COMMENTARY

Angiogenic neovessels promote tissue hypoxia

Anusha Mishra[a,1]

The generation of blood vessels is a key early event during embryonic development that ensures the proper formation of the vasculature to supply oxygen and nutrients to the tissue. This occurs via two processes: vasculogenesis, whereby vessels are formed de novo, and angiogenesis, whereby new vessels sprout from preexisting ones (1). Angiogenic neovascularization continues to occur in the adult, especially in the context of tissue repair and bone morphogenesis (1). However, angiogenesis is also a property of pathologies, such as tumors and retinopathies, where it aberrantly contributes to disease progression.

Although pathological angiogenesis shares similar molecular signaling mechanisms as physiological and reparative angiogenesis, it is often uncontrolled and leads to progression of the disease rather than resolving it (1). In tumors, angiogenic neovessels grow into the mass of dividing cells to increase its energy supply, allowing it to grow further (2). In retinopathies, these neovesels grow abnormally into the vitreous, blocking the light path to the retina and often resulting in sight-damaging hemorrhages (3, 4). Scientists have long sought after antiangiogenic drugs to limit neovascularization to limit the progression of these pathologies (2). In PNAS, Puro et al. (5) report an intriguing new finding about the properties of these neovessels using a model of retinal pathological angiogenesis: that these neovessels have a suprahyperpolarized membrane potential, which spreads to neighboring segments of the normal vasculature and reverses their vasomotor response to hypoxic stimuli, causing them to constrict and thus promoting further hypoxia.

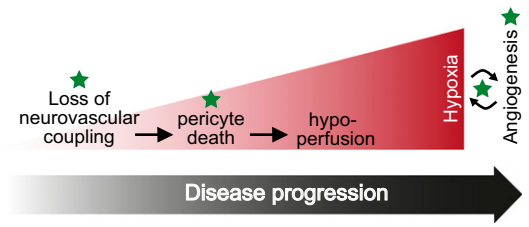

Fig. 1. Hypothetical model of hypoxia-induced pathology in diabetic retinopathy. A loss of neurovascular coupling reduces the supply of oxygen and nutrients to active cells, giving rise to a hypoxic state (denoted by the red gradient). This process causes pericytes to die, resulting in capillary hypoperfusion and further increasing tissue hypoxia. This leads to the angiogenesis, but because of the pathological properties of these angiogenic neovessels, hypoxia is further promoted and the tissue goes into a positive feedback cycle of hypoxia and neovascularization, contributing to disease progression. Green stars represent tentative loci of therapeutic targeting for nonsurgical treatment of diabetic retinopathy with early-stage interventions, like normalizing neurovascular coupling and saving pericytes from dying, and late-stage interventions, like inhibiting angiogenesis and interfering with the hypoxia–neovascularization cycle.

The retina is a protrusion of the CNS and, given its ease of access, provides an excellent preparation for the discovery of cellular and molecular biomarkers of CNS diseases. Furthermore, the retina has a very organized vascular anatomy and clinically presents with pronounced neovascularization in several disease states, including retinopathy of prematurity and diabetic retinopathy (4). Puro et al. (5) used two different animal models of oxygen-induced retinal neovascularization and characterized the new vessels that subsequently sprouted from existing retinal vessels into the vitreous. The authors find that mural cells on these neovessels are much more hyperpolarized than those on normal vessels in healthy retinas. These neovessels were electrotonically contiguous with the retinal vasculature and, therefore, the mural cells on the vessels within the retina that are in close proximity to neovascular complexes were also suprahyperpolarized. A similar suprahyperpolarization was also recorded from neovascular complexes excised from diabetic retinopathy patients (5).

Puro et al. (5) also demonstrate that the hyperpolarized state of the vascular cells is explained by three factors: an increased basal conductance through ATP-sensitive K^+ (K_{ATP}) channels, a reversal of the Na^+/Ca^{2+} exchangers (NCX), and reduced activity of nonspecific cation channels. The vasomotor response to physiologically relevant stimuli is determined by the resting membrane potential of vascular mural cells, which can alter Ca^{2+} entry and mobilization properties (6).

[a]Department of Neuroscience, Physiology, and Pharmacology, University College London, London WC1E 6BT, United Kingdom

Author contributions: A.M. performed research and wrote the paper.

The author declares no conflict of interest.

See companion article on page 9934 in issue 35 of volume 113.

[1]Email: a.mishra@ucl.ac.uk.

www.pnas.org/cgi/doi/10.1073/pnas.1612427113

Accordingly, when presented with a hypoxic challenge, these abnormally hyperpolarized retinal vessels constricted rather than dilated, the reverse of what has been observed in healthy control vessels. Puro et al. explored the role of some putative vasoactive molecules in mediating this response reversal and found that lactate, acting via the monocarboxylate transporter (MCT) and NCX, is to blame (5). This finding is perhaps because the low membrane potential of these cells (close to the K^+ reversal potential) prevents any change in voltage as a result of K^+ channel activation, while also reversing NCX activity, which allows Ca^{2+} to enter the cells when activated instead of leaving, therefore resulting in constrictions. This finding implies that the pathologically hyperpolarized membrane properties of angiogenic neovessels promote vasoconstriction via the same lactate–MCT–NCX signaling pathway that normally results in vasodilation (7).

