Angiopoietin-2

The Key to Vascular Stabilization in Retinal Health and Disease

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Acknowledgements

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Introduction

Vascular growth is a critical and tightly regulated biological process. While angiogenesis, (also called neovascularization) — the development of new vasculature — is critical for embryonic development, it still occurs physiologically in adults, though less frequently. Still, biological processes, such as (but not limited to) the menstrual cycle, long-term sustained exercise, pregnancy, and wound healing all require neovascularization. Thus, neovascularization is relevant throughout the human lifespan. Given the importance of this system in various tissues and organ systems, vascular growth control is unsurprisingly complex, involving many factors, pathways, and processes (Figure 1).

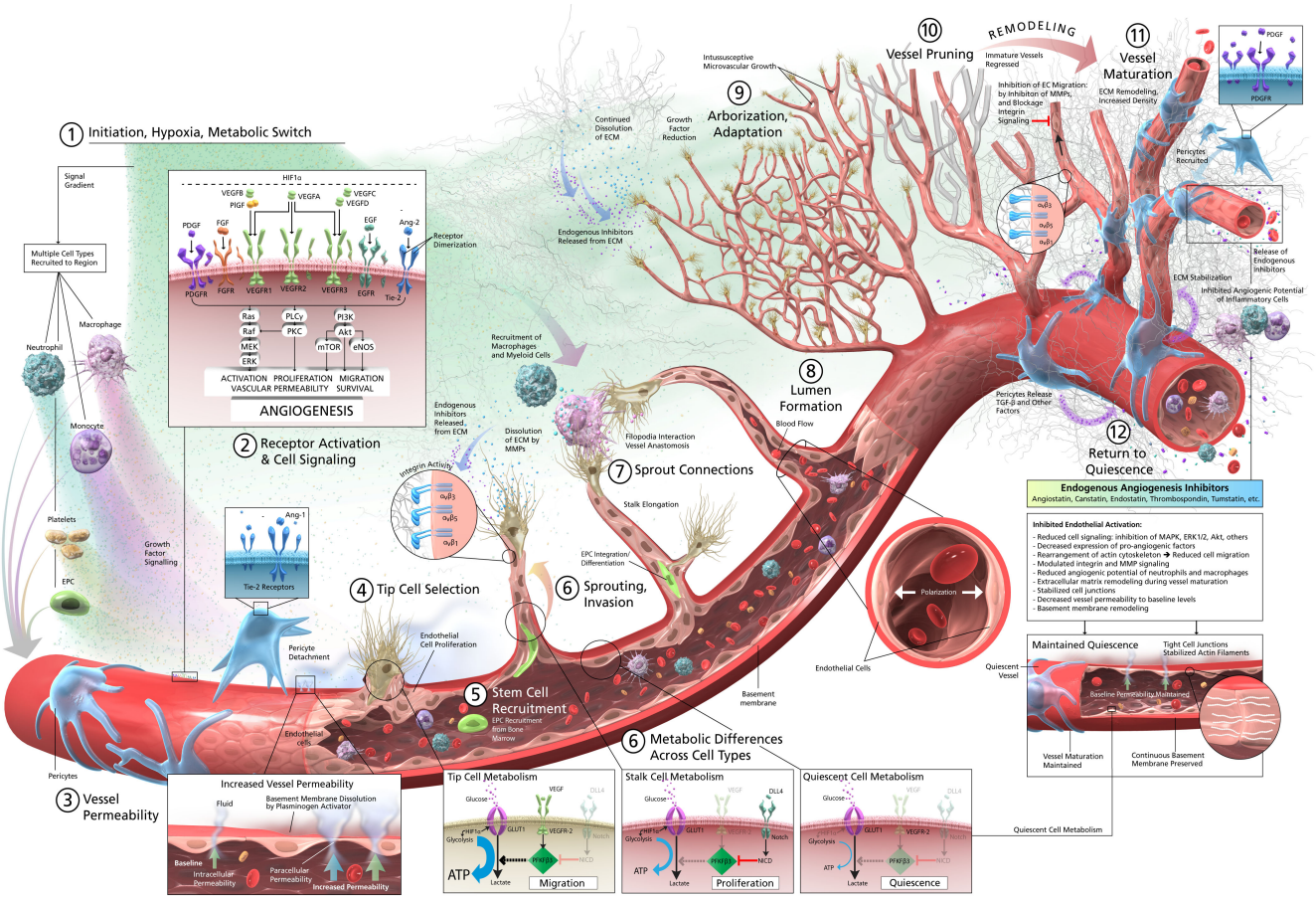

Figure 1. The process of angiogenesis.

A typical, stable blood vessel is largely comprised of a tube of endothelial cells in a tight-junction network, sheathed in pericytes which provide structural stability. A key characteristic of physiological vascular function is the control of vessel permeability. Vessels must remain structurally impermeable to function properly and avoid leakage.

