Essentials in Ophthalmology
Series Editor: Arun D. Singh

Andreas Stahl *Editor*

Anti-Angiogenic Therapy in Ophthalmology

Springer

Essentials in Ophthalmology

Series Editors
Arun D. Singh

More information about this series at http://www.springer.com/series/5332

Andreas Stahl
Editor

Anti-Angiogenic Therapy in Ophthalmology

Springer

Editor
Andreas Stahl, MD
Eye Center at the Medical Center
University of Freiburg
Freiburg, Germany

ISSN 1612-3212 ISSN 2196-890X (electronic)
Essentials in Ophthalmology
ISBN 978-3-319-24095-4 ISBN 978-3-319-24097-8 (eBook)
DOI 10.1007/978-3-319-24097-8

Library of Congress Control Number: 2016937532

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

In 2006, intraocular anti-VEGF therapy for exudative age-related macular degeneration (AMD) was ranked among the top 10 breakthroughs of the year by Science Magazine. Since then, antiangiogenic therapy has broadened its impact from AMD treatment to various other diseases of the eye like macular oedema in diabetic retinopathy or retinal vein occlusion. In other areas, for example, retinopathy of prematurity (ROP), antiangiogenic therapy is just beginning to find its place and is currently being evaluated in clinical studies that weigh its benefit against potential risks. As a third category, there are indications like macular telangiectasia where antiangiogenic therapy has after initial hopeful use become to be seen as potentially unfavourable in the long run.

Due to the broad use of antiangiogenic therapies in these fundamentally different ocular diseases, it is crucial for the treating physician to understand both the underlying principles of angiogenic eye diseases and the available clinical data on therapies and outcome. This book therefore combines an overview over retinal vascular physiology with a detailed analysis of the available clinical data on antiangiogenic therapy in various ocular disorders. The authors are all experts in their respective fields and have achieved to combine concise but crucial pathophysiologic background information with detailed clinical data reflecting our current state of knowledge on antiangiogenic therapy in ophthalmology.

Freiburg, Germany — Andreas Stahl, MD

Contents

Contributors

Hansjürgen T. Agostini, MD Eye Center, Albert-Ludwigs-University Freiburg, Freiburg im Breisgau, Germany

Björn Bachmann, MD Department of Ophthalmology, University of Cologne, Cologne, Germany

James Bainbridge, PhD, FRCOphth Department of Genetics, UCL Institute of Ophthalmology, London, UK

Felix Bock, PhD Department of Ophthalmology, University of Cologne, Cologne, Cologne, Germany

Jing Chen, PhD Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

Emily Y. Chew, MD National Institutes of Health, National Eye Institute, Bethesda, MD, USA

Claus Cursiefen, MD Department of Ophthalmology, University of Cologne, Cologne, Germany

Nicolas Feltgen Universitätsmedizin Göttingen, University Eye Hospital, Göttingen, Germany

Robert P. Finger, MD, MIH, PhD Department of Ophthalmology, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, VIC, Australia

Dujon Fuzzard, MBBS/BMedSc Department of Ophthalmology, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, VIC, Australia

Salvatore Grisanti, MD Department of Ophthalmology, University of Lübeck, Lübeck, Schleswig-Holstein, Germany

Robyn H. Guymer, MBBS, PhD, FRANZCO Department of Ophthalmology, Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, VIC, Australia

Ann Hellström, MD, PhD Department of Ophthalmology, Institute of Neuroscience and Physiology, The Queen Silvia Children's Hospital, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

Frank G. Holz, MD Department of Ophthalmology, University of Bonn, Bonn, Germany

Deniz Hos, MD Department of Ophthalmology, University of Cologne, Cologne, Cologne, Germany

Peter Charbel Issa, MD, DPhil Department of Ophthalmology, University of Bonn, Bonn, Germany

Bernd Junker, MD Hannover Medical School, University Eye Hospital, Hannover, Germany

Tim U. Krohne, MD, FEBO Department of Ophthalmology, University of Bonn, Bonn, Germany

Clemens Lange, MD, PhD Eye Center, University Hospital Freiburg, Freiburg, Germany

Chi-Hsiu Liu, PhD Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

Julia Lüke, MD, PD Department of Ophthalmology, University of Lübeck, Lübeck, Schleswig-Holstein, Germany

Matthias Lüke, MD, PD Department of Ophthalmology, University of Lübeck, Lübeck, Schleswig-Holstein, Germany

Carsten H. Meyer, MD, FEBO, FMH Department of Ophthalmology, Pallas Clinic, Aargau, Switzerland

Amelie Pielen, MD Hannover Medical School, University Eye Hospital, Hannover, Germany

