laminin 10, and osteopontin (114). Even with its diverse binding preferences, α5β1 integrin is primarily known as a fibronectin receptor (114). Interestingly, recent research has shown that αvβ3 integrin competitively binds to fibronectin, creating cross-talk signals that recruit α5β1 integrin at an opposite, unspecified site on fibronectin (14). Ultimately, these interactions increase the adhesion of both αvβ3 and α5β1 integrins to fibronectin (14). Unlike with integrin αvβ3′s nondetectable expression in adult microvasculature, after embryogenesis, α5β1 integrin's expression decreases but is still present at low levels in adult vasculature (57, 95, 114). The relevance of α5β1 integrin in development is apparent, as multiple α5 knockout mice models do not survive past E11 due to neural tube defects, lack of angiogenesis, and leaky blood vessels (29, 44, 45, 152).

The α5β1 integrin may play a key role in many different diseases. Notably, targeting α5β1 in cancer has been highly studied as it is significantly upregulated in tumorigenesis, with functions in tumor development, angiogenesis, and progression (114). This increase is not limited to cancer but is observed in models of experimental ischemic stroke, increasing in the ischemic penumbra around day 4 until peak expression at 7 days poststroke, the same pattern of expression as αvβ3 integrin and brain endothelial cell proliferation (57). Unlike with αvβ3, there is a compensatory increase in α5β1 integrin when αvβ3 integrin is inhibited (76). Furthermore, α5β1 is closely linked to VEGF receptor 1 (as seen by a solid-phase binding assay), and upon cross-talk, enhances cell migration, proliferation, and adhesion, processes that are blocked upon use of integrin α5 antibodies and knockout mice (77, 137a). Because of its strong angiogenic ties, α5β1 integrin has been highly targeted as a means for therapeutic intervention in inhibiting angiogenesis for many decades with varying results (refer to Ref. 9 for further information), but its role in angiogenesis and stroke pathology has been largely ignored until recently.

Roberts et al. (108) studied the effects of α5β1 integrin in endothelial specific knockout mice after MCAO with surprising results. These mice, unlike the previously discussed pan-knockouts, are not embryonically lethal and have no obvious vascular or developmental changes (108, 133). Surprisingly, these knockout mice suffered significantly smaller infarcts compared with their wild-type controls through apparent stabilization of the BBB as shown by an absence of IgG (150 kDa) extravasation into the brain parenchyma (108). This is suited toward the hypothesis that inhibition of angiogenesis, in this case through inhibition of α5β1 integrin, prevents a remodeling of neurovasculature initiated by vasogenic edema. As embryonic pan-deletion of α5 integrin contributes to abnormal angiogenesis and leaky blood vessels (44, 45, 152), inhibition of α5β1 integrin therapeutically in already developed, adult vasculature could be an avenue for future ischemic stroke intervention.

α6β4 Integrin. α6β4 integrin is mainly expressed on astrocytes, but also on endothelial cells, though it is not expressed during embryogenesis, but rather during adult vasculogenesis due to the switch from fibronectin- to laminin-driven angiogenesis (87, 88, 137). Initially, α6β4 decreases within 2–4 h after MCAO, but begins to increase at day 4 and continues to peak expression by day 14 (87, 88, 126, 137). Increased α6β4 expression also correlates to increasing brain endothelial cell proliferation (reaching its peak at day 7), which swaps with astrocytic proliferation that peaks at day 14 (57, 88). The astrocytic effects after ischemic stroke will be discussed later in this review.

α6β1 Integrin. As with most β1 integrins, α6β1 integrin is expressed on endothelial cells in the brain (7). During embryogenesis, the endothelial integrins α5β1 and αvβ3 are the most highly expressed, but an integrin switch occurs for the laminin binding α6β1 integrin in adulthood (88). The α6β1 continues to be the dominantly expressed β1 integrin in mature vessels, but this is quickly switched for α5β1 after cerebral hypoxia, once angiogenesis is initiated (76). Current research on therapeutically targeting this integrin has focused on blocking it to prevent angiogenesis in solid tumors with very limited study in ischemic stroke.

α1β1 Integrin. The α1β1 integrin is present on endothelial cells and astrocytes, preferably binding to the ECM components collagen IV and perlecan. Embryonically, α1 integrin subunit knockout mice show no lethality and finite defects, limited to cell proliferation complications (29). On the contrary, β1 integrin subunit knockout mice, eliminating all the various integrins with a β1 component, show neonatal lethality at E6 while embryonic stem cells without β1 integrin result in hematopoietic defects (29). After experimental ischemic stroke, α1β1 expression (by probing α1 and β1 separately) decreases by 30% within 2 h, and 75% by 24 h after occlusion (126). Further studies with integrin β1 inhibitory antibodies have resulted in severe deficiencies in BBB stability as well as promoting a decrease in expression of the tight junction protein claudin-5 and shifting its localization away from the extracellular wall, suggesting a significant role in BBB maintenance embryonically and at maturity (98), though no further research has been conducted in this regard in ischemic stroke.

Integrins: conclusion. As described here, the variety of integrins in the brain is expansive in terms of type, function/preferred ligand(s), and expression. The highly reactive nature of integrins suggests a new approach for potential ischemic stroke therapy, as evidenced by a relatively recent increase in the number of studies focused on them. However, the success of modulating these integrins to therapeutic effect is thus far as varied as is the integrins themselves.

Extracellular Matrix Proteins

Collectively, the ECM consists of multimeric proteins that participate in cellular migration and differentiation as well as functioning as a support system for endothelial cells and astrocytes when in complex with integrins and is composed of a combination of proteoglycans, glycoproteins, and collagens (71, 94). The ECM is vital for development, function, and regulation of vasculature, tight junctions, neurons, and astrocytes through cellular signaling and adhesion (10). Defects in any ECM protein can result in serious developmental and functional complications (10). Here, we will discuss the cere-

brovascular extracellular matrix proteins that interact with integrins following ischemic stroke.

Fibronectin. The ECM glycoprotein fibronectin is a disulfide-linked dimer (~250 kDa per monomer) that can exist as a soluble form, plasma fibronectin produced by hepatocytes in the liver, or insoluble form, cellular fibronectin produced by fibroblasts in the basement membrane (64, 87, 101, 132). Developmentally, fibronectin is significantly increased in the basement membrane, driving angiogenesis in developing vasculature through binding to α5β1 and αvβ3 integrins (91, 93). Fibronectin knockout mice are embryonically lethal at E10 because of severe vascular, notochord, and somite deformities, due to genetic manipulation to the fibronectin gene before isoform formation due to splicing can occur (44). This is evident as the cellular fibronectin isoform is necessary for cerebral vascular development, but these vasculature and notochord deformities, in addition to preventing lethality from the null gene, are prevented in the plasma fibronectin-null mouse (87, 112, 140).

Fibronectin is highly reactive after stroke, increasing in the ischemic penumbra until peak expression at 7 days post-MCAO, but the researchers did not separate expression by isoform (57, 75). Previous studies suggest that the increase is due to trafficked plasma fibronectin to the infarct area 2 days post-MCAO (112). To further reinforce this concept, plasma fibronectin-null mice that underwent MCAO had significantly increased infarct volumes and increased TUNEL (apoptotic cells) staining compared with their wild-type controls with no compensation from cellular fibronectin (112, 129).

Not only is fibronectin responsive after stroke, but it is also vulnerable to degradation from proteases, most notably MMPs (65, 146). MMP-9 is increased by 48 h after ischemic stroke in humans (22), but also after intracerebral hemorrhage induced by VEGF injections and IL-1β-induced systemic inflammation by nearly six-fold (74, 84), increasing the degradation of ECM proteins. With this relationship in mind, serum levels of cellular fibronectin and MMP-9 are increased in patients who experience a hemorrhagic transformation following ischemic stroke (18, 19). Interestingly, the higher levels of both cellular fibronectin and MMP-9 predict increased bleeding associated with the hemorrhage.

Laminin. Laminin is a heterotrimeric protein composed of different α, β, and γ subunits to form 15 different isoforms (24, 51, 78, 90) that exists in endothelial cells and astrocytes. While embryonic angiogenesis is driven by fibronectin, this is substituted by laminin during adult angiogenesis (88, 157), though this process will continually alternate during VEGF-stimulated injury (i.e., tumor growth and metastasis, ischemia, wound healing, etc.) (57, 75, 88, 138). Of the 5 α, 4 β, and 3 γ laminin subunits, laminin 8 (α4β1γ1) and 10 (α5β1γ1) are endothelial specific, while laminin 1 (α1β1γ1) and 2 (α2β1γ1) are expressed solely on astrocytes (61, 119, 121, 156). It is important to note that only laminin 8 is expressed during development, while the first detectable levels of laminin 10 are 3–4 wk postbirth in mice (51, 131). The importance of laminin to vascular integrity is obvious as laminin α4 knockout mice and laminin γ1 knockout mice suffer from leaky vasculature and experience intracerebral hemorrhages, leading to lethality at E6 (120, 131). Further study with conditional laminin α4 knockout mice showed similar defects in vasculature that again led to intracerebral hemorrhages (20, 155).

