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Review

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Perlecan Domain V Therapy for Stroke: A Beacon of Hope?

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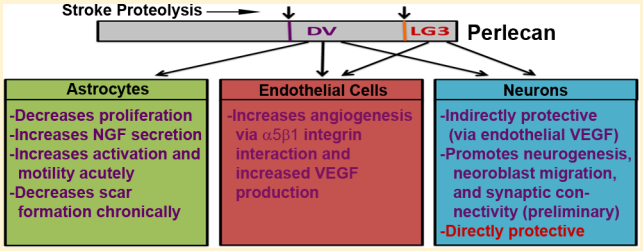
ABSTRACT: The sad reality is that in the year 2012, people are still dying or suffering from the extreme morbidity of ischemic stroke. This tragedy is only compounded by the graveyard full of once promising new therapies. While it is indeed true that the overall mortality from stroke has declined in the United States, perhaps due to increased awareness of stroke symptoms by both the lay public and physicians, it is clear that better therapies are needed. In this regard, progress has been tremendously slowed by the simple fact that experimental models of stroke and the animals that they typically employ, rats and mice, do not adequately represent human stroke. Furthermore, the neuroprotective therapeutic approach, in which potential treatments are administered with the hope of preventing the spread of dying neurons that accompanies a stroke, typically fail for a number of reasons such as there is simply more brain matter to protect in a human than there is in a rodent! For this reason, there has been somewhat of a shift in stroke research away from neuroprotection and toward a neurorepair approach. This too may be problematic in that agents that might foster brain repair could be acutely deleterious or neurotoxic and vice versa, making the timing of treatment administration after stroke critical. Therefore, in our efforts to discover a new stroke therapy, we decided to focus on identifying brain repair elements that were (1) endogenously and actively generated in response to stroke in both human and experimental animal brains, (2) present acutely and chronically after ischemic stroke, suggesting that they could have a role in acute neuroprotection and chronic neurorepair, and (3) able to be administered peripherally and reach the site of stroke brain injury. In this review, I will discuss the evidence that suggests that perlecan domain V may be just that substance, a potential beacon of hope for stroke patients.



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Having a stroke, or any brain injury for that matter, has the potential to rob us of what makes us uniquely human. Whether the injury causes unilateral weakness, an inability to speak or process language, or other severe morbidity, the final outcome can profoundly reduce one's quality of life. Despite this sobering reality, our tools to combat ischemic stroke, that kind of stroke caused by the blockage of a cerebral blood vessel (typically from a blood clot/thrombus), are very limited. If a stroke patient is "lucky" and they are quickly attended to in a capable hospital environment, they may receive tissue plasminogen activator (tPA) within a limited therapeutic window, measured in hours, in an attempt to bust the blood clot causing the stroke, re-establish blood flow to the affected brain area (which is not without its own risks of reperfusion injury[1,2]), and potentially have a good outcome. More recently, it has been demonstrated that tPA administered after a so-called "wake-up" stroke, where a patient has had a stroke while asleep and wakes up with stroke symptoms, may be efficacious even when the exact time of stroke onset cannot be pinpointed (the patient was asleep after all when the stroke occurred!).[3] Of course, tPA is only effective if one suffers a stroke due to a thrombus, which is the case about 85% of the time, the remainder being due to bleeding in the brain (hemorrhagic stroke), thereby necessitating that health care providers prove the type of stroke, ischemic versus hemorrhagic, before tPA can be administered. Furthermore, tPA carries the risk of so-called "hemorrhagic transformation", that is, causing a brain bleed, with potentially lethal consequences (the risks and benefits of tPA therapy for ischemic stroke are nicely reviewed in ref 4). Finally, for exceptionally large clots that tPA would not be able to completely lyse or when the therapeutic window for tPA is missed, the option to mechanically retrieve the clot is often available (review in ref 5). Again, this runs the inherent risk of reperfusion injury in a patient that is already hemodynamically unstable. Clearly, additional and better stroke treatment options are needed.