Przemyslaw Sapieha, PhD Departments of Ophthalmology and Biochemistry, Maisonneuve-Rosemont Hospital Research Center, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, QC, Canada

Lois E.H. Smith, MD, PhD Department of Ophthalmology, Harvard Medical School, Boston Children's Hospital, Boston, MA, USA

Andreas Stahl, MD Eye Center at the Medical Center, University of Freiburg, Freiburg, Germany

David J. Valent, DO National Institutes of Health, National Eye Institute, Bethesda, MD, USA

Focke Ziemssen, MD Center for Ophthalmology, Eberhard-Karl University Tübingen, Tübingen, Germany

Retinal Vascular Development

1

Jing Chen, Chi-Hsiu Liu, and Przemyslaw Sapieha

1.1 Anatomy of Blood Vessel Networks in the Eye

To aid in understanding retinal vascular development, we will first describe the origins of ocular blood vessels. The orbital vascular anatomy is highly complex in human. The ophthalmic artery, the first major branch of the internal carotid artery, is the main source of the arterial supply to the orbit and its derived arterial structures. It passes beneath the optic nerve and accompanies the nerve through the optic canal into the inner wall of the orbit. The central retinal artery, the first branch of the ophthalmic artery, pierces the optic nerve sheath inferiorly about 8–15 mm (in humans) behind the globe, and occupies a central position within the optic nerve when entering the retina. Other branches of ophthalmic artery, including the posterior ciliary arteries, serve the optic nerve head, choroid, ciliary body, and iris (Gray 2008; Paul Riordan-Eva 2011; Hayreh 2006).

J. Chen (✉) • C.-H. Liu
Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School, 300 Longwood Avenue, Boston, Boston, MA 02115, USA
e-mail: Jing.Chen@childrens.harvard.edu; Chi-Hsiu.Liu@childrens.harvard.edu

P. Sapieha
Departments of Ophthalmology and Biochemistry, Maisonneuve-Rosemont Hospital Research Centre, Maisonneuve-Rosemont Hospital, University of Montreal, 5415 Assomption Boulvard, Montreal, QC, Canada, H1T 2M4
e-mail: Mike.Sapieha@umontreal.ca

1.1.1 Retinal Vessels

The retina is one of the most structurally intricate and metabolically active tissues in the body. It receives its blood supply from two sources: (1) the central retinal artery and its three branched plexi, which supplies the inner two-thirds of the retina; and (2) the choriocapillaris (choriocapillary layer) adjacent to the Bruch's membrane which supplies the outer retina. The central retinal artery and its accompanying vein run along the inferior margin of the optic nerve sheath and enter the eye through the optic disk. The vessel branches then immediately bifurcate into the superior nasal and temporal, or the inferior nasal and temporal branches, each supplying a distinct quadrant of the retina. The branching pattern of the vessels is either dichotomous or at right angles to the original vessel (Gray 2008; Paul Riordan-Eva 2011; Netter 2006). Branches from the central retinal artery then dive into the retina towards photoreceptors forming a capillary plexus which provides nutrients to the inner retinal layers. The overall structure of retinal vessels is composed of three distinct capillary layers, one in the nerve fiber layer and the other two along each sides of the inner nuclear layer (Fig. 1.1).