Laminin's main role after ischemic stroke appears to be more aligned with its endothelial cell interactions. Within 24 h after MCAO, increases in laminin in both endothelial cells (laminin 8 and 10) and astrocytes (laminin 1 and 2) in the ischemic penumbra are observed (61, 125). Alternatively, there is a decrease in endothelial laminin located in the ischemic core due to loss of vasculature from ischemic conditions (75). Endothelial laminin, specifically laminin 10, is also essential for BBB integrity after in vitro oxygen glucose deprivation by regulating occludin and ZO-1 expression and localization to the extracellular cell wall, decreasing paracellular resistance through the endothelial cells (62). Finally, activation of α2β1 integrin by its binding to endothelial laminin halts endothelial proliferation (86), a process that is slowed around 7 days post-MCAO (59) when astrocytic proliferation takes over (57, 88).

Chronic repair has been implicated in laminin-initiated mechanisms driving neurogenesis after MCAO. Endothelial laminin promotes neurite growth in vitro (99) but has yet to be detected in in vivo studies. Recent studies have shown laminin in conjunction with β1 integrin in providing scaffolds, a neural chain that promotes neuronal migration toward injury, 16 days after occlusion (41). Although laminin-mediated neurogenesis after MCAO is poorly understood, the impact that this glycoprotein has on neurogenesis could be significant.

Perlecan. Perlecan is a heparan sulfate proteoglycan that contains a protein core with five different domains (domain I–V) and three glycosaminoglycan chains at the NH_2 terminus and is most commonly found in hyaline cartilage, but it is also located in basement membranes throughout the body (67, 85, 142). Overall, perlecan plays a major role in cellular migration, proliferation, and differentiation, but the individual domains have functions and binding domains unique to their sequence and structure (35, 142). Perlecan, like fibronectin and laminin, is upregulated during embryogenesis around cerebral vasculature to provide maintenance and assemble the developing basement membranes (25, 54). When absent from the basement membrane, complete perlecan knockout mice result in embryonic lethality at E12 resulting from cardiac failure and exencephaly, with surviving embryos soon succumbing to their skeletal, cardiac, and cerebral defects (25, 40, 54). On the other hand, a second type of transgenic mouse with truncated perlecan (producing 10% of total perlecan, perlecan hypomorphs) yields surviving neonates that have a normal basement membrane but are still susceptible to exencephaly as well as exhibiting truncated skeletal features (25, 109). Collectively, the loss of perlecan embryonically and in maturation is significant in both transgenic mouse models, indicating its high importance in basement membrane maintenance in both development and adulthood, and although perlecan is mostly known for its functions in hyaline cartilage, it has shown interesting promise as a target for ischemic stroke therapy (67, 90).

Perlecan is decreased by 43–63% within 2 h of reperfusion after MCAO and is continually degraded through day 7 by cathepsin (caspase) B and L (23, 76). Cleavage by cathepsin L on perlecan releases the COOH-terminal fragment domain v within hours after experimental ischemic stroke, resulting in a strong increase by 24 h postreperfusion that is sustained through 7 days (73). Treatment with exogenous recombinant domain V after MCAO not only is neuroprotective in wild-type mice, but rescues pathology in perlecan-deficient mice (i.e.,

perlecan hypomorphs), thus reducing infarct volumes and improving functional outcomes (neuroprotection and neurorepair) (15, 23, 73). Furthermore, the neuroprotective effects of domain V were blocked upon administration of an anti-α5β1 antibody. Interestingly, α5β1 integrin is a potential receptor of domain V, but not perlecan (73). Collectively, these results suggest that perlecan, and its domain V in particular, play a fundamental role in the brain's response to ischemic stroke injury.

Collagen IV. Collagens are an abundant basal lamina protein expressed on all tissue types in the body (102). Of the variety of collagens, the most abundant in the basement membrane in the body is collagen IV, a nonfibrillary collagen (102). Even though collagen IV is one of the three main components of the basement membrane (laminin and heparan sulfate proteoglycans composing the rest), embryonically knocked out collagen IV results in no cerebral deficits, but rather only appearing to affect renal development (29, 102). Interestingly, mature mutations can result in ischemic strokes in young patients, potentially resulting in a significant role in poststroke pathology (130). Studies in collagen IV expression have been contradictory. One study using Western blot analysis shows a reduction in collagen IV after experimental ischemic stroke that corresponds to a decrease in cerebral vasculature (52). Although, more recent studies have observed the opposite phenomenon when analyzing by immunohistochemistry (55). Here, the authors themselves admit the potential false-positive in the results because of a potential increase in degradation (i.e., more proteins available for targeting) or as a result of the neurovasculature overcompensating to stabilize damaged vessels (55). These conflicting results need more investigation and improvement before any determination of collagen IV's impact on stroke severity or poststroke recovery can be made.

Extracellular matrix proteins: conclusion. The proteins in the extracellular matrix surrounding endothelial cells and astrocytes are primarily composed of collagen IV, laminin, heparan sulfate proteoglycans, and fibronectin. This diverse group has a variety of reactions following stroke, but overall are attributed to the BBB stability, an important target to reduce the expansion of damage and edema. The interest in targeting these proteins has been limited at best, most likely due to a decrease in accessibility on the basal side of the endothelium. Current focus is on modulating these proteins through other mechanisms, such as reducing MMPs, targeting integrins, and understanding the full reactive mechanisms after ischemic stroke.

CELLULAR COMPONENTS OF THE BBB

As discussed, integrins and ECM proteins are not limited to endothelial cells but span all cell types in the neurovascular unit including astrocytes and pericytes. When ischemia and the following inflammatory, vasogenic, etc. mechanisms influence these BBB components, it can directly affect BBB permeability (113). Below, we briefly describe the rest of the cells that comprise the BBB with a particular focus on how their interactions with the ECM via integrins affect the BBB after ischemic stroke.

Endothelial Cells

Endothelial cells are responsible for the first layer of the BBB, providing a scaffold for TJs, junctional adhesion molecules, and the extracellular matrix (as discussed throughout this review). Heavy scrutiny has been placed on paracellular permeability, permeability between endothelial cells, as the main regulator for poststroke edema (68). Until recently, little importance has been placed on transcellular permeability, permeability through an endothelial cell. It has now been shown that endothelial cells undergo a four-step process after ischemic stroke, localized to the core (49). First, endothelial cells swell, potentially attributed to activation of connexin-43, a gap junction protein (68), Next, endothelial cells gain a permeable surface. Here, an increase in transcytotic, particularly caveolin-1, and pinocytotic vesicles begins to compromise the endothelial cells and cause an increase in BBB permeability at 4–6 h (49, 66, 68, 69). Interestingly, at this time, there appears to be limited compromise of the tight junctions. This process leads to free movement of molecules across the endothelial cell, eventually resulting in a loss of endothelial integrity (68). Finally, this is followed by a loss of endothelial integrity, Once the extracellular matrix is exposed, endothelial cell loss occurs (69). The endothelial role in BBB permeability is shown to be resolved 24 h poststroke (49). Because of this process, the early damage done to endothelial cells following ischemic stroke could be a potential therapeutic target of acute treatment.

Astrocytes

Astrocytes, particularly their extended endfeet surrounding the ECM and pericytes, are the last line of defense to prevent unwanted proteins, molecules, etc. (3). The endfeet are also stabilizers of the BBB by releasing paracrine signaling (as determined by neurons), but they can modulate cerebral blood flow, neuronal functions, and tight junction formations as well (5, 53, 82, 89, 145, 158). Astrocytic dysfunction, as seen by the separation of astrocytic endfeet with the basement membrane, occurs early after MCAO, within 2 h (44, 116). This process is referred to as astrocytic swelling and corresponds to increases in BBB permeability by way of cytotoxic edema, the loss of two astrocytic-endothelial cell anchoring proteins, α1β1 and α6β1 integrins, and an increase in excretion of MMPs (74, 84, 126). Overall, this acute mechanism after ischemia is responsible for cellular death of astrocytes, exacerbating cytotoxic edema and BBB dysfunction and thus the damage after ischemic stroke (126, 127, 129).

Interestingly, expression of the astrocytic-associated integrins has been the focus of poststroke research, but little attention has been placed on their potential as a therapeutic target (see above). A possible explanation for this may be due to the loss of astrocytes inducing demyelination in the white matter that is absent in gray matter (80). Previous research has described a higher contact between the astrocytes and endothelium in the white matter (20). Because rodents possess significantly less white matter than humans, this increases the difficulty and potential lack of translation of any therapy targeting astrocytic associated integrins.

