Basement membrane and blood–brain barrier

Lingling Xu, Abhijit Nirwane, Yao Yao

To cite: Xu L, Nirwane A, Yao Y. Basement membrane and blood–brain barrier. *Stroke and Vascular Neurology* 2019;4: e000198. doi:10.1136/svn-2018-000198

Received 19 October 2018
Accepted 16 November 2018
Published Online First
5 December 2018

ABSTRACT

The blood–brain barrier (BBB) is a highly complex and dynamic structure, mainly composed of brain microvascular endothelial cells, pericytes, astrocytes and the basement membrane (BM). The vast majority of BBB research focuses on its cellular constituents. Its non-cellular component, the BM, on the other hand, is largely understudied due to its intrinsic complexity and the lack of research tools. In this review, we focus on the role of the BM in BBB integrity. We first briefly introduce the biochemical composition and structure of the BM. Next, the biological functions of major components of the BM in BBB formation and maintenance are discussed. Our goal is to provide a concise overview on how the BM contributes to BBB integrity.

INTRODUCTION

The blood–brain barrier (BBB) is a highly complex and dynamic structure, mainly composed of brain microvascular endothelial cells (BMECs), pericytes, astrocytes and a non-cellular component—the basement membrane (BM).^{1–3} By tightly regulating what enters the brain, the BBB functions to maintain the homeostasis of the central nervous system.^{4–6} Consistent with this important role, BBB disruption has been found in a variety of neurological disorders.^{2,7,8} The vast majority of BBB research, however, focuses on its cellular constituents, including BMECs, pericytes and astrocytes. It has been shown that BMECs contribute to BBB's barrier property via forming tight junctions at the intercellular space⁹ and limiting transcellular transport (transcytosis).^{10,11} Pericytes, mural cells that cover capillaries in the vasculature, play important roles in the formation, maturation and maintenance of the BBB.¹² Astrocytes, by interacting with pericytes and BMECs through their endfeet,¹³ participate in BBB maintenance and ion/water transport.^{14,15} The readers are referred to the following references for more information on the functions of these cells in BBB integrity.^{16–21}

Unlike the cellular constituents of the BBB, the BM is largely understudied probably due to its intrinsic complexity. Recent studies suggest that the BM also contributes substantially to vascular barrier function.^{22–25} In this review, we summarise recent findings on the

function of the BM in BBB integrity. First, we briefly introduce the biochemical composition and structure of the BM. Next, we discuss the function of each major component of the BM in BBB formation and maintenance.

BASEMENT MEMBRANE

The BM is a unique form of the extracellular matrix (ECM) found predominantly underneath endothelial and epithelial cells. It exerts many important functions, including structural support, cell anchoring and signalling transduction.^{26–28} In the brain, two types of BM are found: an endothelial BM and a parenchymal BM (figure 1), which are separated by pericytes.^{29–31} Under physiological conditions, the two BM layers are indistinguishable and look like one in areas without pericytes (figure 1). Structurally, the BM is a highly organised protein sheet with a thickness of 50–100 nm.^{32–34} Biochemically, the BM consists of four major ECM proteins: collagen IV, laminin, nidogen and perlecan. These ECM proteins are synthesised predominantly by BMECs, pericytes and astrocytes at the BBB. The functional significance of each BM component in BBB integrity is discussed below.

COLLAGEN IV

Collagen IV, the most abundant component of the BM, is a trimeric protein containing three α -chains. Currently, six collagen IV α -chains (COL4A1–6) have been identified.^{35–37} Unlike COL4A3–6, which are more spatially and temporally restricted, COL4A1 and COL4A2 are present in almost all BMs and are highly conserved across species.³⁸ It has been shown that ablation of COL4A1/2 results in abnormal BM structure and embryonic lethality at E10.5–E11.5, although BM formation during early development is unaffected,³⁹ suggesting that collagen IV is required for the maintenance but not formation of the BM. In addition, mice with splice mutation lacking exon 41 of COL4A1 in both alleles die during embryogenesis, whereas those with such mutation in one allele show

© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, Georgia, USA

Correspondence to

Dr. Yao Yao, Department of Pharmaceutical and Biomedical Sciences, University of Georgia, 240 West Green Street, Athens GA 30602, USA; yyao@uga.edu

Figure 1 Schematic illustration of the blood–brain barrier. BM, basement membrane.

cerebrovascular defects, including porencephaly and intracerebral haemorrhage.^{40–42} To examine the relative contribution of each cell type at the BBB, exon 41 of COL4A1 was ablated in BMECs, pericytes and astrocytes, respectively. Although loss of exon 41 of COL4A1 in astrocytes caused very mild intracerebral haemorrhage, such mutation in BMECs or pericytes resulted in fully penetrant intracerebral haemorrhage and incompletely penetrant porencephaly.⁴² These results suggest that loss of exon 41 of COL4A1 in both BMECs and pericytes contributes to cerebrovascular defects. Consistent with these reports, various missense mutations in COL4A1/2 lead to brain malformation and intracerebral haemorrhage with different severity.^{43–45} Together, these results suggest a crucial role of collagen IV in vascular integrity. The major findings in these studies have been summarised in [table 1](#).

LAMININ

Laminin is a T-shaped or cruciform-shaped trimeric protein composed of α , β and γ chains. So far, five α , four β and three γ chains have been identified.^{46–47} Various combinations of these subunits generate a large number of laminin isoforms. Although BMECs, pericytes and astrocytes all make laminin at the BBB, they synthesise different laminin isoforms. For example, BMECs generate laminin- $\alpha4\beta1\gamma1$ (-411) and laminin-511,^{29–48} astrocytes predominately make laminin-211,^{29–49} whereas pericytes mainly synthesise laminins containing $\alpha4$, $\alpha5$ and $\gamma1$.^{50–51} Due to this cell-specific expression pattern, laminin shows differential distribution between endothelial and parenchymal BMs.³⁰ Specifically, astrocyte-derived laminin-211 is predominantly found in parenchymal BM, whereas endothelial cell-derived laminin-411 and laminin-511 are mainly located in endothelial BM.

To investigate laminin's function in BBB integrity, a variety of laminin loss-of-function mutants have been generated. Global knockout of most laminin subunits,

including $\alpha5$,^{52–54} $\beta1$ ⁵⁵ or $\gamma1$,^{55–57} leads to embryonic lethality, preventing investigation of their functions in BBB integrity. To overcome this limitation and enable investigation of laminin's function in a cell-specific manner, we generated a series of conditional knockout lines targeting the laminin $\gamma1$ chain, a common subunit found in almost all laminin isoforms at the BBB. In a previous study, we showed that loss of astrocyte-derived laminin (laminin-211) led to age-dependent BBB breakdown and intracerebral haemorrhage.¹ Consistent with our finding, laminin $\alpha2$ null mutants displayed postnatal BBB disruption.⁵⁸ These results suggest an indispensable role of astrocytic laminin in BBB maintenance.

In addition, we also generated transgenic mice with laminin deficiency in vascular smooth muscle cells (vSMCs, termed SKO hereafter)^{51–59} and mural cells (vSMCs and pericytes, termed PKO hereafter).^{51–60} In a mixed genetic background, the PKO mice demonstrated BBB breakdown and hydrocephalus, and usually died within 4 months.⁵¹ None of these deficits were observed in SKO mice,⁵¹ suggesting that it is the loss of pericyte-derived rather than vSMC-derived laminin that causes these changes. Given that hydrocephalus itself can cause BBB compromise, it remains unclear whether BBB disruption in PKO mice is due to loss of pericytic laminin or secondary to hydrocephalus. Based on that hydrocephalus is highly genetic background dependent,^{61–67} we hypothesise that we can eliminate or reduce hydrocephalus by crossing the PKO mice into different backgrounds. We are currently testing this hypothesis in our laboratory.