A. Stahl (ed.), *Anti-Angiogenic Therapy in Ophthalmology*, Essentials in Ophthalmology, DOI 10.1007/978-3-319-24097-8_1

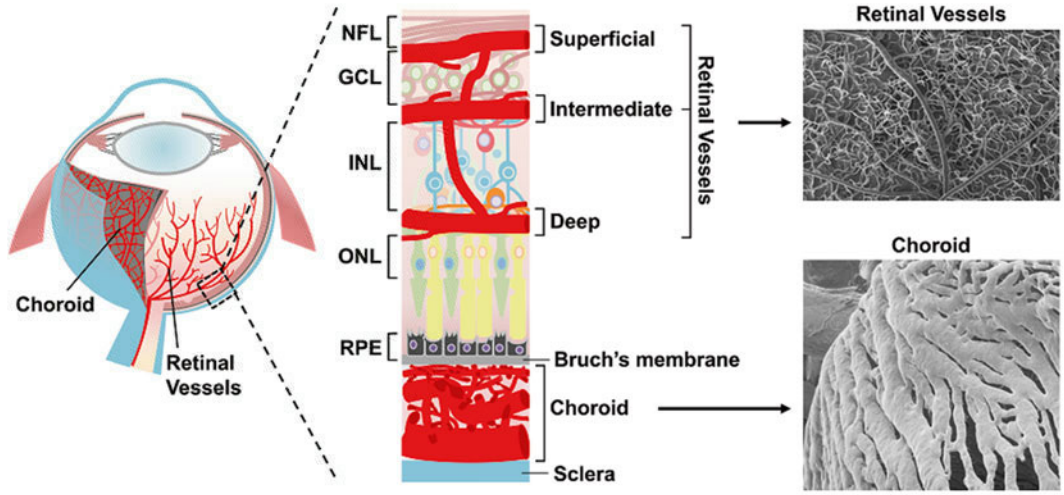

Fig. 1.1 A schematic illustration of the ocular vasculature. *Left*: A schematic cross-section through an eye showing the retinal vasculature lining the inner surface of the retina and the choroid vessels. *Right*: An enlarged cross-sectional illustration of the eye showing detailed structure of the retinal and choroidal vasculature. Three layers of retinal vessels are embedded among retinal neurons: the superficial retinal vasculature lies in the NFL; the intermediate and deep retinal vascular networks align along each sides of the INL. The choroidal vessels between RPE and sclera serve to supply blood to the outer portion of the retina. *GCL* ganglion cell layer, *INL* inner nuclear layer, *NFL* nerve fiber layer, *ONL* outer nuclear layer, *RPE* retinal pigment epithelium. Enlarged images on the *right* depict retinal and choroidal vascular cast from mouse eyes

Retinal vessels have a non-fenestrated endothelium forming the inner blood–retinal barrier. In addition, branches of the central retinal artery are terminal arteries that do not anastomose with each other (Gray 2008; Netter 2006). Contrary to the inner retinal layers, the photoreceptor layer is avascular without blood vessels from the central retinal artery. Thus, it relies on the choriocapillaris to supply oxygen and nutrient by diffusion from choroidal vessels.

1.1.2 Choroidal Vessels

The choroid, a thin highly vascular membrane, lies between the retina and the sclera and invests the posterior five-sixths of the globe in human. The choroid vessels originate from two groups of branches of the ophthalmic artery: (1) the short posterior ciliary arteries, which supply the posterior portion of choroid; (2) the long posterior ciliary arteries, which supply the anterior choroid, ciliary body, and iris. They are distinguished in three layers of choroidal vasculature: the innermost choriocapillaris, the intermediate Sattler's layer, and the outermost Haller's layer (Hartnett 2013). The more outer the vessels are located in the choroid, the bigger the size of their lumens. While the outermost choroidal layer is composed mainly of small arteries and veins, the innermost choriocapillaris is characterized by an exceedingly fine capillary plexus adjacent to the Bruch's membrane (Fig. 1.1) (Paul Riordan-Eva 2011; Ross and Pawlina 2005). In humans, the capillaries of the choriocapillaris are approximately 3–18 μm in diameter and oval shaped in the posterior eye, becoming gradually wider (approximately 6–36 μm in diameter) and longer (36–400 μm in length) as they move towards the equatorial region. The choriocapillaris is a sinusoidal vascular plexus with highly fenestrated endothelium, and as the site of the greatest blood flow in the body (Henkind et al. 1979), it provides 65–85 % of the blood volume in the eye. Through diffusion, it nourishes the cells in the outer portion of the retina (Bela et al. 2011), including the retinal pigment epithelium (RPE) and photoreceptors, as well as the fovea, which contains only photoreceptors for high acuity central vision and is devoid of other retinal neurons.

Interestingly, some mammalian species such as echidnas, guinea pigs, and rabbits lack retinal vasculature, with their oxygen and nutrient supply to the retina being solely provided by diffusion from the choriocapillaris. It appears that the thickness of the retina is directly related to their evolutionary vascularization state. These avascular retinas are typically thinner than the theoretical oxygen diffusion maximum of 143 μm, whereas vascularized retinas are approximately twice as thick, yet their avascular portion are still within the oxygen diffusion limit (Chase 1982; Buttery et al. 1991; Dreher et al. 1992).

1.1.3 Hyaloidal Vessels

The hyaloid vasculature is a transient embryonic vascular bed which develops during embryonic and fetal stages to provide blood supply to the developing eye. The hyaloid artery originates from the ophthalmic artery. It enters the embryonic fissure and extends through the vitreous to the lens. In the developing eye, the hyaloid vasculature plays an important role in many aspects. It supplies the inner part of the retina with oxygen and nutrients; it is also involved in the development and maturation of the lens and makes up the primary vitreous (Hartnett 2013; Fruttiger 2007). During human fetal development, the hyaloid vasculature is first seen at the fourth week of gestation and reaches its maximum prominence during the ninth week. During mid-gestation, the hyaloid vasculature regresses and the retinal vasculature contemporaneously develops. Regression of the hyaloid artery leaves a central extension from the optic disk to the posterior lens surface, called the hyaloid canal or Cloquet's canal (Hartnett 2013).