In order to sprout *new* vessels, pre-existing vessels must be permeable. Thus, tightly controlled regulatory systems exist to manage vessel integrity. These regulatory pathways include the critical proteins: vascular endothelial growth factor (VEGF), tyrosine kinase receptor 2 (Tie2), as well as angiopoietins -1 and -2 (Ang-1/Ang-2). Together, these factors execute a balancing act to regulate neovascularization and vessel permeability.

Expression of angiopoietin-1 (known as Ang-1) is robust and stable in mature, healthy tissues. Ang-1 primarily functions to maintain vascular quiescence.

Ang-1 is predominantly expressed by perivascular cells, including vascular smooth muscle cells (VSMCs) and pericytes, but also in mural cells and platelets.[1,2] Ang-1 functions as an agonist for Tie2, a receptor expressed on the endothelial cell membrane.[3] Ang-1 binding and activation (via phosphorylation) of Tie2 inhibits apoptosis and promotes endothelial survival.[4] It also triggers the recruitment of pericytes, and facilitates their stable attachment to the external walls of blood vessels.[5] Pericytes reinforce the vasculature, maintaining stability and inhibiting neovascularization and leakage.[6] Additionally, pericytes also express low levels of Tie2, and Ang-1 activation of Tie2 in these cells promotes anti-migratory behavior, further enforcing vasculature stabilization.[7]

Collectively, Ang-1 activation of Tie2 supports homeostasis of the vasculature. They maintain pericyte attachment and endothelial cell cohesion and survival (Figure 2).