Pericytes

Pericytes exist between astrocytes and the ECM to form a support system (scaffold) for endothelial cells as well as to send paracrine signals by direct contact to the endothelium (13, 143). Additionally, pericytes are essential for regulation

of ECM proteins, particularly astrocytic laminins 1 and 2 (6). After ischemia, pericyte detachment occurs within 2 h postreperfusion, inducing hypoperfusion. Increases in post-stroke VEGF levels facilitate the detachment of pericytes from the endothelium, promoting an increase in MMP-9 expression (increasing ECM degradation) and caveolae-mediated transcytosis (increased BBB permeability) in both in vivo and in vitro models (8, 11, 32, 42, 47, 143, 151, 154). Furthermore, these pericytic-induced increases in BBB permeability are region specific, occurring more prominently in the cortex, striatum, and hippocampus (136). Administration of a VEGF inhibitor in vitro reversed the BBB permeability effects (8). Pericytes are also composed of actin and myosin filaments, generating smooth muscle type actions (9, 16). Contraction of the pericytes, decreasing the diameter of capillary vessels, is due to the acute ATP depletion following ischemic reperfusion, resulting in a reduced or complete lack of cerebral blood flow even after removal of a thrombus ("no-reflow" phenomenon) (27, 50, 107, 156).

FUTURE CONSIDERATIONS

Integrins, ECM constituents, and the rest of the components of the BBB are significantly impacted after ischemic stroke. From the influence of integrin-ECM complexes, growth factors, TJ remodeling, MMPs, etc. there are many different targets that one could turn their attention to for potential breakthroughs in understanding stroke pathophysiology and developing new therapies, although some may have more promise than others (Fig. 3).

The importance of these components, particularly integrins and ECM proteins, in embryonic development conveys their fundamental necessity for cerebral vasculature development and maintenance as demonstrated by embryonic lethality of nearly every knockout. Because of this, genetic changes (mutations, deletions, etc.) may influence the probability of experiencing a stroke and/or a stroke's severity, whether from BBB dysfunction or clot formation (i.e., $\alpha 2\beta 1$). For example, recent investigation has found that a polymorphism in the $\alpha 2$ subunit at C807T increases the probability of stroke by 1.266 times (79).

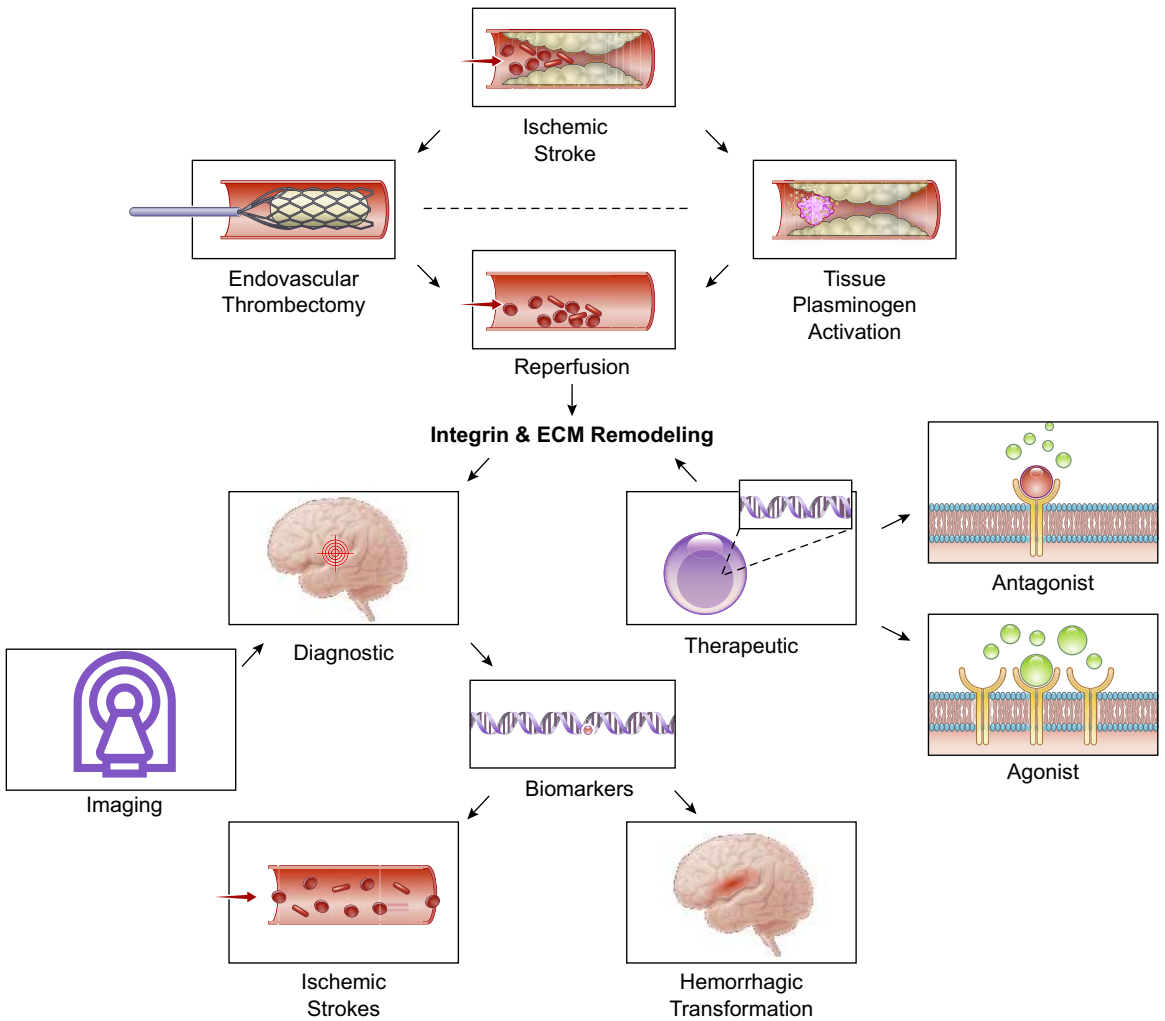

Fig. 3. Proposed future applications for integrins and extracellular matrix (ECM) in ischemic stroke.

This discovery is limited to clot formation, most likely due to increases in cholesterol in these patients (79), but the significance of potential genetic alterations cannot be understated.

As discussed, it is proposed that vasogenic edema after ischemic stroke is driven by angiogenesis (56, 113, 115) and the reorganization of BBB that must occur during this process. Angiogenesis after ischemic stroke is separated into three stages, initiation, migration and stabilization of new vasculature, and maturation (48, 122). Previous studies that have focused on increasing angiogenesis after ischemic stroke, based on the fact that patients with increased cerebrovasculature experience better outcomes, have had limited success (70, 92). Instead, our focus may need to turn toward inhibiting proangiogenic integrins (α5β1 and αvβ3) and ECM proteins (fibronectin) to prevent early angiogenesis. This appears counterintuitive; why prevent the growth of new blood vessels to an area that has experienced significant loss of cerebral blood flow and damaged vasculature in the ischemic core? As discussed in this review, mechanisms following ischemic stroke increase proangiogenic proteins, but also growth factors (VEGF) that in turn facilitate the release of pericytes from vasculature, and MMPs, increasing BBB breakdown. Furthermore, the novel finding that α5 endothelial specific knockout mice have smaller infarcts and less BBB disruption after MCAO (108) reinforces the idea that early angiogenesis, by destabilizing the BBB, is detrimental to acute stroke injury and could be a therapeutic target in the future.

Furthermore, because components of the BBB are highly reactive after ischemic stroke, novel imaging techniques could be used to visualize the ischemic core and penumbra days after the initial injury. Efforts in this direction have been made, specifically with gadolinium-tagged αvβ3 in both ischemic stroke and myocardial infarction (Ga-PRGD2) under computed tomography (CT) scans (124). Interestingly, researchers were able to detect αvβ3 differences between control patients and injury patients, up to 14 yr poststroke, but significant differences were observable only up to 3 wk poststroke (124). Furthermore, the amount of αvβ3 correlated to the severity of the injury, thus the more severe the injury, the more αvβ3 was detectable by CT with Ga-PRGD2 (124). This technique is in early development, especially for stroke, as most significant differences were observed in patients who experienced a myocardial infarction (124), but this establishes precedent for targeting proteins that are upregulated after stroke.