1.2 Development of Retinal Vasculature

Among the three vascular beds in the eye, the retinal vasculature is the most extensively studied. The development of the retinal vasculature has served as an excellent model for elucidating the mechanisms of vascular development, remodeling, and maturation. Studies on retinal vasculature over the past several decades have greatly expanded our understanding of the fundamental processes governing normal and pathologic vascularization including the relationship between hypoxia and vessel growth, as well as the contribution of neurovascular interaction in vascular homeostasis.

1.2.1 Angiogenesis Is the Dominant Process in Retinal Vascular Development

Blood vessels are generally composed of several distinct cell layers with a single layer of endothelial cells forming the lumen in the innermost part of the vessel. In large macrovessels such as aortae, the inner endothelial cell layer is covered by a central layer of mural cells/smooth muscle cells, and usually an external layer consisting of connective tissue lined with small vessels and nerves. In microvessels and capillaries, which constitute most of the retinal vessels, the endothelial cell layer is covered externally by a noncontiguous single layer of pericytes/mural cells, allowing close interaction of vascular endothelial cells with surrounding neurons, glia, and inflammatory cells to coordinate the process of vascular growth, remodeling, and repair.

The developmental vascularization process in the retina is mediated primarily via angiogenesis (Fruttiger 2002), similarly as some other tissues such as the kidney and the brain. In angiogenesis, vascular endothelial cells sprout and proliferate from preexisting blood vessels, usually venules, and develop into new vessels with fully functional lumen. During this process, local increases in growth factors destabilize a portion of the preexisting vessels, allowing the activation of pericytes and remodeling of extracellular matrix. Endothelial cell migration and proliferation subsequently occurs to form new vessels. Angiogenesis is also considered the dominant process governing new blood vessel growth during the wound healing process and in pathologic retinal vessel growth such as in tumors and retinopathies (Saint-Geniez and D'Amore 2004).

This mechanism of angiogenic development is in contrast with vasculogenesis where dispersed primitive vascular precursor cells or hemangioblasts cluster together and form into tube-like endothelial structures, in the absence of existing vessels. Vasculogenesis occurs during the embryonic development of the circulatory system and gives rise to the heart and the first primitive vascular plexus such as the yolk sac circulation. It was suggested that the very initial process of vascular development in the retina results from vasculogenesis from resident angioblasts (McLeod et al. 2006), then angiogenesis becomes dominant to form the rest of retinal vasculature. Yet with increasing evidences of circulating endothelial precursor cells from bone marrow modifying developing and injured retinal blood vessels (Grant et al. 2002; Sengupta et al. 2003; Dorrell et al. 2004), the precise distinction between angiogenesis and vasculogenesis is becoming blurry.

1.2.2 Temporal and Spatial Development of Three Layers of Retinal Vasculature

In humans, retinal vascularization starts in utero at about 16 weeks of gestational age and is completed at approximately 40 weeks of gestation, right before birth. Developmental retinal vascularization occurs concurrently as the hyaloid vessels regress. The retina is vascularized first in the most superficial (i.e., innermost) layer on the vitreous side, starting from the optic nerve head and then progressing centrifugally outwards towards the ora serrata, the peripheral edge of the retina. This superficial primary plexus reaches the nasal side of the ora serrata at about 36 weeks gestational age, and the temporal retina at approximately 40 weeks gestational age. As the superficial layer is nearing completion, retinal vessels dive into the retina to form first a deep and then an intermediate layer along with a well-organized network of inter-connecting vessels to complete three vascular layers: a superficial vascular layer which lies in the inner part of the nerve fiber layer, an intermediate layer in the inner plexiform layer, and a deep layer in the outer plexiform layer (Dorrell et al. 2002) (Fig. 1.1).

In other mammals such as primates and rodents, the conserved pattern of three retinal vascular layers forms over varying timescales (Gariano and Gardner 2005). In mice, one of the most studied model systems of retinal vascular development, the superficial vascular plexus starts to develop during the first week after birth, with radial growth as seen similarly in humans (Fig. 1.2). During the second week, angiogenic sprouts start to form from the superficial layer and grow perpendicular to the primary vascular plexus into the retina to create two deeper layers of capillary networks. A complete vascular system is formed by the end of three weeks after birth (Stahl et al. 2010a). Studies in the mouse retina have shed light on the cross talk among multiple cell types that function together to direct vascular growth in the retina, and identified the important roles of oxygen and oxygen-mediated growth factors in this process.