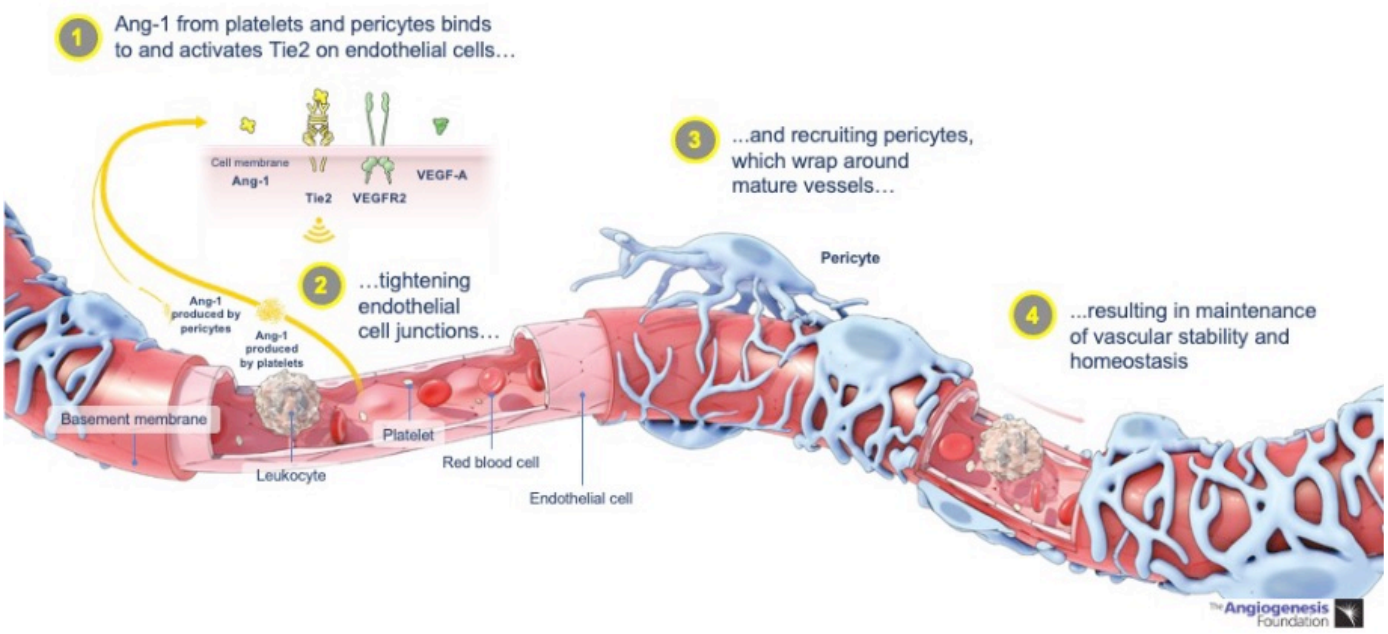

Figure 2. Ang-1 Maintains Vascular Quiescence

Ang-2 Destabilizes the Vasculature and Drives Neovascularization

Another of the angiopoietins, Ang-2, functions in diametric opposition to Ang-1. Levels are typically low in mature, homeostatic vasculature.[8] Ang-2 is primarily expressed by endothelial cells, which sequester Ang-2 inside endothelial storage granules, called Weibel–Palade bodies.[9] Ang-2 can also bind to the Tie2 receptor, and in doing so, acts as an antagonist.

Upon stimulatory input, Ang-2 is rapidly released from the Weibel–Palade bodies, and outcompetes Ang-1 for Tie2 binding, as an antagonist. With Tie2 inactivated, the VEGF receptor (VEGFR) becomes accessible to its agonist, VEGF-A.[10] VEGF signaling results in destabilization of the vasculature and promotes angiogenic behavior. Endothelial tight junctions, which are critical to the integrity of the vasculature, become disrupted. This is believed to lead to weakened cell-cell connections and hyperpermeable vessels.[11,12] Pericytes detach from the outer walls of the vasculature, further compromising vessel stability, and through these weakened, permeabilized vessels, leukocytes escape from

within the vessel. Once they are outside of the circulation, they trigger extravascular release of inflammatory cytokines.[13,14]

Collectively, this environment enables vascular sprouting to occur in neovascularization. Newly created vessels are initially structurally weak and prone to leakage (Figure 3). Thus, Ang-2 is a major effector of vascular instability.