A PLACE FOR EVERYTHING, AND EVERYTHING IN ITS PLACE

Until quite recently, the major emphasis on developing new stroke therapies has been on protecting at risk neurons. Unfortunately, a great many very promising experimental neuroprotective therapies have failed in clinical trials. Clearly, a small mouse or rat brain, animals that are typically used in preclinical stroke studies, is inadequate to represent the human brain. Besides the issue of size, where there is simply more

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brain tissue to protect in a human than in a rodent, there is the simple fact that these rodents seem to have more resilient brains in response to injury in the first place. This, coupled with the largely artificial means by which stroke is induced experimentally, whether a cerebral blood vessel is clamped externally, stopped up from within with a filament (or even with an injected blood clot), or made to close chemically (say with endothelin-1[6,7]), results in experimental therapies that are "lost in translation". Regardless of the reason, neuroprotective therapies for stroke have by and large been a losing proposition.

What's worse, in ischemic stroke, there is a spatiotemporal gradient[8] in which potentially neuroprotective therapies have a limited window for administration before they might actually become detrimental to brain repair. For example, modulators of glutamate excitotoxicity might be acutely neuroprotective immediately after stroke but entirely detrimental to brain repair mechanisms that would require glutamate signaling for the establishment of new neuronal connections.[8] Unfortunately, this works both ways. For example, vascular endothelial growth factor (VEGF) can enhance neuroreparative processes including angiogenesis (the development of new blood vessels from preexisting vasculature) and neurogenesis (the development of new neurons) and even offer some neuroprotection but worsens the breakdown of the blood–brain barrier if administered acutely (i.e., less than 24 h) after stroke.[9−11] So, if neuroprotective therapies can only be given more or less acutely after stroke, and neuroreparative therapies can only be given subacutely or chronically after stroke, perhaps the obvious solution is to discover a therapy that is both neuroprotective and neuroreparative.

THE GAME IS AFOOT

In pursuit of just such a potential stroke therapy, we reasoned that the ideal candidate might be derived from the brain's own attempts at neuroprotection and neurorepair. We also reasoned that an ideal candidate might be (1) endogenously and actively generated in response to stroke in experimental animal and human brains, (2) present both acutely and chronically after ischemic stroke, and (3) able to be administered peripherally and reach the site of stroke brain injury. Why?...

Endogenous Brain Generation in Response to Experimental and Human Stroke. Our bodies more often than not try to compensate for injury, regardless of the ultimate success of such compensations. It is reasonable, then, that the brain has its own neuroprotective and neuroreparative mechanism(s) for responding to stroke, which are ultimately overwhelmed in the face of significant injury. Importantly, in looking for a new stroke therapy candidate, we were looking for something that was specifically generated in the brain by stroke, not something that was always present. Furthermore, if such a factor(s) could be identified in both people and experimental animals, perhaps the odds of such a factor successfully translating from preclinical to human trials could be improved. Lastly, we reasoned that a substance produced endogenously after stroke might be relatively safe (with minimal side effects) when administered therapeutically.

Present Both Acutely and Chronically after Ischemic Stroke. In the context of stroke research, the poststroke generation and cellular release of proteases is largely perceived as a bad thing (reviewed in ref 12). Among other activities, these proteases degrade extracellular components of the neurovascular niche, primarily various basement membranes and the extracellular matrix, contributing to the breakdown of the blood–brain barrier in stroke affected areas, ultimately resulting in edema formation, etc. (see ref 13 for review). However, because the extracellular matrix is more than inert extracellular glue but rather is composed of complex substances whose biology is very much influenced by proteolytic processing, it is reasonable to conclude that such stroke-induced proteolysis might "liberate" potentially beneficial components. Unfortunately, such proteolytic processing or "degradation" of the matrix is likely to do just that, that is, quickly generate and then perhaps just or nearly as quickly degrade potentially significant matrix fragments. For this reason, brain extracellular matrix proteolysis is likely to result in the production of relatively short-lived extracellular matrix fragments with subsequent limited potential to impact more chronic stroke repair and recovery processes. For example, in a number of experimental stroke models, endostatin, the antiangiogenic c-terminal portion of the extracellular matrix component collagen XVIII, is present for no more than 2−3 days as a cleaved protein fragment after stroke.[14] This may be due to a decrease in the presence or activity of specific proteases that can cleave endostatin from collagen XVIII with time, a decrease in the levels of collagen XVIII "source material" for endostatin generation, or the additional presence of other proteases that further degrade the endostatin after it is generated.