Unlike laminin $\alpha5$ global knockout mice, laminin $\alpha4$ null mutants are viable.⁶⁸ They show compromised vascular integrity and haemorrhage at perinatal stage but not in adulthood.⁶⁸ Since laminin $\alpha5$ expression in the vasculature starts after birth,^{48–69} it is believed that loss of laminin $\alpha4$ is compensated by laminin $\alpha5$, which rescues the haemorrhagic phenotype in adulthood. Recently, mice with laminin $\alpha5$ deficiency in endothelial cells were generated.^{70–71} These mutants fail to display any obvious defects under homeostatic conditions,^{70–71} again suggesting potential compensation between laminin $\alpha4$ and $\alpha5$. Due to this mutual compensation, the role of endothelial laminin in BBB integrity remains largely unknown. These loss-of-function studies are summarised in [table 1](#).

NIDOGEN

Nidogen, also known as entactin, functions to stabilise the collagen IV and laminin networks. Two nidogen isoforms (nidogen-1 and nidogen-2) have been identified in mammals.⁷² Interestingly, mice deficient for nidogen-1 or nidogen-2 are grossly normal, except that a mild alteration in brain capillary BM is observed in nidogen-1 mutants.^{72–74} In addition, although nidogen-1 expression is unchanged in nidogen-2 null mice,⁷⁵ redistribution and upregulation of nidogen-2 have been observed in nidogen-1 null mice.⁷⁶ These results indicate the existence

Table 1 Loss-of-function studies on major BM components

Genes	Knockout/mutation	Cre promoter	Knockout phenotype	References
Collagen IV				
Collagen 4A1/2	Global knockout	–	BM structural deficiencies and embryonic lethality (E10.5–E11.5)	39
Collagen 4A1	Lacking exon 41 in both alleles	–	Embryonic lethality	45
Collagen 4A1	Lacking exon 41 in one allele	–	Intracerebral haemorrhage and porencephaly	40 41
Collagen 4A1	Conditional knockout	Rosa26-CreER, Tie2-Cre, Pdgfrb-Cre, Gfap-Cre	Intracerebral haemorrhage and porencephaly with different severity	42
Collagen 4A1/2	Missense mutations	–	Vascular defects and brain malformations	43 44
Collagen 4A2	Missense mutations	–	Intracerebral haemorrhage	45
Laminin				
Laminin α 2	Global knockout	–	BBB disruption	58
Laminin α 4	Global knockout	–	Haemorrhage during embryonic/neonatal stage	68
Laminin α 5	Global knockout	–	Embryonic lethality (~E17) and defects in neural tube closure and neural crest cell migration	52–54
	Conditional knockout	Tie2-Cre (endothelium)	No gross CNS abnormalities under homeostatic conditions	70 71
Laminin β 1	Global knockout	–	Embryonic lethality (E5.5–E6.5)	55
Laminin γ 1	Global knockout	–	Embryonic lethality (E5.5–E6.5)	55–57
	Conditional knockout	Nestin-Cre (neural progenitors)	BBB breakdown and intracerebral haemorrhage	1 87
	Conditional knockout	Pdgfrb-Cre (mural cells)	Hydrocephalus and BBB breakdown	51
Nidogen				
Nidogen-1	Global knockout	–	Mild BM alteration in brain capillaries	72–74
Nidogen-2	Global knockout	–	No effect on BM formation	75
Nidogen-1 and nidogen-2	Global knockout	–	BM defect and perinatal lethality	77–79
Perlecan				
Perlecan	Global knockout	–	Embryonic lethality (E10–E12)	84–86

BBB, blood–brain barrier; BM, basement membrane; CNS, central nervous system.

of compensatory mechanism between nidogen-1 and nidogen-2. Consistent with this speculation, deletion of both nidogen-1 and nidogen-2 leads to severe BM defects and perinatal lethality.^{77–79} It remains unclear how nidogens contribute to BBB integrity.