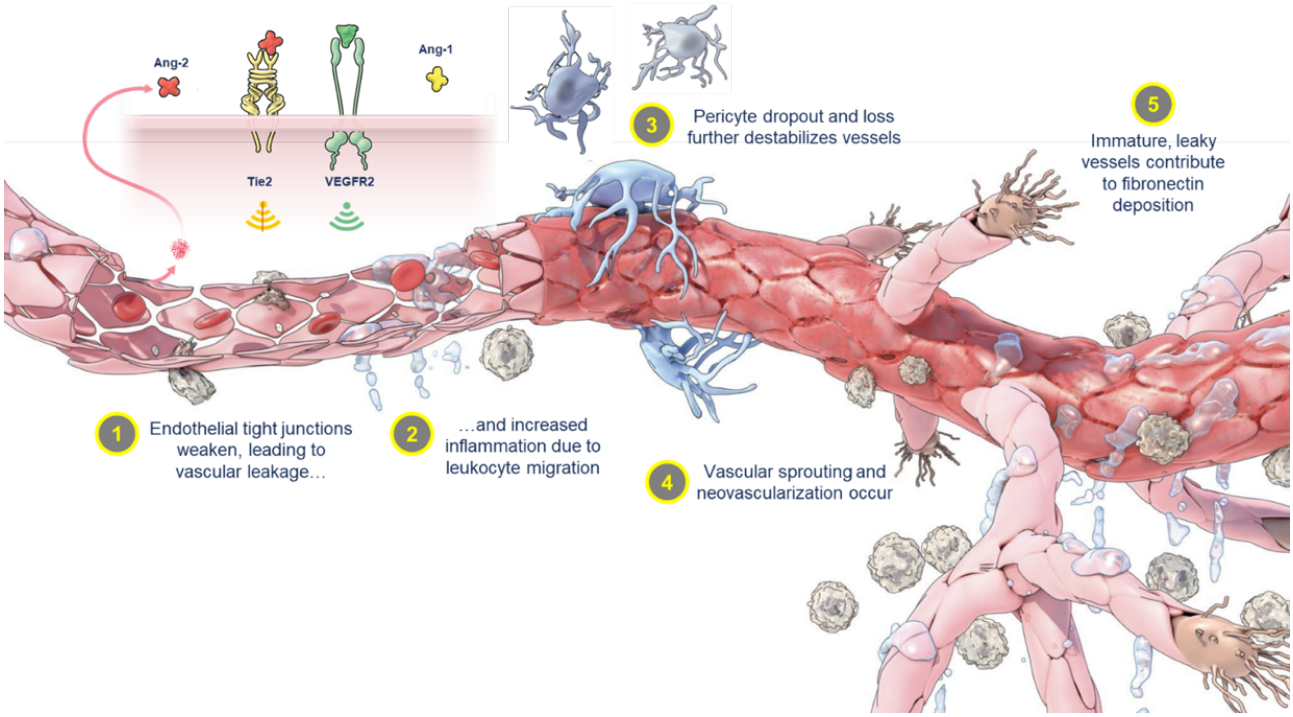

Figure 3. Ang-2 Expression Destabilizes the Vasculature and Drives Neovascularization.

Ang-2 and VEGF in Retinal Pathology

The introduction of anti-VEGF therapies represented a breakthrough in treating vascular instability in the eye.[15,16] Therefore, research has investigated novel therapeutic targets based on the discovery that Ang-2 and VEGF may work synergistically, rather than sequentially, to drive neovascularization (Figure 4). Preclinical studies have revealed that loss of Ang-2 can attenuate vascular leakage induced by overexpression of VEGF. Furthermore, both Ang-2 and VEGF function in synergy to break down endothelial tight

junctions, inducing vascular leakage.[17,18] Co-overexpression of VEGF and Ang-2 also results in loss of pericytes that compromises the structure of the neovasculature, demonstrating the close functional relationship between these two factors.[19] However, in mice models, Ang-2 does not rely entirely on VEGF to change the vasculature. Ang-2 overexpression is sufficient to compromise neovascular function and architecture, and VEGF inhibition can trigger upregulation of Ang-2, which is sufficient to increase vascular permeability.[20,21] Taken together, this suggests that dual targeting of both Ang-2 and VEGF may have synergistic effects. Indeed, dual inhibition in pre-clinical eye models demonstrated reduced vascular leakage and choroidal neovascularization.[18]

Pre-clinical models suggest blocking Ang-2 suppresses permeability, neovascularization, and inflammation. Moreover, overexpression of Ang-2 may contribute to progression of retinal vascular disease. Ultimately, Ang-2 facilitates vascular permeability in concert with VEGF and, although more research is needed, there is an emerging body of evidence that Ang-2 is critical to retinal vascular pathology.