Finally, biomarkers for stroke severity and/or BBB dysfunction are highly sought after as an inexpensive, quick diagnostic. As previously discussed, increased levels of cellular fibronectin and MMP-9 in the serum could predict hemorrhagic transformation and severity of bleeding in ischemic stroke patients. Additionally, the degraded portions of TJ proteins enter the lumen of the vasculature and can be analyzed in a time-dependent manner. Specifically, levels of the TJ protein occludin increase in the serum by 4.5 h after ischemic stroke and continue 20 h later (24 h after ischemia) (100). Collectively, these potential serum biomarkers could predict the risk of hemorrhagic transformation after ischemic stroke, specifically when determining the use of tissue plasminogen activator (t-PA).

CONCLUSION

Taken together, ischemic stroke has a complex multifactorial and spatiotemporal impact on the BBB. Because of this, previous hopes that targeting any singular aspect of BBB stroke pathophysiology might produce a "magic bullet" for ischemic stroke treatment are unlikely to bear fruit. Instead, an effective therapeutic target(s) is likely to require multifactorial mechanisms of action on the BBB and multiple treatments may need to be used in combination to affect such benefit. Consequently, interest in the BBB, integrins, and the ECM, in the context of stroke, has never been higher and will undoubtedly lead to novel discoveries and new stroke therapies in the not too distant future.

ACKNOWLEDGMENTS

The authors thank Tom Dolan and the Medical Illustration team at the University of Kentucky for their expertise.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.E. prepared figures; D.E. drafted manuscript; D.E. and G.J.B. edited and revised manuscript; D.E. and G.J.B. approved final version of manuscript.

REFERENCES

1. **Abo-Ramadan U, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, Soinne L, Strbian D, Tatlisumak T.** Post-ischemic leakiness of the blood-brain barrier: a quantitative and systematic assessment by Patlak plots. *Exp Neurol* 219: 328–333, 2009. doi:10.1016/j.expneurol.2009.06.002.
2. **Abumiya T, Lucero J, Heo JH, Tagaya M, Koziol JA, Copeland BR, del Zoppo GJ.** Activated microvessels express vascular endothelial growth factor and integrin αvβ3 during focal cerebral ischemia. *J Cereb Blood Flow Metab* 19: 1038–1050, 1999. doi:10.1097/00004647-199909000-00012.
3. **Ahsan MS, Yamazaki M, Maruyama S, Kobayashi T, Ida-Yonemochi H, Hasegawa M, Henry Ademola A, Cheng J, Saku T.** Differential expression of perlecan receptors, α-dystroglycan and integrin β1, before and after invasion of oral squamous cell carcinoma. *J Oral Pathol Med* 40: 552–559, 2011. doi:10.1111/j.1600-0714.2010.00990.x.
4. **Alghisi GC, Ponsonnet L, Rüegg C.** The integrin antagonist cilengitide activates αvβ3, disrupts VE-cadherin localization at cell junctions and enhances permeability in endothelial cells. *PLoS One* 4: e4449, 2009. doi:10.1371/journal.pone.0004449.
5. **Alvarez JI, Katayama T, Prat A.** Glial influence on the blood brain barrier. *Glia* 61: 1939–1958, 2013. doi:10.1002/glia.22575.
6. **Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C.** Pericytes regulate the blood-brain barrier. *Nature* 468: 557–561, 2010. doi:10.1038/nature09522.
7. **Baeten KM, Akassoglou K.** Extracellular matrix and matrix receptors in blood-brain barrier formation and stroke. *Dev Neurobiol* 71: 1018–1039, 2011. doi:10.1002/dneu.20954.
8. **Bai Y, Zhu X, Chao J, Zhang Y, Qian C, Li P, Liu D, Han B, Zhao L, Zhang J, Buch S, Teng G, Hu G, Yao H.** Pericytes contribute to the disruption of the cerebral endothelial barrier via increasing VEGF expression: implications for stroke. *PLoS One* 10: e0124362, 2015. doi:10.1371/journal.pone.0124362.
9. **Bandopadhyay R, Orte C, Lawrenson JG, Reid AR, De Silva S, Allt G.** Contractile proteins in pericytes at the blood-brain and blood-retinal barriers. *J Neurocytol* 30: 35–44, 2001. doi:10.1023/A:1011965307612.
10. **Barros CS, Franco SJ, Müller U.** Extracellular matrix: functions in the nervous system. *Cold Spring Harb Perspect Biol* 3: a005108, 2011. doi:10.1101/cshperspect.a005108.
11. **Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic BV.** Pericytes control key neurovascular functions and neuro-

nal phenotype in the adult brain and during brain aging. *Neuron* 68: 409–427, 2010. doi:10.1016/j.neuron.2010.09.043.