Perlecan, a heparan sulfate proteoglycan, is a prominent component of vascular basement membranes and in this capacity is found throughout the brain (see ref 15 for a review of perlecan). Previous studies have demonstrated that its 82 kDa C-terminal protein portion, termed domain V (DV), is antiangiogenic once it is proteolytically processed from full length perlecan.[16] Importantly, perlecan appears to be profoundly sensitive to proteolytic processing (by as yet unknown proteases) within a few hours after experimental stroke in nonhuman primates.[17] As an extension of this observation, we demonstrated that DV was actively generated in the brains of stroked mice and rats from 1 to 7 days after stroke[18] and at least as long as 15 days after stroke (unpublished observations). Further preliminary studies have demonstrated that DV is also generated in human stroked brain tissue (unpublished observations). Therefore, DV has at least tentatively met our first two requirements for a novel stroke therapy.

Reaching the Site of Stroke Injury. Of course, a potential stroke therapy is only as good as its ability to reach, either directly or indirectly, the affected brain tissue. This is further influenced by the potential for side effects with systemic administration, the relative instability of the stroke patient for more invasive intrathecal or intraventricular routes of administration, etc. Perhaps an ideal therapy might be one that could be administered systemically, have little or no systemic side effects, and home to the site of injury. Because previous studies have demonstrated that DV is antiangiogenic but angiogenesis is considered to be an important component of the brain's repair response to stroke, we were pleasantly surprised that human recombinant DV enhanced, rather than inhibited, brain endothelial cell angiogenesis in vitro.[18] This paradoxical activity appeared to be due to brain microvascular endothelial cells lacking the previously identified antiangiogenic $\alpha2\beta1$ integrin DV receptor coupled with the presence of a DV proangiogenic $\alpha5\beta1$ receptor on these brain endothelial cells. Because it had also been previously shown that DV was capable of homing to the site of a solid tumor xenograft on an animal's

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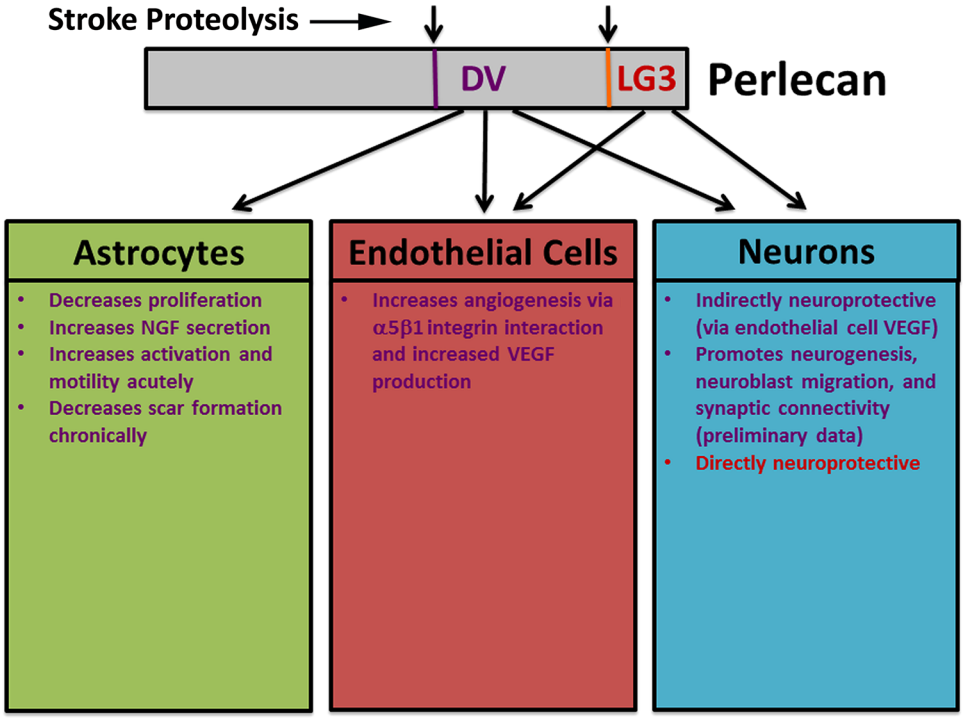