1.2.3 Oxygen and VEGF in Retinal Vascular Development

1.2.3.1 Lack of Oxygen Drives Blood Vessel Growth in the Eye

A hypothesized role of oxygen in retinal vascular development originates from early observations that capillaries grow more profusely near venules than around arteries (Michaelson 1948; Ashton 1966; Wise 1961). Observed retinal vascular patterns from some eye diseases also support this notion. In retinopathy of prematurity and diabetic retinopathy, an initial lack of retinal vessels with resulting retinal ischemia precedes pathologic vessel growth, supporting the idea of hypoxia as a critical stimulator of new blood vessel growth (Gariano and Gardner 2005). During development, as retinal neurons and glial cells differentiate and mature, their metabolic demands increase, creating a radial wave of hypothesized "physiologic hypoxia" that leads the development of new vessels from the center towards the periphery of the retina (Chan-Ling et al. 1995) (Fig. 1.2).

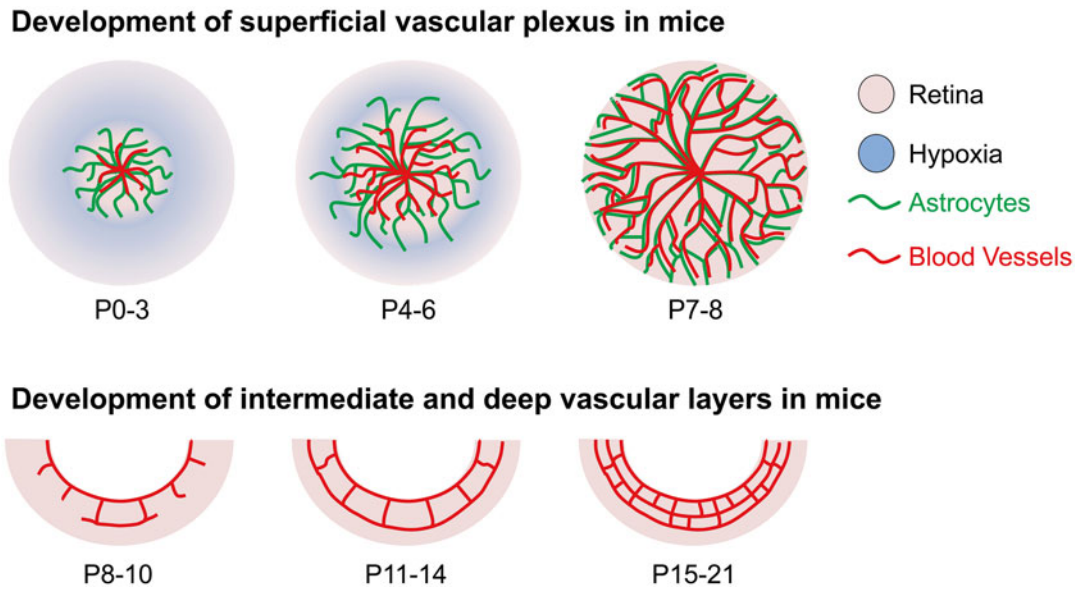

Fig. 1.2 A schematic illustration of the retinal vascular development in mice. *Top panel*: A scheme of retinal vascular growth that originates from the optic nerve head after birth and grows radially towards the peripheral of the retina, and reaches the edge of the retina around postnatal day (P) 8. The growth of the superficial vascular plexus of retinal vessels (*red*) follows a hypothesized physiologic hypoxia wave (*blue*) and an astrocytic template (*green*). *Bottom panel*: After completion of the superficial vascular layer, blood vessels dive down towards the outer retina during the second week after birth, forming a deeper layer of vessels first, and an intermediate vascular layer last. The whole retinal vascular growth process is completed approximately three weeks after birth in mice. In humans, this process occurs prenatally during in utero development

After new vessels have formed, bringing oxygen and alleviating hypoxia, the vascular growth continues radially towards the peripheral retina.

1.2.3.2 VEGF Is A Dominant Growth Factor Governing Retinal Vascular Growth

The effect of oxygen on vascular growth is mediated in large part by vascular endothelial growth factor (VEGF), a hypoxia-induced growth factor. VEGF is expressed in the developing retina in a pattern that coincides with retinal blood vessel development both temporally and spatially (Stone et al. 1995; Pierce et al. 1995, 1996). VEGF and other hypoxia-induced growth factors are induced through the transcription factor hypoxia-inducible factor (HIF), a global regulator of O_2 homeostasis (Wang et al. 1995; Wang and Semenza 1993a, b), composed of an oxygen-sensitive α unit and a constitutively expressed transcription activating β subunit. Under normoxic conditions, HIF1α or HIF2α are hydroxylated on a proline residue by the prolyl hydroxylases, enabling its interaction with von Hippel–Lindau protein, resulting in its ubiquitylation and proteasomal degradation (Jaakkola et al. 2001; Ohh et al. 2000; Epstein et al. 2001). Under hypoxic conditions, HIF1α or HIF2α escapes prolyl hydroxylation and interacts with HIF1β to form a heterodimer, which then translocates to the nucleus and activates target genes such as VEGF by binding to a hypoxia-responsive element in their promoter region (Wang et al. 1995). While a gradient of VEGF is found preceding the superficial vascular plexus in the retina, it is also postulated that the relative hypoxia of the deeper retinal layers during development also results in a VEGF gradient that favors sprouting from the superficial layer downwards, resulting in the formation of first the deep and then the intermediate layers of capillary networks (Stone et al. 1995; Pierce et al. 1995).