12. **Benjamin EJ. Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jimenez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JHY, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee.** Heart Disease and Stroke Statistics—2017 Update: A Report From the American Heart Association. *Stroke* 137: 1–459, 2017. doi:10.1161/CIR.0000000000000485.
13. **Bergers G, Song S.** The role of pericytes in blood-vessel formation and maintenance. *Neuro-oncol* 7: 452–464, 2005. doi:10.1215/S1152851705000232.
14. **Bharadwaj M, Strohmeyer N, Colo GP, Helenius J, Beerenwinkel N, Schiller HB, Fässler R, Müller DJ.** αV-class integrins exert dual roles on α5β1 integrins to strengthen adhesion to fibronectin. *Nat Commun* 8: 14348, 2017. doi:10.1038/ncomms14348.
15. **Bix GJ, Gowing EK, Clarkson AN.** Perlecan domain V is neuroprotective and affords functional improvement in a photothrombotic stroke model in young and aged mice. *Transl Stroke Res* 4: 515–523, 2013. doi:10.1007/s12975-013-0266-1.
16. **Boado RJ, Pardridge WM.** Differential expression of alpha-actin mRNA and immunoreactive protein in brain microvascular pericytes and smooth muscle cells. *J Neurosci Res* 39: 430–435, 1994. doi:10.1002/jnr.490390410.
17. **Bounds JV, Wiebers DO, Whisnant JP, Okazaki H.** Mechanisms and timing of deaths from cerebral infarction. *Stroke* 12: 474–477, 1981. doi:10.1161/01.STR.12.4.474.
18. **Castellanos M, Leira R, Serena J, Blanco M, Pedraza S, Castillo J, Dávalos A.** Plasma cellular-fibronectin concentration predicts hemorrhagic transformation after thrombolytic therapy in acute ischemic stroke. *Stroke* 35: 1671–1676, 2004. doi:10.1161/01.STR.0000131656.47979.39.
19. **Castellanos M, Sobrino T, Millán M, García M, Arenillas J, Nombela F, Brea D, Perez de la Ossa N, Serena J, Vivancos J, Castillo J, Dávalos A.** Serum cellular fibronectin and matrix metalloproteinase-9 as screening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. *Stroke* 38: 1855–1859, 2007. doi:10.1161/STROKEAHA.106.481556.
20. **Chen ZL, Yao Y, Norris EH, Kruyer A, Jno-Charles O, Akhmerov A, Strickland S.** Ablation of astrocytic laminin impairs vascular smooth muscle cell function and leads to hemorrhagic stroke. *J Cell Biol* 202: 381–395, 2013. doi:10.1083/jcb.201212032.
21. **Clark EA, Brugge JS.** Integrins and signal transduction pathways: the road taken. *Science* 268: 233–239, 1995. doi:10.1126/science.7716514.
22. **Clark AW, Krekoski CA, Bou SS, Chapman KR, Edwards DR.** Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. *Neurosci Lett* 238: 53–56, 1997. doi:10.1016/S0304-3940(97)00859-8.
23. **Clarke DN, Al Ahmad A, Lee B, Parham C, Auckland L, Fertala A, Kahle M, Shaw CS, Roberts J, Bix GJ.** Perlecan domain V induces VEGf secretion in brain endothelial cells through integrin α5β1 and ERK-dependent signaling pathways. *PLoS One* 7: e45257, 2012. doi:10.1371/journal.pone.0045257.
24. **Colognato H, Yurchenco PD.** Form and function: the laminin family of heterotrimers. *Dev Dyn* 218: 213–234, 2000. doi:10.1002/(SICI)1097-0177(200006)218:2<213::AID-DVDY1>3.0.CO;2-R.
25. **Costell M, Gustafsson E, Aszódi A, Mörgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fässler R.** Perlecan maintains the integrity of cartilage and some basement membranes. *J Cell Biol* 147: 1109–1122, 1999. doi:10.1083/jcb.147.5.1109.
26. **Cox D, Brennan M, Moran N.** Integrins as therapeutic targets: lessons and opportunities. *Nat Rev Drug Discov* 9: 804–820, 2010. doi:10.1038/nrd3266.
27. **Dalkara T, Arsava EM.** Can restoring incomplete microcirculatory reperfusion improve stroke outcome after thrombolysis? *J Cereb Blood Flow Metab* 32: 2091–2099, 2012. doi:10.1038/jcbfm.2012.139.
28. **Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Davis GE, Hill MA, Meininger GA.** Integrins and mechanotransduction of the vascular myogenic response. *Am J Physiol Heart Circ Physiol* 280: H1427–H1433, 2001. doi:10.1152/ajpheart.2001.280.4.H1427.
29. **De Arcangelis A, Georges-Labouesse E.** Integrin and ECM functions: roles in vertebrate development. *Trends Genet* 16: 389–395, 2000. doi:10.1016/S0168-9525(00)02074-6.
30. **Dávalos A, Toni D, Iweins F, Lesaffre E, Bastianello S, Castillo J.** Neurological deterioration in acute ischemic stroke: potential predictors and associated factors in the European cooperative acute stroke study (ECASS) I. *Stroke* 30: 2631–2636, 1999. doi:10.1161/01.STR.30.12.2631.
31. **Dirnagl U, Iadecola C, Moskowitz MA.** Pathobiology of ischaemic stroke: an integrated view. *Trends Neurosci* 22: 391–397, 1999. doi:10.1016/S0166-2236(99)01401-0.
32. **Duz B, Oztas E, Erginay T, Erdogan E, Gonul E.** The effect of moderate hypothermia in acute ischemic stroke on pericyte migration: an ultrastructural study. *Cryobiology* 55: 279–284, 2007. doi:10.1016/j.cryobiol.2007.08.009.
33. **Eliceiri BP, Cheresh DA.** Adhesion events in angiogenesis. *Curr Opin Cell Biol* 13: 563–568, 2001. doi:10.1016/S0955-0674(00)00252-0.
34. **Faraci FM.** Protecting against vascular disease in brain. *Am J Physiol Heart Circ Physiol* 300: H1566–H1582, 2011. doi:10.1152/ajpheart.01310.2010.
35. **Feigin VL, Norrving B, George MG, Foltz JL, Roth GA, Mensah GA.** Prevention of stroke: a strategic global imperative. *Nat Rev Neurol* 12: 501–512, 2016. doi:10.1038/nrneurol.2016.107.
36. **Feigin VL, Roth GA, Naghavi M, Parmar P, Krishnamurthi R, Chugh S, Mensah GA, Norrving B, Shiue I, Ng M, Estep K, Cercy K, Murray CJL, Forouzanfar MH; Global Burden of Diseases, Injuries and Risk Factors Study 2013 and Stroke Experts Writing Group.** Global burden of stroke and risk factors in 188 countries, during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet Neurol* 15: 913–924, 2016. doi:10.1016/S1474-4422(16)30073-4.
37. **Fischer S, Wiesnet M, Marti HH, Renz D, Schaper W.** Simultaneous activation of several second messengers in hypoxia-induced hyperpermeability of brain derived endothelial cells. *J Cell Physiol* 198: 359–369, 2004. doi:10.1002/jcp.10417.
38. **Flammer AJ, Lüscher TF.** Three decades of endothelium research: from the detection of nitric oxide to the everyday implementation of endothelial function measurements in cardiovascular diseases. *Swiss Med Wkly* 140: w13122, 2010. doi:10.4414/smw.2010.13122.
39. **Fogal B, Pober JS.** Vascular endothelial cells as immunological targets in atherosclerosis. In: *Inflammation and Atherlosclerosis*, edited by Wick G, Grundtman C. Vienna: Springer, 2012, p. 87–114. doi:10.1007/978-3-7091-0338-8_6.
40. **Ford-Perriss M, Turner K, Guimond S, Apedaile A, Haubeck HD, Turnbull J, Murphy M.** Localisation of specific heparan sulfate proteoglycans during the proliferative phase of brain development. *Dev Dyn* 227: 170–184, 2003. doi:10.1002/dvdy.10298.
41. **Fujioka T, Kaneko N, Ajioka I, Nakaguchi K, Omata T, Ohba H, Fässler R, García-Verdugo JM, Sekiguchi K, Matsukawa N, Sawamoto K.** β1 integrin signaling promotes neuronal migration along vascular scaffolds in the post-stroke brain. *EBioMedicine* 16: 195–203, 2017. doi:10.1016/j.ebiom.2017.01.005.
42. **Fukuda S, Fini CA, Mabuchi T, Koziol JA, Eggleston LL Jr, del Zoppo GJ.** Focal cerebral ischemia induces active proteases that degrade microvascular matrix. *Stroke* 35: 998–1004, 2004. doi:10.1161/01.STR.0000119383.76447.05.
43. **Garcia JH, Liu K-F, Yoshida Y, Chen S, Lian J.** Brain microvessels: factors altering their patency after the occlusion of a middle cerebral artery (Wistar rat). *Am J Pathol* 145: 728–740, 1994.
44. **George EL, Georges-Labouesse EN, Patel-King RS, Rayburn H, Hynes RO.** Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development* 119: 1079–1091, 1993.
45. **Goh KL, Yang JT, Hynes RO.** Mesodermal defects and cranial neural crest apoptosis in alpha5 integrin-null embryos. *Development* 124: 4309–4319, 1997.
46. **Golestanian E, Liou JI, Smith MA.** Long-term survival in older critically ill patients with acute ischemic stroke. *Crit Care Med* 37: 3107–3113, 2009. doi:10.1097/CCM.0b013e3181b079b2.
47. **Gonul E, Duz B, Kahraman S, Kayali H, Kubar A, Timurkaynak E.** Early pericyte response to brain hypoxia in cats: an ultrastructural study. *Microvasc Res* 64: 116–119, 2002. doi:10.1006/mvre.2002.2413.