Figure 1. Perlecan DV and LG3 therapeutic effects on neurons, brain endothelial cells, and astrocytes after ischemic stroke. The schematic summarizes the currently known therapeutic effects of DV and its LG3 C-terminal portion when administered via intraperitoneal injection. DV and LG3 are also proteolytically generated from endogenous perlecan in the brain after stroke as indicated.

flank when administered via intraperitoneal (i.p.) injection as a potential cancer therapy,[19] we hypothesized that that the same could occur with i.p. DV administration after experimental stroke. Indeed, when human recombinant DV was i.p. administered 24 h after experimental stroke in mice and rats, it tracked to both the ischemic core and peri-infarct region, crossed the blood–brain barrier, and deposited in a perivascular distribution within 4 h of administration.[18] DV was able to reach the infarcted brain tissue, as well as peri-infarct brain regions, because a *transient* middle cerebral artery (mca) occlusion model was used, which allows for vascular reperfusion to the stroked brain region after 1 h of mca occlusion.

DV AS A NEW STROKE THERAPY

Importantly, perlecan DV appears to have multiple potentially beneficial effects when administered after experimental stroke. Collectively, we have so far demonstrated that poststroke DV treatment results in smaller mean infarct volumes, dramatically improved or recovered motor function, increased peri-infarct angiogenesis, increased peri-infarct astrocyte activation and motility (acutely) but decreased chronic astrogliosis, and, in preliminary studies, increased peri-infarct neurogenesis, neuroblast migration, and new synapse formation (summarized in Figure 1).[18,20] Importantly, these studies were performed using multiple stroke models (tandem ipsilateral common carotid artery and mca occlusion, endothelin-1 stereotactic injection, photothrombosis) in both rats and mice. Furthermore, *human* recombinant DV was used in all cases, which although very similar to mouse and rat DV is not identical, suggesting the possibility that if effective in rodents, it might be even more effective in human patients.

Just as DV's beneficial effects are multiple, its mechanisms of action are novel and surprising. As mentioned above, in the absence of the antiangiogenic $\alpha2\beta1$ integrin on brain microvascular endothelial cells, DV appears to exert its activity via the $\alpha5\beta1$ integrin. This interaction ultimately results in increased production and release of VEGF. VEGF, in turn, is neuroprotective and enhances angiogenesis. Curiously, just as DV has the opposite activity in nonbrain endothelial cells, so too does it suppress rather than enhance VEGF signaling outside of the brain.[21,22] Also, unlike in human umbilical vein endothelial cells and porcine aortic endothelial cells, where DV suppressed VEGFA transcription, DV had no effect on astrocyte VEGF production.[20] Clearly, DV's alternative, functionally descriptive name "endorepellin" describes only a small part of DV's activity; it does not take into consideration its differing activity on endothelial cells of brain versus nonbrain origin or its effects on other cell types.

Indeed, DV appears to act through multiple receptors in astrocytes, including $\alpha2\beta1$, $\alpha5\beta1$, and α-dystroglycan, to inhibit astrocyte proliferation (integrin mediated) and cause NGF release (α-dystroglycan), which was also antiproliferative.[20] Furthermore, while full length DV was only indirectly neuroprotective via endothelial cell produced VEGF, DV's LG3 fragment appears to be directly neuroprotective via an as yet undetermined mechanism of action.[23] This result is particularly intriguing in that it has been speculated that LG3 is the "business end" activity-wise of full length DV,[16,24] but to the best of our knowledge, it has never been shown to have distinctly different activity from full length DV. Therefore, DV and its LG3 portion, which is also endogenously generated after experimental stroke,[23] may have different but synergistic beneficial roles poststroke.

The importance of brain endothelial cell $\alpha5\beta1$ integrin for DV's poststroke activity is also intriguing in that this receptor appears to have a relatively unique temporal expression profile in brain endothelial cells. Liberally expressed during brain development when the process of angiogenesis is robust, it is

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subsequently downregulated in the mature/adult brain (where angiogenesis is minimal) in favor of $\alpha6\beta1$ expression. However, soon after stroke, this receptor is again upregulated in brain endothelial cells, a so-called "integrin receptor switch".[25] In this fashion, both DV and its key receptor are specifically increased after stroke. For this reason, DV stroke therapy may only be maximally effective when brain microvascular endothelial cell $\alpha5\beta1$ integrin expression is also maximal.[18] However, this codependence is not without some advantage. Specifically, acute (within 6 h after stroke and prior to significant upregulation of $\alpha5\beta1$ integrin expression) DV treatment does not result in increased breakdown of the blood−brain barrier and subsequent hemorrhage secondary to VEGF release.[18]