In addition to VEGF, erythropoietin (Epo) is another hypoxia-induced growth factor that plays an important role during retinal blood vessel homeostasis (Chen et al. 2008, 2009; Watanabe et al. 2005). As a growth hormone that is best known for its role in stimulating erythropoiesis, Epo also has potent neuroprotective and pro-angiogenic effects (Caprara and Grimm 2012), and was suggested important for the formation of the intermediate plexus of retina vessels in an HIF-dependent manner (Caprara et al. 2011). In the vitreous of patients with diabetic retinopathy (Watanabe et al. 2005; Katsura et al. 2005), retinopathy of prematurity (Sato et al. 2009) or retinal vein occlusion (Stahl et al. 2010b), Epo is upregulated along with VEGF. These oxygen-regulated hypoxia-responsive factors are therefore considered as some of the master regulators of both normal retinal vessel development and proliferative retinopathies (Stone et al. 1995; Aiello et al. 1995; Smith et al. 1997).

Besides these hypoxia-regulated growth factors, some other non-oxygen-regulated growth factors are also important for retinal vascular development, in part through modulation of the VEGF response. For example, the Tie1–Tie2 receptors are related tyrosine kinase receptors selectively expressed on vascular endothelial cells and required for embryonic vascular development (Dumont et al. 1994; Sato et al. 1995). Angiopoietin2, a Tie2 ligand, promotes retinal angiogenesis through increasing sensitivity to the angiogenic effects of VEGF in retinal vessels (Hackett et al. 2002; Oshima et al. 2004), and the balance between the counteracting effects of angiopoietin1 and angiopoietin2 dictates the consequent vascular response. In addition, the significant role of insulin-like growth factor-1 (IGF-1) in retinal vascular development was supported by the finding that IGF-1 is required for maximum VEGF activation of vascular endothelial cell proliferation and survival pathways (Smith et al. 1997, 1999). In premature infants, low IGF-1 levels are associated with an increased risk of retinopathy of prematurity (Hellstrom et al. 2001, 2002, 2003; Smith 2005; Lofqvist et al. 2006), characterized by slowed, deficient development of retinal vessels in its initial stage.

1.2.3.3 Retinal Neuroglia Serves as the Template of Retinal Vascular Growth

In order to form the stereotyped and precisely organized pattern of retinal vasculature, the diffused gradients of oxygen and hypoxia alone would be insufficient. Striking structural alignment exists among retinal vessels, astrocytes, and neurons (Fig. 1.3a), suggesting the contribution of cellular guidance mechanisms during retinal angiogenesis. Astrocytes are a type of glial cells formed from precursor cells that migrated into the retina from the optic nerve head during embryonic development (Chu et al. 2001), prior to the formation of retinal blood vessels. Astrocyte growth in the retina follows the radially oriented ganglion cell axons (Dorrell et al. 2002; Gariano et al. 1996), which synthesize platelet-derived growth factor (PDGF) to stimulate the growth and alignment of astrocytes (Fruttiger et al. 1996), that express PDGF receptor alpha (PDGFR-α). A complex interconnected astrocytic network forms at the inner surface of the retina after birth just preceding the vessels. The spatially and temporally overlapping pattern of astrocyte network preceding vascular growth led to the hypothesis that astrocytes secrete VEGF and other growth factors to guide the filopodia of sprouting endothelial cells towards the right direction in an R-cadherin-dependent manner (Dorrell et al. 2002; Gariano et al. 1996; Watanabe and Raff 1988; Gerhardt et al. 2003) (Fig. 1.3b). However, some recent findings challenge this indispensable role of astrocytes in retinal vascular development (Weidemann et al. 2010; Scott et al. 2010), suggesting that astrocytic VEGF is perhaps more critical for vascular maintenance in pathologic conditions. This indicates the possibility that other cells surrounding blood vessels, such as retinal neurons and inflammatory cells, may also be crucial in governing retinal vascular development. Both cell types are capable of secreting angiogenic factors including VEGF and will be discussed in the next sections.