PERLECAN

Perlecan, also known as heparan sulfate proteoglycan 2 (HSPG2), is an extremely large protein present in most BMs.⁸⁰ It has various domains (I–V) and motifs, which enable them to interact with a large number of molecules,^{81–83} such as ECM proteins and heparin-binding growth factors. Loss-of-function studies demonstrated that perlecan-deficient mice died at E10–E12. In addition,

many complex phenotypes in multiple tissues/organs were found in these mutants, although BM formation was not affected.^{84–86} These results suggest that perlecan is dispensable for BM formation but required for embryogenesis. Due to this early embryonic lethality, the function of perlecan in BBB integrity remains unknown.

CONCLUDING REMARKS

The BBB plays essential roles in brain homeostasis under physiological conditions and disease pathogenesis/progression under pathological conditions. Recent studies strongly suggest that the BM also actively participates in BBB regulation. However, how exactly the BM regulates BBB integrity at the molecular and cellular

levels is largely unknown due to its intrinsic complexity and the lack of research tools. With the advancement in genetics and biochemistry, we are starting to answer this important question. This knowledge will widen/deepen our understanding of BBB regulation and promote the development of innovative therapies for neurological disorders with BBB disruption.

Contributors LX and AN did the literature search. All authors wrote the manuscript. YY edited the manuscript.

Funding This work was partially supported by the American Heart Association Scientist Development Grant (16SDG29320001).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