As mentioned above, DV treatment after experimental stroke appears to acutely increase peri-infarct astrocyte activation, while prolonged DV treatment reduces the extent of the gliotic scar.[20] Acute enhancement of astrocyte activation may serve to hasten the initial cleanup of the stroke affected region, that is, removal of cellular debris. Likewise, while there is much debate about the pros and cons of chronic glial scar formation, it is clearly not functional brain tissue and likely serves as a physical barrier to newborn migrating neurons that are attempting to repopulate the ischemic infarct. For this reason, we hypothesize that DV's effects on astrocyte function collectively remove impediments to and allow for sustained neuronal restoration after stroke, an area of active investigation in our laboratory.

PUTTING DV STROKE THERAPY IN CONTEXT

Perhaps unsurprisingly, other endogenous extracellular matrix proteins may have a similar beneficial effect in stroke. For example, the extracellular phosphorylated glycoprotein osteopontin is upregulated in the ischemic hemisphere of neonatal (from 12 h to 5 days) and adult (from 2 to 10 days) mice that underwent hypoxic ischemic brain injury.[26,27] Osteopontin, like DV, interacts with several integrins and appears to be both neuroprotective (when administered intraventricularly)[28,29] and an enhancer of brain cell proliferation and neurorepair as evidenced by deficient neurorepair in stroked osteopontin null mice[26] and enhancement of poststroke lateral neuroblast migration.[30] Additionally, thrombin-cleaved osteopontin may be even more neuroprotective and therapeutic after intranasal administration.[31] Likewise, fibronectin, another extracellular matrix glycoprotein and ligand for the $\alpha5\beta1$ integrin (like DV), may be neuroprotective after stroke.[32,33] Therefore, the potential for the brain extracellular matrix to harbor effective stroke therapies is not without precedent.

THE FUTURE FOR DV IN STROKE THERAPY

The history of basic and translational stroke research has clearly had more than its fair share of ups and downs. The advent of tPA and other mechanical blood clot retrieval systems has made a difference in the prognosis of many stroke patients. Increased public awareness of the signs and symptoms of stroke, coupled with improvements in cardiovascular risk factors, have had a significant impact on stroke incidence in the United States. However, the failure of the majority of experimental stroke therapies to translate to human patients remains a major concern. In this regard, we have attempted to use a few guiding principles to aid our search for a new stroke therapy with a greater chance for translational success. Namely, we believe that a successful stroke therapy may result from a better understanding of the brain's own, albeit limited, attempts to repair itself. It is these efforts that led us to the discovery that perlecan DV, an extracellular matrix fragment with proven angiomodulatory activity, is rapidly and persistently generated in the brains of stroked animals and human patients. Further investigation of DV in stroke has revealed that DV is unexpectedly proangiogenic rather than antiangiogenic, neuroprotective, antigliotic, and, in preliminary studies, proneurogenic. Therefore, DV appears to have multimodal therapeutic benefits after stroke. Perhaps this multimodal efficacy could increase the likelihood that DV will ultimately be effective in human stroke patients.

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Notes

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REFERENCES

(1) Palomares, S. M., and Cipolla, M. J. (2011) Vascular protection following cerebral ischemia reperfusion. *J. Neurol. Neurophysiol.* No. S1, No. 004.

(2) Yamashgita, T., Kamiya, T., Deguchi, K., Inaba, T., Zhang, H., Shang, J., Miyazaki, K., Ohtsuka, A., Katayama, Y., and Abe, K. (2009) Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. *J. Cereb. Blood Flow Metab. 29*, 715−725.

(3) Manawadu, D.; Bodla, S.; Jarosz, J.; Kalra, L. Thrombolysis in selected patients with wake up stroke is feasible with similar safety as thrombolysis in 0−4.5 h. Presented at the International Stroke Conference, Abstract, 2012.