1.2.3.4 Dynamics of Vascular Growth

As described above, retinal vessels elaborate their networks throughout the retina by angio-

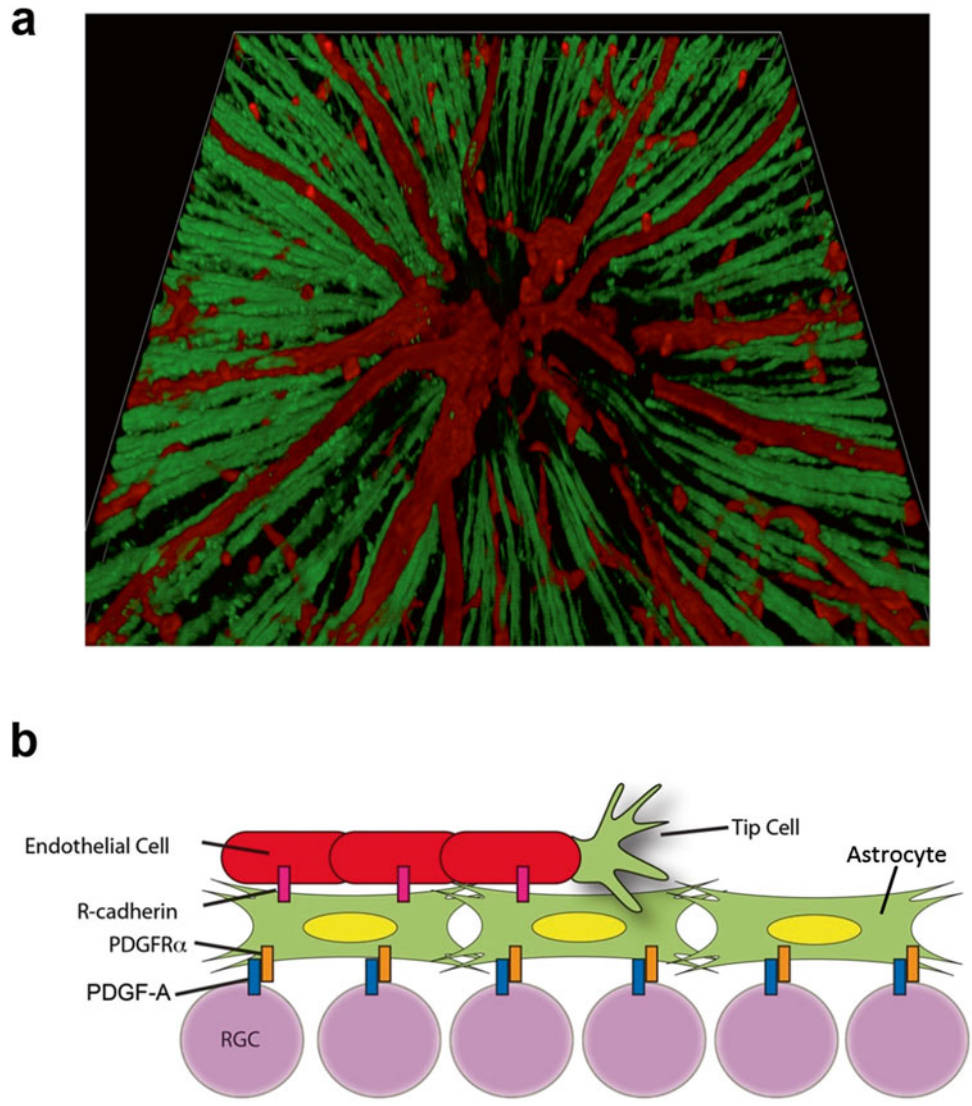

Fig. 1.3 Structural alignment of retinal neuroglia and vessels. (**a**) Three-dimensional reconstruction of an adult mouse retinal flatmount. The image depicts the inner retinal vascular plexus (*red*; stained with Isolectin B4) interwoven with retinal ganglion neurons and their axons (*green*; stained with β-III tubulin). (**b**) Model of vascular growth during retinal development. Astrocytes, express platelet-derived growth factor alpha-receptor (PDGFR-α) and invade the developing retina from the optic nerve head, ahead of the vascular front. They travel on top of PDGF-α-expressing retinal ganglion cells (RGCs). Nascent vessels follow the astrocytic template and form R-cadherin junctions with proximal astrocytes. Adapted from Sapieha, Blood, 2012

genesis, a coordinated process involving endothelial cell proliferation, migration, and assembly into tube-like structures containing a lumen (Provis et al. 1997; Dorrell and Friedlander 2006). Typically, vessel growth is stimulated by angiogenic factors such as VEGF, fibroblast growth factors, angiopoietin2, and/or other chemokines, through a process modulated in part by microglia (Fantin et al. 2010; Rymo et al. 2011). As a consequence of angiogenic signaling, angiopoietin2 stimulates pericyte detachment from the vessel walls, leading to basement membrane degradation by matrix metalloproteinases (MMPs). Endothelial cells then slacken their VE-cadherin