48. **Greenberg DA.** Cerebral angiogenesis: a realistic therapy for ischemic disease? *Methods Mol Biol* 1135: 21–24, 2014. doi:10.1007/978-1-4939-0320-7_2.
49. **Haley MJ, Lawrence CB.** The blood-brain barrier after stroke: structural studies and the role of transcytotic vesicles. *J Cereb Blood Flow Metab* 37: 456–470, 2017. doi:10.1177/0271678X16629976.
50. **Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D.** Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508: 55–60, 2014. doi:10.1038/nature13165.
51. **Hallmann R, Horn N, Selg M, Wendler O, Pausch F, Sorokin LM.** Expression and function of laminins in the embryonic and mature vasculature. *Physiol Rev* 85: 979–1000, 2005. doi:10.1152/physrev.00014.2004.
52. **Hamann GF, Liebetrau M, Martens H, Burggraf D, Kloss CU, Bültemeier G, Wunderlich N, Jäger G, Pfefferkorn T.** Microvascular basal lamina injury after experimental focal cerebral ischemia and reperfusion in the rat. *J Cereb Blood Flow Metab* 22: 526–533, 2002. doi:10.1097/00004647-200205000-00004.
53. **Hamby ME, Sofroniew MV.** Reactive astrocytes as therapeutic targets for CNS disorders. *Neurotherapeutics* 7: 494–506, 2010. doi:10.1016/j.nurt.2010.07.003.
54. **Handler M, Yurchenco PD, Iozzo RV.** Developmental expression of perlecan during murine embryogenesis. *Dev Dyn* 210: 130–145, 1997. doi:10.1002/(SICI)1097-0177(199710)210:2<130::AID-AJA6>3.0.CO;2-H.
55. **Härtig W, Mages B, Aleithe S, Nitzsche B, Altmann S, Barthel H, Krueger M, Michalski D.** Damaged neocortical perineuronal nets due to experimental focal cerebral ischemia in mice, rats and sheep. *Front Integr Nuerosci* 11: 15, 2017. doi:10.3389/fnint.2017.00015.
56. **Heo JH, Han SW, Lee SK.** Free radicals as triggers of brain edema formation after stroke. *Free Radic Biol Med* 39: 51–70, 2005. doi:10.1016/j.freeradbiomed.2005.03.035.
57. **Huang Q, Chen B, Wang F, Huang H, Milner R, Li L.** The temporal expression patterns of fibronectin and its receptors-α5β1 and αvβ3 integrins on blood vessels after cerebral ischemia. *Restor Neurol Neurosci* 33: 493–507, 2015. doi:10.3233/RNN-140491.
58. **Hynes RO.** Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 69: 11–25, 1992. doi:10.1016/0092-8674(92)90115-S.
59. **Hynes RO.** Integrins: bidirectional, allosteric signaling machines. *Cell* 110: 673–687, 2002. doi:10.1016/S0092-8674(02)00971-6.
60. **Johnson BH, Bonafede MM, Watson C.** Short- and longer-term healthcare resource utilization and costs associated with acute ischemic stroke. *Clinicoecon Outcomes Res* 8: 53–61, 2016. doi:10.2147/CEOR.S95662.
61. **Jucker M, Bialobok P, Kleinman HK, Walker LC, Hagg T, Ingram DK.** Laminin-like and laminin-binding protein-like immunoreactive astrocytes in rat hippocampus after transient ischemia. Antibody to laminin-binding protein is a sensitive marker of neural injury and degeneration. *Ann N Acad Sci* 679: 245–252, 1993. doi:10.1111/j.1749-6632.1993.tb18304.x.
62. **Kangwantas K, Pinteaux E, Penny J.** The extracellular matrix protein laminin-10 promotes blood-brain barrier repair after hypoxia and inflammation in vitro. *J Neuroinflammation* 13: 25, 2016. doi:10.1186/s12974-016-0495-9.
63. **Kim C, Ye F, Ginsberg MH.** Regulation of integrin activation. *Annu Rev Cell Dev Biol* 27: 321–345, 2011. doi:10.1146/annurev-cellbio-100109-104104.
64. **Kim SH, Turnbull J, Guimond S.** Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol* 209: 139–151, 2011. doi:10.1530/JOE-10-0377.
65. **Klein T, Bischoff R.** Physiology and pathophysiology of matrix metalloproteases. *Amino Acids* 41: 271–290, 2011. doi:10.1007/s00726-010-0689-x.
66. **Knowland D, Arac A, Sekiguchi KJ, Hsu M, Lutz SE, Perrino J, Steinberg GK, Barres BA, Nimmerjahn A, Agalliu D.** Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron* 82: 603–617, 2014. doi:10.1016/j.neuron.2014.03.003.
67. **Knox SM, Whitelock JM.** Perlecan: how does one molecule do so many things? *Cell Mol Life Sci* 63: 2435–2445, 2006. doi:10.1007/s00018-006-6162-z.
68. **Krueger M, Bechmann I, Immig K, Reichenbach A, Härtig W, Michalski D.** Blood-brain barrier breakdown involves four distinct stages of vascular damage in various models of experimental focal cerebral ischemia. *J Cereb Blood Flow Metab* 35: 292–303, 2015. doi:10.1038/jcbfm.2014.199.
69. **Krueger M, Härtig W, Reichenbach A, Bechmann I, Michalski D.** Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. *PLoS One* 8: e56419, 2013. doi:10.1371/journal.pone.0056419.
70. **Krupiński J, Kałuza J, Kumar P, Kumar S, Wang JM.** Some remarks on the growth-rate and angiogenesis of microvessels in ischemic stroke. Morphometric and immunocytochemical studies. *Patol Pol* 44: 203–209, 1993.
71. **Kular JK, Basu S, Sharma RI.** The extracellular matrix: structure, composition, age-related differences, tools for analysis and applications for tissue engineering. *J Tissue Eng* 5: 2041731414557112, 2014. doi:10.1177/2041731414557112.
72. **Kwon I, Kim EH, del Zoppo GJ, Heo JH.** Ultrastructural and temporal changes of the microvascular basement membrane and astrocyte interface following focal cerebral ischemia. *J Neurosci Res* 87: 668–676, 2009. doi:10.1002/jnr.21877.
73. **Lee B, Clarke D, Al Ahmad A, Kahle M, Parham C, Auckland L, Shaw C, Fidanboylu M, Orr AW, Ogunshola O, Fertala A, Thomas SA, Bix GJ.** Perlecan domain V is neuroprotective and proangiogenic following ischemic stroke in rodents. *J Clin Invest* 121: 3005–3023, 2011. doi:10.1172/JCI46358.
74. **Lee CZ, Xue Z, Zhu Y, Yang GY, Young WL.** Matrix metalloproteinase-9 inhibition attenuates vascular endothelial growth factor-induced intracerebral hemorrhage. *Stroke* 38: 2563–2568, 2007. doi:10.1161/STROKEAHA.106.481515.
75. **Li L, Liu F, Welser-Alves JV, McCullough LD, Milner R.** Upregulation of fibronectin and the α5β1 and αvβ3 integrins on blood vessels within the cerebral ischemic penumbra. *Exp Neurol* 233: 283–291, 2012. doi:10.1016/j.expneurol.2011.10.017.
76. **Li L, Welser JV, Milner R.** Absence of the alpha v beta 3 integrin dictates the time-course of angiogenesis in the hypoxic central nervous system: accelerated endothelial proliferation correlates with compensatory increases in alpha 5 beta 1 integrin expression. *J Cereb Blood Flow Metab* 30: 1031–1043, 2010. doi:10.1038/jcbfm.2009.276.
77. **Li L, Welser-Alves J, van der Flier A, Boroujerdi A, Hynes RO, Milner R.** An angiogenic role for the α5β1 integrin in promoting endothelial cell proliferation during cerebral hypoxia. *Exp Neurol* 237: 46–54, 2012. doi:10.1016/j.expneurol.2012.06.005.
78. **Li S, Edgar D, Fässler R, Wadsworth W, Yurchenco PD.** The role of laminin in embryonic cell polarization and tissue organization. *Dev Cell* 4: 613–624, 2003. doi:10.1016/S1534-5807(03)00128-X.
79. **Lu JX, Lu ZQ, Zhang SL, Zhi J, Chen ZP, Wang WX.** Polymorphism in integrin ITGA2 is associated with ischemic stroke and altered serum cholesterol in Chinese individuals. *Balkan Med J* 31: 55–59, 2014. doi:10.5152/balkanmedj.2013.7993.
80. **Lutz SE, Zhao Y, Gulinello M, Lee SC, Raine CS, Brosnan CF.** Deletion of astrocyte connexins 43 and 30 leads to a dysmyelinating phenotype and hippocampal CA1 vacuolation. *J Neurosci* 29: 7743–7752, 2009. doi:10.1523/JNEUROSCI.0341-09.2009.
81. **Maki T, Hayakawa K, Pham L-DD, Xing C, Lo EH, Arai K.** Biphasic mechanisms of neurovascular unit injury and protection in CNS diseases. *CNS Neurol Disord Drug Targets* 12: 302–315, 2013. doi:10.2174/18715271131203000 4.
82. **Maragakis NJ, Rothstein JD.** Mechanisms of disease: astrocytes in neurodegenerative disease. *Nat Clin Pract Neurol* 2: 679–689, 2006. doi:10.1038/ncpneuro0355.
83. **McCarty JH, Lacy-Hulbert A, Charest A, Bronson RT, Crowley D, Housman D, Savill J, Roes J, Hynes RO.** Selective ablation of alphav integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. *Development* 132: 165–176, 2005. doi:10.1242/dev.01551.
84. **McColl BW, Rothwell NJ, Allan SM.** Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice. *J Neurosci* 28: 9451–9462, 2008. doi:10.1523/JNEUROSCI.2674-08.2008.
85. **Melrose J, Roughley P, Knox S, Smith S, Lord M, Whitelock J.** The structure, location, and function of perlecan, a prominent pericellular proteoglycan of fetal, postnatal, and mature hyaline cartilages. *J Biol Chem* 281: 36905–36914, 2006. doi:10.1074/jbc.M608462200.
86. **Mettouchi A, Klein S, Guo W, Lopez-Lago M, Lemichez E, Westwick JK, Giancotti FG.** Integrin-specific activation of Rac controls

progression through the G_1 phase of the cell cycle. *Mol Cell* 8: 115–127, 2001. doi:10.1016/S1097-2765(01)00285-4.