(4) Armstead, W. M., Ganguly, K., Kiessling, J. W., Riley, J., Chen, X. H., Smith, D. H., Stein, S. C., Higazi, A. A., Cines, D. B., Bdeir, K., Zaitsev, S., and Muzykantov, V. R. (2010) Signaling, delivery and age as emerging issues in the benefit\risk ratio outcome of tPA for treatment of CNS ischemic disorders. *J. Neurochem. 113*, 303−312.

(5) Ansari, S., Rahman, M., McConnell, D. J., Waters, M. F., Hoh, B. L., and Mocco, J. (2011) Recanalization therapy for acute ischemic stroke, part 2: Mechanical intra-arterial technologies. *Neurosurg. Rev. 34*, 11−20.

(6) Sharkey, J., Richie, I., and Kelley, P. (1993) Perivascular microapplication of endothelin-1: A new model of focal cerebral ischaemia in the rat. *J. Cereb. Blood Flow Metab. 13*, 865−871.

(7) Sharkey, J., and Butcher, S. (1995) Characterisation of an experimental model of stroke produced by intracerebral microinjection of endothelin-1 adjacent to the rat middle cerebral artery. *J. Neurosci. Methods 60*, 125−131.

(8) Lo, E. H. (2008) A new penumbra: Transitioning from injury into repair after stroke. *Nat. Med. 14*, 497−500.

(9) Zhang, Z. G., Zhang, L., Jiang, Q., Zhang, R., Davies, K., Powers, C., Bruggen, N., and Chopp, M. (2000) VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J. Clin. Invest. 106*, 829−838.

(10) Hansen, T., Moss, A., and Brindle, N. (2008) Vascular endothelial growth factor and angiopoietins in neurovascular