1. Yao Y, Chen ZL, Norris EH, *et al.* Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nat Commun* 2014;5:3413.
2. Zlokovic BV. The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008;57:178–201.
3. He Y, Yao Y, Tsirka SE, *et al.* Cell-culture models of the blood–brain barrier. *Stroke* 2014;45:2514–26.
4. Persidsky Y, Ramirez SH, Haorah J, *et al.* Blood–brain barrier : structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol* 2006;1:223–36.
5. Abbott NJ, Patabendige AA, Dolman DE, *et al.* Structure and function of the blood–brain barrier. *Neurobiol Dis* 2010;37:13–25.
6. Campos-Bedolla P, Walter FR, Veszelka S, *et al.* Role of the blood–brain barrier in the nutrition of the central nervous system. *Arch Med Res* 2014;45:610–38.
7. Bell RD, Winkler EA, Sagare AP, *et al.* Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. *Neuron* 2010;68:409–27.
8. Montagne A, Barnes SR, Sweeney MD, *et al.* Blood–brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85:296–302.
9. Kniessel U, Wolburg H. Tight junctions of the blood–brain barrier. *Cell Mol Neurobiol* 2000;20:57–76.
10. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* 2004;84:869–901.
11. Fenstermacher J, Gross P, Sposito N, *et al.* Structural and functional variations in capillary systems within the brain. *Ann N Y Acad Sci* 1988;529:21–30.
12. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell* 2011;21:193–215.
13. Wong AD, Ye M, Levy AF, *et al.* The blood–brain barrier: an engineering perspective. *Front Neuroeng* 2013;6:7.
14. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol* 2010;119:7–35.
15. Montgomery DL. Astrocytes: form, functions, and roles in disease. *Vet Pathol* 1994;31:145–67.
16. Abbott NJ. Blood–brain barrier structure and function and the challenges for CNS drug delivery. *J Inherit Metab Dis* 2013;36:437–49.
17. Daneman R, Prat A. The blood–brain barrier. *Cold Spring Harb Perspect Biol* 2015;7:a020412.
18. Hawkins BT, Davis TP. The blood–brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* 2005;57:173–85.
19. Stamatovic SM, Johnson AM, Keep RF, *et al.* Junctional proteins of the blood–brain barrier: new insights into function and dysfunction. *Tissue Barriers* 2016;4:e1154641.
20. Weiss N, Miller F, Cazaubon S, *et al.* The blood–brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* 2009;1788:842–57.
21. Zhao Z, Nelson AR, Betsholtz C, *et al.* Establishment and dysfunction of the blood–brain barrier. *Cell* 2015;163:1064–78.
22. Ohashi KL, Tung DK, Wilson J, *et al.* Transvascular and interstitial migration of neutrophils in rat mesentery. *Microcirculation* 1996;3:199–210.
23. Yadav R, Larbi KY, Young RE, *et al.* Migration of leukocytes through the vessel wall and beyond. *Thromb Haemost* 2003;90:598–606.
24. Hoshi O, Ushiki T. Neutrophil extravasation in rat mesenteric venules induced by the chemotactic peptide N-formyl-methionyl-leucylphenylalanine (fMLP), with special attention to a barrier function of the vascular basal lamina for neutrophil migration. *Arch Histol Cytol* 2004;67:107–14.
25. Bixel MG, Petri B, Khandoga AG, *et al.* A CD99-related antigen on endothelial cells mediates neutrophil but not lymphocyte extravasation in vivo. *Blood* 2007;109:5327–36.
26. Kim SH, Turnbull J, Guimond S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J Endocrinol* 2011;209:139–51.
27. Baeten KM, Akassoglou K. Extracellular matrix and matrix receptors in blood–brain barrier formation and stroke. *Dev Neurobiol* 2011;71:1018–39.
28. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 2009;326:1216–9.
29. Sixt M, Engelhardt B, Pausch F, *et al.* 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* 2001;153:933–46.
30. Hallmann R, Horn N, Selg M, *et al.* Expression and function of laminins in the embryonic and mature vasculature. *Physiol Rev* 2005;85:979–1000.
31. Owens T, Bechmann I, Engelhardt B. Perivascular spaces and the two steps to neuroinflammation. *J Neuropathol Exp Neurol* 2008;67:1113–21.
32. Ruben GC, Yurchenco PD. High resolution platinum-carbon replication of freeze-dried basement membrane. *Microsc Res Tech* 1994;28:13–28.
33. Vracko R, Benditt EP. Capillary basal lamina thickening. Its relationship to endothelial cell death and replacement. *J Cell Biol* 1970;47:281–5.
34. Yurchenco PD. Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb Perspect Biol* 2011;3:a004911.
35. Hudson BG, Reeders ST, Tryggvason K, *et al.* Type IV collagen: structure, gene organization, and role in human diseases. Molecular basis of Goodpasture and Alport syndromes and diffuse leiomyomatosis. *J Biol Chem* 1993;268:26033–6.
36. Filie JD, Burbelo PD, Kozak CA. Genetic mapping of the alpha 1 and alpha 2 (IV) collagen genes to mouse chromosome 8. *Mamm Genome* 1995;6:487–87.
37. Sado Y, Kagawa M, Naito I, *et al.* Organization and expression of basement membrane collagen IV genes and their roles in human disorders. *J Biochem* 1998;123:767–76.
38. Sado Y, Kagawa M, Kishiro Y, *et al.* Establishment by the rat lymph node method of epitope-defined monoclonal antibodies recognizing the six different alpha chains of human type IV collagen. *Histochem Cell Biol* 1995;104:267–75.
39. Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, *et al.* Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 2004;131:1619–28.
40. Gould DB, Phalan FC, van Mil SE, *et al.* Role of COL4A1 in small-vessel disease and hemorrhagic stroke. *N Engl J Med* 2006;354:1489–96.
41. Gould DB, Phalan FC, Breedveld GJ, *et al.* Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science* 2005;308:1167–71.
42. Jeanne M, Jorgensen J, Gould DB. Molecular and genetic analyses of collagen type IV mutant mouse models of spontaneous intracerebral hemorrhage identify mechanisms for stroke prevention. *Circulation* 2015;131:1555–65.
43. Favor J, Gloeckner CJ, Janik D, *et al.* Type IV procollagen missense mutations associated with defects of the eye, vascular stability, the brain, kidney function and embryonic or postnatal viability in the mouse, *Mus musculus*: an extension of the Col4a1 allelic series and the identification of the first two Col4a2 mutant alleles. *Genetics* 2007;175:725–36.
44. Kuo DS, Labelle-Dumais C, Mao M, *et al.* Allelic heterogeneity contributes to variability in ocular dysgenesis, myopathy and brain