The vasculature exists to supply oxygen and nutrients to fuel the body's metabolism, and so it is not surprising that oxygen levels, in turn, regulate vascular tone as well as growth. Indeed, hypoxia is one of the main inducers of both physiological and pathological angiogenesis. This process is assumed to be mediated by the transcriptional action of hypoxia-inducible factors (8), leading to the downstream activation of many angiogenic factors, such as fibroblast growth factors, vascular endothelial growth factors (VEGFs), platelet-derived growth factor, and so forth (8). Although these mechanisms lead to functional neovascularization under physiological and reparative angiogenesis to meet the oxygen and nutritional demands of the tissue, it appears from the data presented by Puro et al. (5) that pathological angiogenesis may not help overcome this hypoxia but rather exacerbate it, and therefore contribute to continued neovascularization.

The energy demand of the CNS is high and, because of a lack of energy stores in the nervous tissue, increases in neuronal activity result in an increase in blood supply to meet the higher energy demand via a process termed neurovascular coupling. In diabetic patients, a loss of retinal neurovascular coupling is observed long before the onset of overt clinical retinopathy (9, 10). This loss is also detectable in animal models of diabetes (11), and predates the stage when capillary hypoperfusion is observed. It is expected that a loss of neurovascular coupling will result in tissue hypoxia. Microvascular cells appear to be highly sensitive to hypoxia, as shown by previous work in both the retina (12) and the cortex (13). Perhaps the loss of neurovascular coupling gives rise to a hypoxic state in the diabetic retina, leading to the loss of pericytes (the vascular mural cells on capillaries) and reduction in capillary perfusion. This, in turn, is likely to increase the hypoxia further and induce compensatory neovascularization, but because of the pathological membrane properties of these retinal neovessels (5), hypoxia may be maintained, resulting in a vicious cycle of hypoxia–neovascularization (Fig. 1). Although these findings fit together, future research should focus on establishing the causality and temporal sequence of these maladaptive processes to better understand the relationship between hypoxia, neurovascular coupling, capillary death, and neovascularization in retinal diseases; this could be done using in vitro preparations like those used by Puro et al. (5) and also corroborated using in vivo retinal preparations (14).

The suggestion that the reversal of the hypoxia-evoked vasomotor response might contribute to a state of continued hypoxia is an interesting hypothesis advanced by Puro et al. (5). On the other hand, the resting suprahyperpolarization is expected to alter the basal tone of these vascular smooth-muscle cells and therefore to increase the luminal diameter of both the neovessels and the retinal vessels proximal to the neovessels. This may, in part, contribute to the edema and hemorrhage observed in late-stage proliferative diabetic retinopathy (3, 4). It is also assumed that an increase in basal diameter of the vessels will increase blood flow and deliver more oxygen to the region that they supply, at least at rest. The interaction between this expected increase in basal oxygen tension and the decrease expected as a result of hypoxia-induced vasoconstriction on local oxygen levels and angiogenic signals should be further explored by combining pharmacology with oximetric analysis of neovascular retinas. Given the difficulties of studying these neovessels in situ (3, 4), computational modeling might also be used to extend our understanding of the minutiae of these processes. This is especially important given the controversy over whether the angiogenesis observed in diabetic retinopathy is induced by hypoxia or the lack of some other nutrient or accumulation of some by-product of metabolism (4).

Puro et al. raise the intriguing suggestion that pathological angiogenesis attempts to self-promote by maintaining a hypoxic state around it, and suggest many new avenues of research—both in the fields of retinopathy as well as cancer—in understanding and possibly interrupting this process with therapeutic intent.

Currently, surgical laser photocoagulation therapy remains the most widely used treatment in diabetic retinopathy; however, this is only applied to prevent sight loss after neovascularization (3, 4). Antiangiogenic treatments like anti-VEGF are being investigated for use in advanced proliferative diabetic retinopathy to prevent neovascularization, but more research is needed to explore the possibility of arresting the progression of retinopathy at earlier stages. Normalization of neurovascular coupling has been suggested as a potential early-stage therapeutic approach to prevent further development of retinopathy following diabetes (15) by preventing the development of retinal hypoxia. Given the new evidence reported by Puro et al. (5), it may also be possible to generate other drugs that repolarize the neovessels to their natural membrane potential or normalize the response of these vessels to hypoxia and, therefore, interrupt the maladaptive hypoxia–neovascularization cycle. Some likely candidates were explored by Puro et al.: for example, NCX inhibitors (5). When combined with antiangiogenic treatments like anti-VEGF, such drugs could have huge potential in nonsurgically limiting neovascularization-related blindness in retinal pathologies, especially in patients with late-stage diabetic retinopathy.