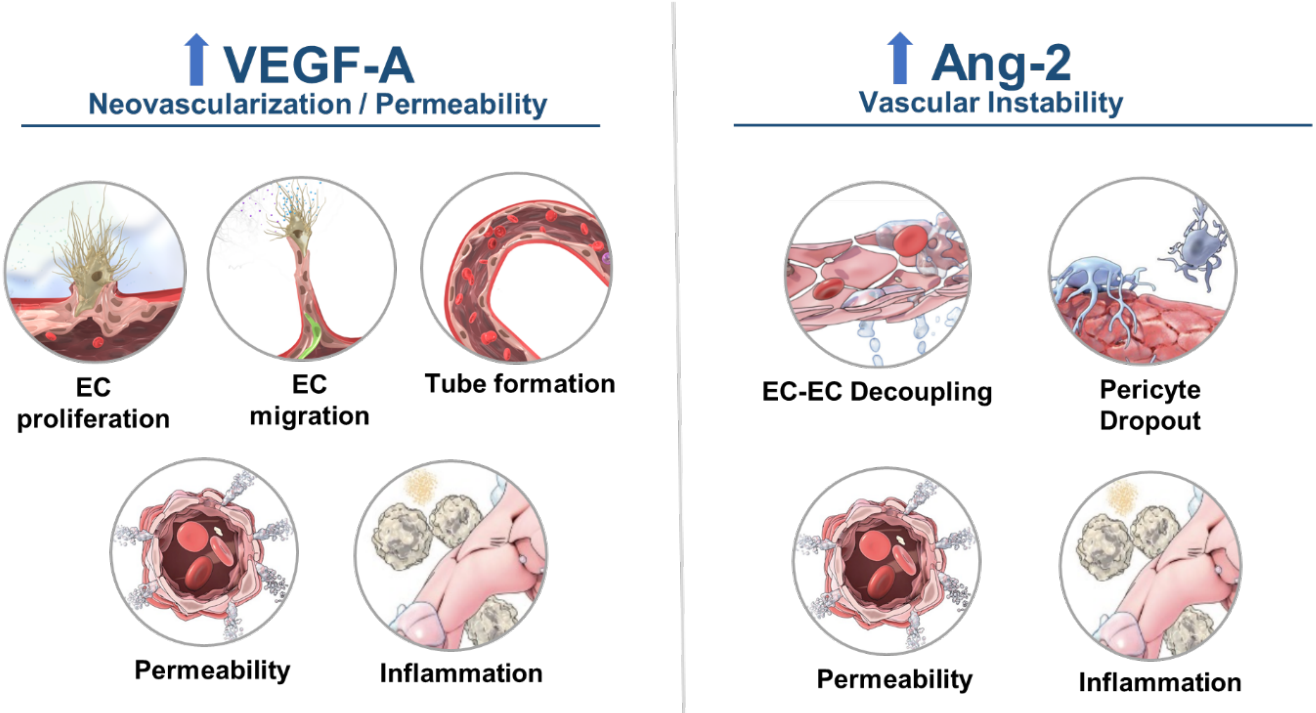

Figure 4. VEGF-A and Ang-2 have Synergistic Functions in Neovascularization.

The Ang/Tie System and its Role in Vascular Stabilization and Pathology Throughout the Body

The importance of controlling vascular homeostasis and neovascularization is not limited to the eye. This mechanism is critical for all tissues. Overexpression of Ang-2 causes vascular instability throughout the body. This is associated with pathology in many disease states, including, but not limited to, retinal neovascularization.

One of the most common pathologies that involve atypical neovascularization is tumor pathology. Overexpression of Ang-2 is found in many cancers, such as hepatocellular carcinoma, non-small cell lung cancer, and neuroendocrine tumors. In these diseases, high Ang-2 is associated with disease progression and poorer outcomes.[22–24] Tumors can become highly vascularized, but the vasculature is abnormal and dysfunctional, such as with glioblastomas.[25] Clarification of the fine points of stability will be necessary to better understand the angiogenic drive in these cancers.