87. **Milner R, Campbell IL.** Developmental regulation of β1 integrins during angiogenesis in the central nervous system. *Mol Cell Neurosci* 20: 616–626, 2002. doi:10.1006/mcne.2002.1151.
88. **Milner R, Campbell IL.** Increased expression of the β4 and α5 integrin subunits in cerebral blood vessels of transgenic mice chronically producing the pro-inflammatory cytokines IL-6 or IFN-α in the central nervous system. *Mol Cell Neurosci* 33: 429–440, 2006. doi:10.1016/j.mcn.2006.09.004.
89. **Milner R, Hung S, Wang X, Spatz M, del Zoppo GJ.** The rapid decrease in astrocyte-associated dystroglycan expression by focal cerebral ischemia is protease-dependent. *J Cereb Blood Flow Metab* 28: 812–823, 2008. doi:10.1038/sj.jcbfm.9600585.
90. **Miner JH, Yurchenco PD.** Laminin functions in tissue morphogenesis. *Annu Rev Cell Dev Biol* 20: 255–284, 2004. doi:10.1146/annurev.cellbio.20.010403.094555.
91. **Mongiat M, Andreuzzi E, Tarticchio G, Paulitti A.** Extracellular matrix, a hard player in angiogenesis. *Int J Mol Sci* 17: E1822, 2016. doi:10.3390/ijms17111822.
92. **Navaratna D, Guo S, Arai K, Lo EH.** Mechanisms and targets for angiogenic therapy after stroke. *Cell Adhes Migr* 3: 216–223, 2009. doi:10.4161/cam.3.2.8396.
93. **Nicosia RF, Bonanno E, Smith M.** Fibronectin promotes the elongation of microvessels during angiogenesis in vitro. *J Cell Physiol* 154: 654–661, 1993. doi:10.1002/jcp.1041540325.
94. **Novak U, Kaye AH.** Extracellular matrix and the brain: components and function. *J Clin Neurosci* 7: 280–290, 2000. doi:10.1054/jocn.1999.0212.
95. **Okada Y, Copeland BR, Hamann GF, Koziol JA, Cheresh DA, del Zoppo GJ.** Integrin alphavbeta3 is expressed in selected microvessels after focal cerebral ischemia. *Am J Pathol* 149: 37–44, 1996.
97. **Orecchia A, Lacal PM, Schietroma C, Morea V, Zambruno G, Failla CM.** Vascular endothelial growth factor receptor-1 is deposited in the extracellular matrix by endothelial cells and is a ligand for the alpha 5 beta 1 integrin. *J Cell Sci* 116: 3479–3489, 2003. doi:10.1242/jcs.00673.
98. **Osada T, Gu YH, Kanazawa M, Tsubota Y, Hawkins BT, Spatz M, Milner R, del Zoppo GJ.** Interendothelial claudin-5 expression depends on cerebral endothelial cell-matrix adhesion by $β_1$-integrins. *J Cereb Blood Flow Metab* 31: 1972–1985, 2011. doi:10.1038/jcbfm.2011.99.
99. **Pákáski M, Kása P, Joó F, Wolff JR.** Cerebral endothelial cell-derived laminin promotes the outgrowth of neurites in CNS neuronal cultures. *Int J Dev Neurosci* 8: 193–198, 1990. doi:10.1016/0736-5748(90)90010-Y.
100. **Pan R, Yu K, Weatherwax T, Zheng H, Liu W, Liu KJ.** Blood occludin level as a potential biomarker for early blood brain barrier damage following ischemic stroke. *Sci Rep* 7: 40331, 2017. doi:10.1038/srep40331.
101. **Pankov R, Yamada KM.** Fibronectin at a glance. *J Cell Sci* 115: 3861–3863, 2002. doi:10.1242/jcs.00059.
102. **Patino MG, Neiders ME, Andreana S, Noble B, Cohen RE.** Collagen: an overview. *Implant Dent* 11: 280–285, 2002. doi:10.1097/00008505-200207000-00014.
103. **Plate KH, Beck H, Danner S, Allegrini PR, Wiessner C.** Cell type specific upregulation of vascular endothelial growth factor in an MCA-occlusion model of cerebral infarct. *J Neuropathol Exp Neurol* 58: 654–666, 1999. doi:10.1097/00005072-199906000-00010.
104. **Rai AT, Seldon AE, Boo S, Link PS, Domico JR, Tarabishy AR, Lucke-Wold N, Carpenter JS.** A population-based incidence of acute large vessel occlusions and thrombectomy eligible patients indicates significant potential for growth of endovascular stroke therapy in the USA. *J Neurointerv Surg* 9: 722–726, 2017. doi:10.1136/neurintsurg-2016-012515.
105. **Rao NM, Levine SR, Gornbein JA, Saver JL.** Defining clinically relevant cerebral hemorrhage after thrombolytic therapy for stroke: analysis of the National Institute of Neurological Disorders and Stroke tissue-type plasminogen activator trials. *Stroke* 45: 2728–2733, 2014. doi:10.1161/STROKEAHA.114.005135.
106. **Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, White IR, Caulfield MJ, Deanfield JE, Smeeth L, Williams B, Hingorani A, Hemingway H.** Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. *Lancet* 383: 1899–1911, 2014. doi:10.1016/S0140-6736(14)60685-1.
107. **Rezkalla SH, Kloner RA.** No-reflow phenomenon. *Circulation* 105: 656–662, 2002. doi:10.1161/hc0502.102867.
108. **Roberts J, de Hoog L, Bix GJ.** Mice deficient in endothelial α5 integrin are profoundly resistant to experimental ischemic stroke. *J Cereb Blood Flow Metab* 37: 85–96, 2015. doi:10.1177/0271678X15616979.
109. **Rodgers KD, Sasaki T, Aszodi A, Jacenko O.** Reduced perlecan in mice results in chondrodysplasia resembling Schwartz-Jampel syndrome. *Hum Mol Genet* 16: 515–528, 2007. doi:10.1093/hmg/ddl484.
110. **Rothwell PM, Coull AJ, Silver LE, Fairhead JF, Giles MF, Lovelock CE, Redgrave JN, Bull LM, Welch SJ, Cuthbertson FC, Binney LE, Gutnikov SA, Anslow P, Banning AP, Mant D, Mehta Z; Oxford Vascular Study.** Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet* 366: 1773–1783, 2005. doi:10.1016/S0140-6736(05)67702-1.
111. **Ruoslahti E.** RGD and other recognition sequences for integrins. *Annu Rev Cell Dev Biol* 12: 697–715, 1996. doi:10.1146/annurev.cellbio.12.1.697.
112. **Sakai T, Johnson KJ, Murozono M, Sakai K, Magnuson MA, Wieloch T, Cronberg T, Isshiki A, Erickson HP, Fässler R.** Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nat Med* 7: 324–330, 2001. doi:10.1038/85471.
113. **Sandoval KE, Witt KA.** Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis* 32: 200–219, 2008. doi:10.1016/j.nbd.2008.08.005.
114. **Schaffner F, Ray AM, Dontenwill M.** Integrin α5β1, the fibronectin receptor, as a pertinent therapeutic target in solid tumors. *Cancers (Basel)* 5: 27–47, 2013. doi:10.3390/cancers5010027.
115. **Schlaug G, Siewert B, Benfield A, Edelman RR, Warach S.** Time course of the apparent diffusion coefficient (ADC) abnormality in human stroke. *Neurology* 49: 113–119, 1997. doi:10.1212/WNL.49.1.113.
116. **Shen W, Li S, Chung SH, Zhu L, Stayt J, Su T, Couraud PO, Romero IA, Weksler B, Gillies MC.** Tyrosine phosphorylation of VE-cadherin and claudin-5 is associated with TGF-β1-induced permeability of centrally derived vascular endothelium. *Eur J Cell Biol* 90: 323–332, 2011. doi:10.1016/j.ejcb.2010.10.013.
117. **Shimamura N, Matchett G, Solaroglu I, Tsubokawa T, Ohkuma H, Zhang J.** Inhibition of integrin alphavbeta3 reduces blood-brain barrier breakdown in focal ischemia in rats. *J Neurosci Res* 84: 1837–1847, 2006. doi:10.1002/jnr.21073.
118. **Shimamura N, Matchett G, Yatsushige H, Calvert JW, Ohkuma H, Zhang J.** Inhibition of integrin alphavbeta3 ameliorates focal cerebral ischemic damage in the rat middle cerebral artery occlusion model. *Stroke* 37: 1902–1909, 2006. doi:10.1161/01.STR.0000226991.27540.f2.
119. **Sixt M, Engelhardt B, Pausch F, Hallmann R, Wendler O, Sorokin LM.** Endothelial cell laminin isoforms, laminins 8 and 10, play decisive roles in T cell recruitment across the blood-brain barrier in experimental autoimmune encephalomyelitis. *J Cell Biol* 153: 933–946, 2001. doi:10.1083/jcb.153.5.933.
120. **Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, Edgar D.** Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. *J Cell Biol* 144: 151–160, 1999. doi:10.1083/jcb.144.1.151.
121. **Sorokin LM, Pausch F, Frieser M, Kröger S, Ohage E, Deutzmann R.** Developmental regulation of the laminin alpha5 chain suggests a role in epithelial and endothelial cell maturation. *Dev Biol* 189: 285–300, 1997. doi:10.1006/dbio.1997.8668.
122. **Stapor PC, Sweat RS, Dashti DC, Betancourt AM, Murfee WL.** Pericyte dynamics during angiogenesis: new insights from new identities. *J Vasc Res* 51: 163–174, 2014. doi:10.1159/000362276.
123. **Strbian D, Durukan A, Pitkonen M, Marinkovic I, Tatlisumak E, Pedrono E, Abo-Ramadan U, Tatlisumak T.** The blood-brain barrier is continuously open for several weeks following transient focal cerebral ischemia. *Neuroscience* 153: 175–181, 2008. doi:10.1016/j.neuroscience.2008.02.012.
124. **Sun Y, Zeng Y, Zhu Y, Feng F, Xu W, Wu C, Xing B, Zhang W, Wu P, Cui L, Wang R, Li F, Chen X, Zhu Z.** Application of (68)Ga-PRGD2 PET/CT for αvβ3-integrin imaging of myocardial infarction and stroke. *Theranostics* 4: 778–786, 2014. doi:10.7150/thno.8809.
125. **Szabó A, Kálmán M.** Disappearance of the post-lesional laminin immunopositivity of brain vessels is parallel with the formation of gliovascular junctions and common basal lamina. A double-labelling immuno-

histochemical study. *Neuropathol Appl Neurobiol* 30: 169–177, 2004. doi:10.1046/j.0305-1846.2003.00524.x.