- malformations caused by Col4a1 and Col4a2 mutations. *Hum Mol Genet* 2014;23:1709–22.
45. Jeanne M, Labelle-Dumais C, Jorgensen J, et al. COL4A2 mutations impair COL4A1 and COL4A2 secretion and cause hemorrhagic stroke. *Am J Hum Genet* 2012;90:91–101.
 46. Durbecq M. Laminins. *Cell Tissue Res* 2010;339:259–68.
 47. Yao Y. Laminin: loss-of-function studies. *Cell Mol Life Sci* 2017;74:1095–115.
 48. Sorokin LM, Pausch F, Frieser M, et al. Developmental regulation of the laminin alpha5 chain suggests a role in epithelial and endothelial cell maturation. *Dev Biol* 1997;189:285–300.
 49. Jucker M, Tian M, Norton DD, et al. Laminin alpha 2 is a component of brain capillary basement membrane: reduced expression in dystrophic dy mice. *Neuroscience* 1996;71:1153–61.
 50. Stratman AN, Malotte KM, Mahan RD, et al. Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. *Blood* 2009;114:5091–101.
 51. Gautam J, Zhang X, Yao Y. The role of pericytic laminin in blood brain barrier integrity maintenance. *Sci Rep* 2016;6:36450.
 52. Nguyen NM, Miner JH, Pierce RA, et al. Laminin alpha 5 is required for lobar septation and visceral pleural basement membrane formation in the developing mouse lung. *Dev Biol* 2002;246:231–44.
 53. Miner JH, Cunningham J, Sanes JR. Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentopathy in mice lacking the laminin alpha5 chain. *J Cell Biol* 1998;143:1713–23.
 54. Coles EG, Gammill LS, Miner JH, et al. Abnormalities in neural crest cell migration in laminin alpha5 mutant mice. *Dev Biol* 2006;289:218–28.
 55. Miner JH, Li C, Mudd JL, et al. Compositional and structural requirements for laminin and basement membranes during mouse embryo implantation and gastrulation. *Development* 2004;131:2247–56.
 56. Smyth N, Vatansever HS, Meyer M, et al. The targeted deletion of the LAMC1 gene. *Ann N Y Acad Sci* 1998;857:283–6.
 57. Smyth N, Vatansever HS, Murray P, et al. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. *J Cell Biol* 1999;144:151–60.
 58. Menezes MJ, McClenahan FK, Leiton CV, et al. The extracellular matrix protein laminin α 2 regulates the maturation and function of the blood–brain barrier. *J Neurosci* 2014;34:15260–80.
 59. Yao Y, Norris EH, Strickland S. The cellular origin of laminin determines its role in blood pressure regulation. *Cell Mol Life Sci* 2015;72:999–1008.
 60. Yao Y, Norris EH, Mason CE, et al. Laminin regulates PDGFR β (+) cell stemness and muscle development. *Nat Commun* 2016;7:11415.
 61. Ding H, Wu X, Boström H, et al. A specific requirement for PDGF-C in palate formation and PDGFR- α signaling. *Nat Genet* 2004;36:1111–6.
 62. Fredriksson L, Li H, Fieber C, et al. Tissue plasminogen activator is a potent activator of PDGF-CC. *Embo J* 2004;23:3793–802.
 63. Baribault H, Penner J, Iozzo RV, et al. Colorectal hyperplasia and inflammation in keratin 8-deficient FVB/N mice. *Genes Dev* 1994;8:2964–73.
 64. Threadgill DW, Dlugosz AA, Hansen LA, et al. Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype. *Science* 1995;269:230–4.
 65. Bonyadi M, Rusholme SA, Cousins FM, et al. Mapping of a major genetic modifier of embryonic lethality in TGF beta 1 knockout mice. *Nat Genet* 1997;15:207–11.
 66. George EL, Georges-Labouesse EN, Patel-King RS, et al. Defects in mesoderm, neural tube and vascular development in mouse embryos lacking fibronectin. *Development* 1993;119:1079–91.