Dysregulation of the Ang/Tie system is not limited to tumor development. Overexpression of Ang-2 also occurs in chronic kidney disease, where higher levels of Ang-2 correlate with increased renal tube damage and more advanced disease stages.[26,27] Venous leg ulcers, such as those common to elderly patients and those with varicose veins, demonstrate largely increased Ang-2 expression in exudative, non-healing ulcers; in a study of 35 patients, Ang-2 mRNA was found in biopsies from more non-healing ulcers than normal skin and healed ulcers ($p=0.026$).[28] More recently, it has been shown that elevated Ang-2 levels contribute to pulmonary disease severity. [29] This also applies to COVID-19, where elevated Ang-2 levels is hypothesized to be associated with increased in-hospital mortality.[30] Research into other disease states continues as well, where the implications of abnormal Ang/Tie function are observed, but not yet fully understood. One such example is in diseases treated with anti-VEGF therapeutics but complicated by venous occlusions. Veins downstream of an occlusion become unstable and leaky, exhibiting hallmark characteristics of Ang/Tie dysregulation.[31] Further study is needed to parse the involvement of the Ang/Tie axis in this and other diseases.

There are multiple retinal diseases that involve dysregulated Ang-2 expression and in which increasing Ang-2 coincides with worsening disease presentations. In neovascular age-related macular degeneration (nAMD), Ang-2 levels can be increased 5.5-fold, and as with chronic kidney disease, increased levels correlate with increased disease severity.[32] In diabetic macular edema (DME) presentations, increased levels of Ang-2 are associated with poorer glycemic control, and patients who develop active diabetic retinopathy commonly exhibit highly elevated Ang-2.[33]

The Ang/Tie axis may be involved in other retinal vascular diseases, such as sickle cell retinopathy and macular ischemia. The Ang/Tie system could very well be involved in any disease where abnormal blood vessels present in the eye without a clear understanding of the cause.

As it is well-established that control of vascular architecture is essential for any healthy organ to function, understanding the role of vascular stabilization deepens the insight into the pathogenesis of vision loss in retinal vascular diseases, where high Ang-2 is associated with poor visual acuity, macular thickness, and active neovascularization. While it is clear that the Ang/Tie axis plays an important role in homeostasis and pathogenesis of nAMD and DME, further research will continue to reveal insight into its critical role in other vascular pathologies (Figure 5).