regeneration and protection following stroke. *Curr. Neurovasc. Res. 5*, 235−244.
(11) Sun, Y., Jin, K., Xie, L., Childs, J., Mao, X. O., Logvinova, A., and Greenberg, D. A. (2003) VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J. Clin. Invest. 111*, 1843−1851.
(12) Wang, X., Rossell, A., and Lo, E. H. (2008) Targeting extracellular matrix proteolysis for hemorrhagic complications of tPA stroke therapy. *CNS Neurol. Disord.: Drug Targets 7*, 235−242.
(13) Roberts, J., Kahle, M. P., and Bix, G. J. (2012) Perlecan and the blood-brain barrier: Beneficial proteolysis? *Front. Pharmacol. 3*, 1−5.
(14) Tian, H. L., Chen, H., Cui, Y. H., Xu, T., and Zhou, L. F. (2007) Increased protein and mRNA expression of endostatin in the ischemic brain tissue of rabbits after middle cerebral artery occlusion. *Neurosci. Bull. 23*, 35−40.
(15) Bix, G., and Iozzo, R. (2008) Novel interactions of perlecan: Unraveling perlecan's role in angiogenesis. *Microsc. Res. Tech. 71*, 339−348.
(16) Mongiat, M., Sweeney, S., San Antonio, J. D., Fu, J., and Iozzo, R. V. (2003) Endorepellin, a novel inhibitor of angiogenesis derived from the C terminus of perlecan. *J. Biol. Chem. 278*, 4238−4249.
(17) Fukuda, S., Fini, C. A., Mabuchi, T., Koziol, J. A., Eggleston, L. L., Jr., and del Zoppo, G. J. (2004) Focal cerebral ischemia induces active proteases that degrade microvascular matrix. *Stroke 35*, 998−1004.
(18) Lee, B., Clarke, D., Al Ahmad, A., Kahle, M., Parham, C., Auckland, L., Shaw, C., Fidanboylu, M., Orr, A. W., Ogunshola, O., Fertala, A., Thomas, S. A., and Bix, G. (2011) Perlecan domain V is neuroprotective and pro-angiogenic following ischemic brain stroke in rodents. *J. Clin. Invest. 121*, 3005−3023.
(19) Bix, G., Castello, R., Burrows, M., Zoeller, J. J., Weech, M., Iozzo, R. A., Cardi, C., Thakur, M. T., Barker, C. A., Camphausen, K. C., and Iozzo, R. V. (2006) Endorepellin in vivo: Targeting the tumor vasculature and retarding cancer growth and metabolism. *J. Natl. Cancer Inst. 98*, 1634−1646.
(20) Al-Ahmad, A. J., Lee, B., Saini, M., and Bix, G. J. (2011) Perlecan domain V modulates astrogliosis in vitro and after focal cerebral ischemia through multiple receptors and increased nerve growth factor release. *Glia 59*, 1822−1840.
(21) Goyal, A., Poluzzi, C., Smythies, J., Shellard, A., Neill, T., and Iozzo, R. V. (2012) Endorepellin affects angiogenesis by antagonizing diverse VEGFR2-evoked signaling pathways: transcriptional repression of HIF-1a and VEGFA and concurrent inhibition of NFAT activation. *J. Biol. Chem.*, DOI: jbc.M112.401786.
(22) Clarke, D. N., Al Ahmad, A., Lee, B., Parham, C., Auckland, L., Fertala, A., Kahle, M., Shaw, C. S., Roberts, J., and Bix, G. J. (2012) Perlecan domain V induces VEGF secretion in brain endothelial cells through integrin a5b1 and ERK-dependent signlaing pathways. *PLoS One 7*, e45257.
(23) Saini, M., Pinteaux, E., Lee, B., and Bix, G. (2011) Oxygen-glucose deprivation and interleukin-1a trigger the release of perlecan LG3 by cells of neurovascular unit. *J. Neurochem. 119*, 760−771.
(24) Gonzalez, E. M., Reed, C. C., Bix, G., Fu, J., Zhang, Y., Gopalakrishnan, B., Greenspan, D. S., and Iozzo, R. V. (2005) BMP-1/Tolloid-like metalloproteases process endorepellin, the angiostatic C-terminal fragment of perlecan. *J. Biol. Chem. 280*, 7080−7087.
(25) Milner, R., Hung, S., Xiaoyun, W., Berg, G., Spatz, M., and del Zoppo, G. (2008) Responses of endothelial cell and astrocyte matrix-integrin receptors to ischemia mimic those observed in the neurovascular unit. *Stroke 39*, 191−197.
(26) van Velthoven, C. T. J., Heijhen, C. J., van Bel, F., and Kavelaars, A. (2011) Osteopontin enhances endogenous repair after neonatal hypoxic-ischemic brain injury. *Stroke 42*, 2294−2301.
(27) Wang, X., Louden, C., Yue, T. L., Ellison, J. A., Barone, F. C., Solleveld, H. A., and Feuerstein, G. Z. (1998) Delayed expression of osteopontin after focal stroke in the rat. *J. Neurosci. 18*, 2075−2083.
(28) Meller, R., Stevens, S. L., Minami, M., Cameron, J. A., King, S., Rosenzweig, H., Doyle, K., Lessov, N. S., Simon, R. P., and Stenzel-Poore, M. P. (2005) Neuroprotection by osteopontin in stroke. *J. Cereb. Blood Flow Metab. 25*, 217−225.
(29) Chen, W., Ma, Q., Suzuki, H., Hartman, R., Tang, J., and Zhang, J. H. (2011) Osteopontin reduced hypoxia−ischemia neonatal brain injury by suppression of apoptosis in a rat pup model. *Stroke 42*, 764−769.
(30) Yan, Y. P., Lang, B. T., Vemuganti, R., and Dempsey, R. J. (2009) Osteopontin is a mediator of the lateral migration of neuroblasts from the subventricular zone after focal cerebral ischemia. *Neurochem. Int. 55*, 826−832.
(31) Doyle, K. P., Yang, T., Lessov, N. S., Ciesielski, T. M., Stevens, S. L., Simon, R. P., King, J. S., and Stenzel-Poore, M. P. (2008) Nasal administration of osteopontin peptide mimetics confers neuroprotection in stroke. *J. Cereb. Blood Flow Metab. 28*, 1235−1248.
(32) Zhao, L. R., Spellman, S., Kim, J., Duan, W. M., McCarthy, J. B., and Low, W. C. (2005) Synthetic fibronectin peptide exerts neuroprotective effects on transient focal brain ischemia in rats. *Brain Res. 1054*, 1−8.
(33) Sakai, T., Johnson, K., Murozono, M., Sakai, K., Magnuson, M., Wieloch, T., Cronberg, T., Isshiki, A., Erickson, H., and Fassler, R. (2001) 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.