126. **Tagaya M, Haring H-P, Stuiver I, Wagner S, Abumiya T, Lucero J, Lee P, Copeland BR, Seiffert D, del Zoppo GJ.** Rapid loss of microvascular integrin expression during focal brain ischemia reflects neuron injury. *J Cereb Blood Flow Metab* 21: 835–846, 2001. doi:10.1097/00004647-200107000-00009.
127. **Tagaya M, Liu KF, Copeland B, Seiffert D, Engler R, Garcia JH, del Zoppo GJ.** DNA scission after focal brain ischemia. Temporal differences in two species. *Stroke* 28: 1245–1254, 1997. doi:10.1161/01.STR.28.6.1245.
128. **Takada Y, Ye X, Simon S.** The integrins. *Genome Biol* 8: 215, 2007. doi:10.1186/gb-2007-8-5-215.
129. **Tate CC, García AJ, LaPlaca MC.** Plasma fibronectin is neuroprotective following traumatic brain injury. *Exp Neurol* 207: 13–22, 2007. doi:10.1016/j.expneurol.2007.05.008.
130. **Terni E, Giannini N, Brondi M, Montano V, Bonuccelli U, Mancuso M.** Genetics of ischaemic stroke in young adults. *BBA Clin* 3: 96–106, 2014. doi:10.1016/j.bbacli.2014.12.004.
131. **Thyboll J, Kortesmaa J, Cao R, Soininen R, Wang L, Iivanainen A, Sorokin L, Risling M, Cao Y, Tryggvason K.** Deletion of the laminin alpha4 chain leads to impaired microvessel maturation. *Mol Cell Biol* 22: 1194–1202, 2002. doi:10.1128/MCB.22.4.1194-1202.2002.
132. **To WS, Midwood KS.** Plasma and cellular fibronectin: distinct and independent functions during tissue repair. *Fibrogenesis Tissue Repair* 4: 21, 2011. doi:10.1186/1755-1536-4-21.
133. **van der Flier A, Badu-Nkansah K, Whittaker CA, Crowley D, Bronson RT, Lacy-Hulbert A, Hynes RO.** Endothelial alpha5 and alphav integrins cooperate in remodeling of the vasculature during development. *Development* 137: 2439–2449, 2010. doi:10.1242/dev.049551.
134. **Vanhoutte PM, Shimokawa H, Feletou M, Tang EH.** Endothelial dysfunction and vascular disease—a 30th anniversary update. *Acta Physiol (Oxf)* 219: 22–96, 2017. doi:10.1111/apha.12646.
136. **Villaseñor R, Kuennecke B, Ozmen L, Ammann M, Kugler C, Grüninger F, Loetscher H, Freskgård PO, Collin L.** Region-specific permeability of the blood-brain barrier upon pericyte loss. *J Cereb Blood Flow Metab* 37: 3683–3694, 2017. doi:10.1177/0271678X17697340.
137. **Wagner S, Tagaya M, Koziol JA, Quaranta V, del Zoppo GJ.** Rapid disruption of an astrocyte interaction with the extracellular matrix mediated by integrin alpha 6 beta 4 during focal cerebral ischemia/reperfusion. *Stroke* 28: 858–865, 1997. doi:10.1161/01.STR.28.4.858.

137a. **Wang H, Keiser JA.** Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: role of flt-1. *Circ Res* 83: 832–840, 1998. doi:10.1161/01.RES.83.8.832.

138. **Wang N, Butler JP, Ingber DE.** Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260: 1124–1127, 1993. doi:10.1126/science.7684161.
139. **Wang W, Dentler WL, Borchardt RT.** VEGF increases BMEC monolayer permeability by affecting occludin expression and tight junction assembly. *Am J Physiol Heart Circ Physiol* 280: H434–H440, 2001. doi:10.1152/ajpheart.2001.280.1.H434.
140. **Wang Y, Reheman A, Spring CM, Kalantari J, Marshall AH, Wolberg AS, Gross PL, Weitz JI, Rand ML, Mosher DF, Freedman J, Ni H.** Plasma fibronectin supports hemostasis and regulates thrombosis. *J Clin Invest* 124: 4281–4293, 2014. doi:10.1172/JCI74630.
141. **Wei L, Erinjeri JP, Rovainen CM, Woolsey TA.** Collateral growth and angiogenesis around cortical stroke. *Stroke* 32: 2179–2184, 2001. doi:10.1161/hs0901.094282.
142. **Whitelock JM, Melrose J, Iozzo RV.** Diverse cell signaling events modulated by perlecan. *Biochemistry* 47: 11174–11183, 2008. doi:10.1021/bi8013938.
143. **Winkler EA, Bell RD, Zlokovic BV.** Central nervous system pericytes in health and disease. *Nat Neurosci* 14: 1398–1405, 2011. doi:10.1038/nn.2946.
144. **Witt KA, Mark KS, Sandoval KE, Davis TP.** Reoxygenation stress on blood-brain barrier paracellular permeability and edema in the rat. *Microvasc Res* 75: 91–96, 2008. doi:10.1016/j.mvr.2007.06.004.
145. **Wong AD, Ye M, Levy AF, Rothstein JD, Bergles DE, Searson PC.** The blood-brain barrier: an engineering perspective. *Front Neuroeng* 6: 7, 2013. doi:10.3389/fneng.2013.00007.
146. **Woessner JF Jr.** Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 5: 2145–2154, 1991. doi:10.1096/fasebj.5.8.1850705.
147. **Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER III, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; Writing Group Members; American Heart Association Statistics Committee; Stroke Statistics Subcommittee.** Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* 133: e38–e360, 2016. doi:10.1161/CIR.0000000000000350.
148. **Wu X, Reddy DS.** Integrins as receptor targets for neurological disorders. *Pharmacol Ther* 134: 68–81, 2012. doi:10.1016/j.pharmthera.2011.12.008.
149. **Xiao F.** Bench to bedside: brain edema and cerebral resuscitation: the present and future. *Acad Emerg Med* 9: 933–946, 2002. doi:10.1197/aemj.9.9.933.
150. **Yamada KM, Miyamoto S.** Integrin transmembrane signaling and cytoskeletal control. *Curr Opin Cell Biol* 7: 681–689, 1995. doi:10.1016/0955-0674(95)80110-3.
151. **Yamagishi S, Yonekura H, Yamamoto Y, Fujimori H, Sakurai S, Tanaka N, Yamamoto H.** Vascular endothelial growth factor acts as a pericyte mitogen under hypoxic conditions. *Lab Invest* 79: 501–509, 1999.
152. **Yang JT, Rayburn H, Hynes RO.** Embryonic mesodermal defects in alpha 5 integrin-deficient mice. *Development* 119: 1093–1105, 1993.
153. **Yang Q, Tong X, Schieb L, Vaughan A, Gillespie C, Wiltz JL, King SC, Odom E, Merritt R, Hong Y, George MG.** Vital Signs: Recent Trends in Stroke Death Rates—United States, 2000-2015. *MMWR Morb Mortal Wkly Rep* 66: 933–939, 2017. doi:10.15585/mmwr.mm6635e1.
154. **Yang S, Jin H, Zhu Y, Wan Y, Opoku EN, Zhu L, Hu B.** Diverse functions and mechanisms of pericytes in ischemic stroke. *Curr Neuropharmacol* 15: 892–905, 2017. doi:10.2174/1570159X15666170112170226.
155. **Yao Y, Chen ZL, Norris EH, Strickland S.** Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nat Commun* 5: 3413, 2014. doi:10.1038/ncomms4413.
156. **Yemisci M, Gursoy-Ozdemir Y, Vural A, Can A, Topalkara K, Dalkara T.** Pericyte contraction induced by oxidative-nitrative stress impairs capillary reflow despite successful opening of an occluded cerebral artery. *Nat Med* 15: 1031–1037, 2009. doi:10.1038/nm.2022.
157. **Yousif LF, Di Russo J, Sorokin L.** Laminin isoforms in endothelial and perivascular basement membranes. *Cell Adhes Migr* 7: 101–110, 2013. doi:10.4161/cam.22680.
158. **Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann KA, Pozzan T, Carmignoto G.** Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci* 6: 43–50, 2003. doi:10.1038/nn980.