These findings may apply to tumor angiogenesis, something that has yet to be explored. Therefore, from a clinical standpoint, inhibiting this cycle presents an opportunity to "starve" the cancer and therefore stop it from growing further, or stop cancer cells from metastatizing by arresting the growth of a new vascular framework, along which they often migrate (2). This forms the basis for antiangiogenic therapeutics against cancer (2), which has largely focused on inhibiting angiogenic factors (16). Development of drugs that interrupt the hypoxia–neovascularization cycle could be combined with antiangiogenic drugs to develop a more effective, multitargeted treatment approach

in cancer therapeutics. However, the first step in this direction will be to test whether neovessels in tumors have similar biophysical properties as those in the retina.

Neurovascular coupling is, at least in part, mediated by glial cells in both the cortex and the retina (17, 18). In the case of gliomas, one of the most devastating brain cancers, it has been reported that glioma cells insert their processes between the glial endfeet and the vasculature to hijack the control of existing vessels from the surrounding tissue (19). In addition to the pathological cycle of hypoxia–neovascularization, this take-over of vascular control by glioma cells probably contributes to more nutrients being supplied to the cancer rather than the surrounding healthy tissue. Future research in the field of brain cancers should also focus on dissecting how these two separate aberrant pathways—neovascularization and hijacking of neurovascular coupling—interact in advancing cancer cells.

In summary, the findings reported by Puro et al. (5) raise the intriguing suggestion that pathological angiogenesis attempts to self-promote by maintaining a hypoxic state around it, and suggest many new avenues of research—both in the fields of retinopathy as well as cancer—in understanding and possibly interrupting this process with therapeutic intent.

1 Chung AS, Ferrara N (2011) Developmental and pathological angiogenesis. *Annu Rev Cell Dev Biol* 27:563–584.
2 Folkman J (1971) Tumor angiogenesis: Therapeutic implications. *N Engl J Med* 285(21):1182–1186.
3 Stitt AW, et al. (2016) The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res* 51:156–186.
4 Gariano RF, Gardner TW (2005) Retinal angiogenesis in development and disease. *Nature* 438(7070):960–966.
5 Puro DG, Kohmoto R, Fujita Y, Gardner TW, Padovani-Claudio DA (2016) Bioelectric impact of pathological angiogenesis on vascular function. *Proc Natl Acad Sci USA* 113(35):9934–9939.
6 Jackson WF (2000) Ion channels and vascular tone. *Hypertension* 35(1 Pt 2):173–178.
7 Yamanishi S, Katsumura K, Kobayashi T, Puro DG (2006) Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *Am J Physiol Heart Circ Physiol* 290(3):H925–H934.
8 Krock BL, Skuli N, Simon MC (2011) Hypoxia-induced angiogenesis: Good and evil. *Genes Cancer* 2(12):1117–1133.
9 Garhöfer G, et al. (2004) Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. *Br J Ophthalmol* 88(7):887–891.
10 Mandecka A, et al. (2007) Influence of flickering light on the retinal vessels in diabetic patients. *Diabetes Care* 30(12):3048–3052.
11 Mishra A, Newman EA (2010) Inhibition of inducible nitric oxide synthase reverses the loss of functional hyperemia in diabetic retinopathy. *Glia* 58(16):1996–2004.
12 Nakaizumi A, Puro DG (2011) Vulnerability of the retinal microvasculature to hypoxia: Role of polyamine-regulated K(ATP) channels. *Invest Ophthalmol Vis Sci* 52(13):9345–9352.
13 Hall CN, et al. (2014) Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508(7494):55–60.
14 Srienc AI, Kornfield TE, Mishra A, Burian MA, Newman EA (2012) Assessment of glial function in the in vivo retina. *Methods Mol Biol* 814:499–514.
15 Mishra A, Newman EA (2012) Aminoguanidine reverses the loss of functional hyperemia in a rat model of diabetic retinopathy. *Front Neuroenergetics* 3:10.
16 Petrillo M, Scambia G, Ferrandina G (2012) Novel targets for VEGF-independent anti-angiogenic drugs. *Expert Opin Investig Drugs* 21(4):451–472.
17 Attwell D, et al. (2010) Glial and neuronal control of brain blood flow. *Nature* 468(7321):232–243.
18 Newman EA (2015) Glial cell regulation of neuronal activity and blood flow in the retina by release of gliotransmitters. *Philos Trans R Soc Lond B* 370(1672): 20140195.
19 Watkins S, et al. (2014) Disruption of astrocyte-vascular coupling and the blood-brain barrier by invading glioma cells. *Nat Commun* 5:4196.