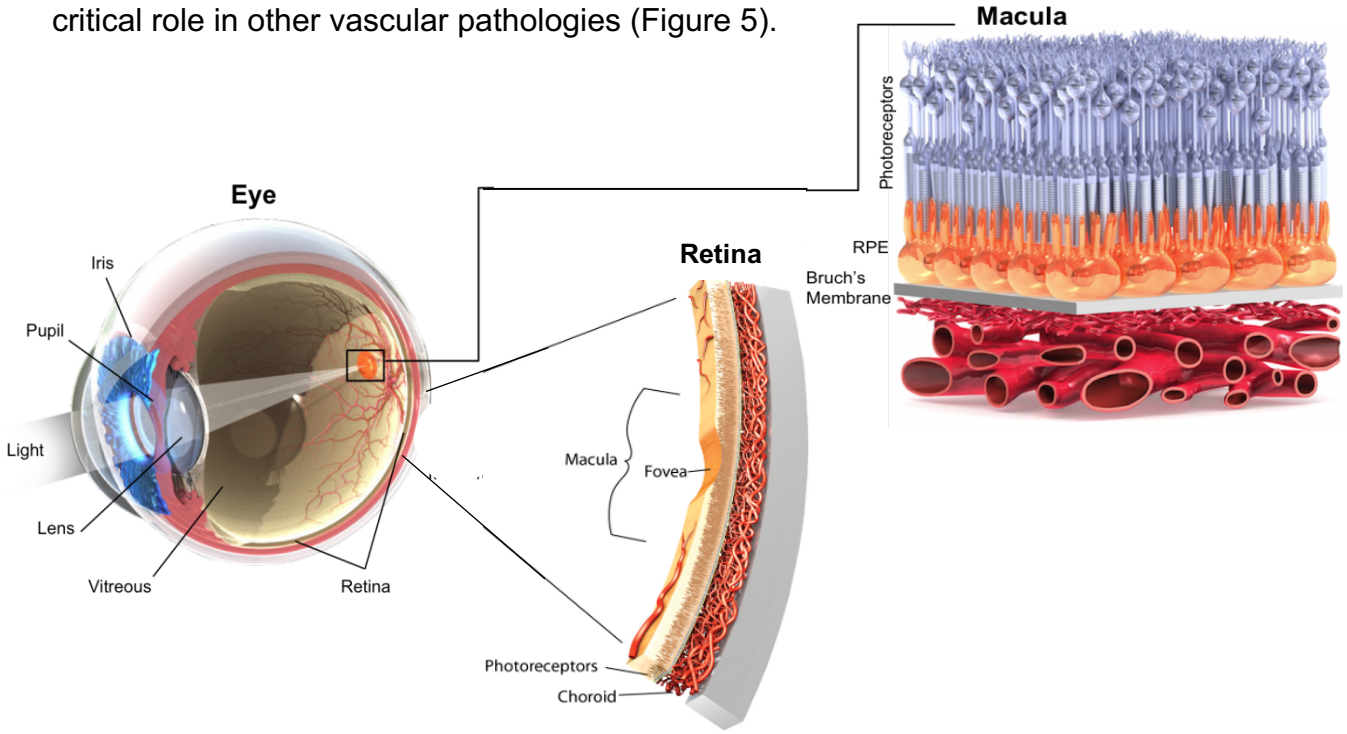

Figure 5. Neovascularization affecting the macula and retina in nAMD and DME.

Conclusions

There is a growing body of scientific evidence elucidating how the Ang/Tie pathway controls vascular stabilization throughout the body. Ang-1 is responsible for recruiting pericytes, tightening cell-cell junctions, and suppressing inflammation. Ang-2 competes with Ang-1 and reverses its effects, leading to pericyte detachment, loosened cell-cell junctions, increased vascular permeability, inflammation, and facilitating neovascularization through VEGF. Despite this, VEGF function is not entirely dependent on Ang-2 expression, nor is VEGF required for Ang-2 to drive vascular instability. Elevated Ang-2 disrupts vascular stability and is present in a diverse multitude of pathologies, from cancer to COVID-19 to vision loss, correlating with disease progression and poor outcomes.

Specific to ophthalmology, pre-clinical and clinical studies continue to support the important role the Ang/Tie axis plays in the homeostasis of the healthy eye and in the pathogenesis of nAMD and DME, and likely other retinal vascular diseases. Continued research will be helpful to fully parse its function and importance to vascular pathologies across the body, but it is clear with the knowledge that we already possess that the Ang/Tie system is of critical relevance to vascular health.

Invited Comments

"[It is] interesting seeing the impact of Ang-2 on so many other aspects of health beyond just the eye. As we know, vision problems often co-exist with other health conditions, so the more we discuss this the more likely we are to improve patient outcomes. Ang-2 and VEGF often – but not always – work hand in hand against vascular stabilization. This is certainly an important factor in improving patient outcomes, as is the fact that interventions that tackle Ang-2 along with VEGF may provide for a longer-term treatment than anti-VEGF alone. Understanding this from a patient perspective would bring added hope that patients living with AMD or DME are often challenged to find."

– Jeff Todd, President and CEO, Prevent Blindness

"The Angiogenesis Foundation has created a beautifully illustrated, thorough, and clear examination of the factors that influence the next frontier in the treatment of retinal vascular diseases: vascular instability. In simplifying the workings of the Ang/Tie pathway, the white paper and tethered webinar series provide a sense of hope – to a broad audience – that science is on the cusp of creating significant new tools in the management of neovascularization."

– Matthew Levine, Director of Advocacy and Partnerships,
American Macular Degeneration Foundation

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