 67. Heiman-Patterson TD, Sher RB, Blankenhorn EA, et al. Effect of genetic background on phenotype variability in transgenic mouse models of amyotrophic lateral sclerosis: a window of opportunity in the search for genetic modifiers. *Amyotroph Lateral Scler* 2011;12:79–86.
 68. Thyboll J, Kortessmaa J, Cao R, et al. Deletion of the laminin alpha4 chain leads to impaired microvessel maturation. *Mol Cell Biol* 2002;22:1194–202.
 69. Patton BL, Miner JH, Chiu AY, et al. Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. *J Cell Biol* 1997;139:1507–21.
 70. Song J, Lokmic Z, Lammermann T, et al. Extracellular matrix of secondary lymphoid organs impacts on B-cell fate and survival. *Proc Natl Acad Sci USA* 2013;110:E2915–E2924.
 71. Song J, Zhang X, Buscher K, et al. Endothelial basement membrane laminin 511 contributes to endothelial junctional tightness and thereby inhibits leukocyte transmigration. *Cell Rep* 2017;18:1256–69.
 72. Kang SH, Kramer JM. Nidogen is nonessential and not required for normal type IV collagen localization in *Caenorhabditis elegans*. *Mol Biol Cell* 2000;11:3911–23.
 73. Dong L, Chen Y, Lewis M, et al. Neurologic defects and selective disruption of basement membranes in mice lacking entactin-1/nidogen-1. *Lab Invest* 2002;82:1617–30.
 74. Murshed M, Smyth N, Miosge N, et al. The absence of nidogen 1 does not affect murine basement membrane formation. *Mol Cell Biol* 2000;20:7007–12.
 75. Schymeinsky J, Nedbal S, Miosge N, et al. Gene structure and functional analysis of the mouse nidogen-2 gene: nidogen-2 is not essential for basement membrane formation in mice. *Mol Cell Biol* 2002;22:6820–30.
 76. Miosge N, Sasaki T, Timpl R. Evidence of nidogen-2 compensation for nidogen-1 deficiency in transgenic mice. *Matrix Biol* 2002;21:611–21.
 77. Bader BL, Smyth N, Nedbal S, et al. Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. *Mol Cell Biol* 2005;25:6846–56.
 78. Böse K, Nischt R, Page A, et al. Loss of nidogen-1 and -2 results in syndactyly and changes in limb development. *J Biol Chem* 2006;281:39620–9.
 79. Mokkapat S, Baranowsky A, Mirancea N, et al. Basement membranes in skin are differently affected by lack of nidogen 1 and 2. *J Invest Dermatol* 2008;128:2259–67.
 80. Knox SM, Whitelock JM. Perlecan: how does one molecule do so many things? *Cell Mol Life Sci* 2006;63:2435–45.
 81. Costell M, Sasaki T, Mann K, et al. Structural characterization of recombinant domain II of the basement membrane proteoglycan perlecan. *FEBS Lett* 1996;396(2–3):127–31.
 82. Dolan M, Horchar T, Rigatti B, et al. Identification of sites in domain I of perlecan that regulate heparan sulfate synthesis. *J Biol Chem* 1997;272:4316–22.
 83. Hopf M, Göhring W, Mann K, et al. Mapping of binding sites for nidogens, fibulin-2, fibronectin and heparin to different IG modules of perlecan. *J Mol Biol* 2001;311:529–41.
 84. Arikawa-Hirasawa E, Watanabe H, Takami H, et al. Perlecan is essential for cartilage and cephalic development. *Nat Genet* 1999;23:354–8.
 85. Costell M, Gustafsson E, Aszódi A, et al. Perlecan maintains the integrity of cartilage and some basement membranes. *J Cell Biol* 1999;147:1109–22.
 86. Rossi M, Morita H, Sormunen R, et al. Heparan sulfate chains of perlecan are indispensable in the lens capsule but not in the kidney. *Embo J* 2003;22:236–45.
 87. Chen ZL, Yao Y, Norris EH, et al. Ablation of astrocytic laminin impairs vascular smooth muscle cell function and leads to hemorrhagic stroke. *J Cell Biol* 2013;202:381–95.