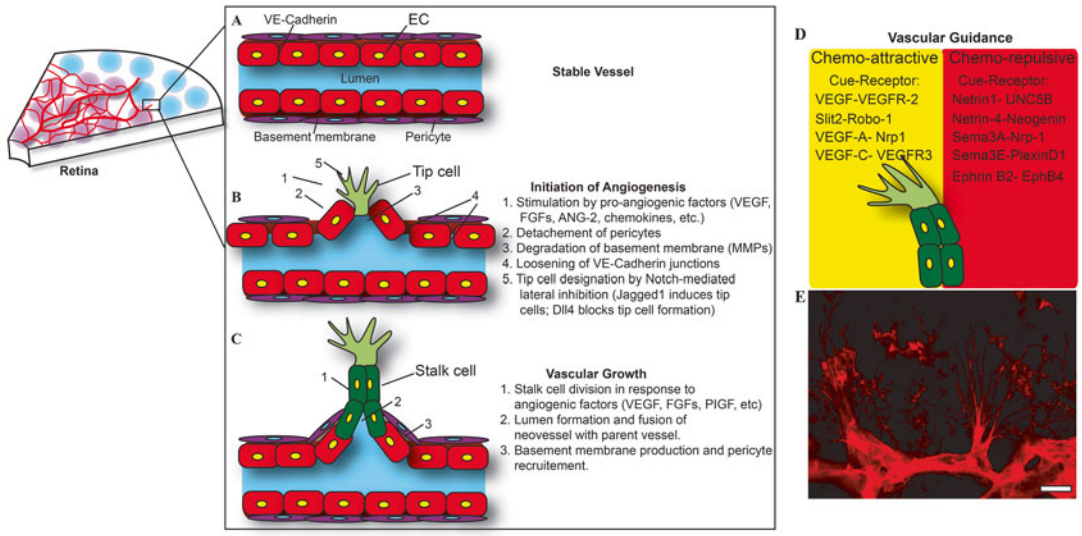

Fig. 1.4 Graphic depiction of the cellular mechanisms governing retinal angiogenesis. (**a**) A stable, quiescent vessel with aligned endothelial cells (ECs), united by VE-cadherin-rich junctions, and covered by pericytes. (**b**) Upon stimulation by angiogenic factors, a cascade of events ensues leading to pericyte detachment, basement membrane degradation and endothelial junction loosening. Determination of stalk versus tip cell phenotypes is achieved through Notch-dependent signaling. VEGF through VEGFR2 induces the Notch ligand Dll4 on tip cells which subsequently activates Notch in adjacent endothelial cells and specifies their stalk cell phenotype. Conversely, the Notch ligand Jagged1 is highly expressed by stalk cells and antagonizes Dll4. This promotes VEGFR2 expression and renders ECs more responsive to VEGF and thus more susceptible to form tip cells. (**c**) Once the vessel has sprouted, stalk cells behind the tip cell divide and assemble to form the lumen of the neovessel. Pericytes are then recruited and basement membrane is laid down. (**d**) To reach its final destination, the growing neovessel must navigate through the tissue by responding to a series of diffusible and membrane-bound attractive and repellent guidance cues. (**e**) Confocal image of sprouting retinal vessels with filopodia-rich tip cells (stained with isolectin B4) from P4 mouse retinas. Scale bar: 10 μm. Adapted from Sapieha, Blood, 2012

and claudin-rich junctions and a leading tip cell protrudes and advances towards the attractant angiogenic gradients (reviewed in (Carmeliet and Jain 2011)) (Fig. 1.4a–c).

Growth of nascent blood vessels occurs via the advancement of leader tip cells (Gerhardt et al. 2003), with the leading tip position sometimes shared by several endothelial cells. Endothelial tip cells, which are induced by VEGF, are non-mitotic and enriched in both receptors for angiogenic factor (such as VEGFR) and other receptors that were initially described to respond to neuronal guidance cues (Huber et al. 2003). The physiological role of tip cells is to probe the tissue environment through their projecting filopodias and guide the nascent vessel towards growth factor gradients or guidance cues to its appropriate final destination. These neuronal guidance receptors include the neuropilins and plexins (for semaphorins), Unc5b, neogenin and DCC (for netrins), the Eph receptors (for ephrins), and roundabouts (for slits) (Adams et al. 1999; Klagsbrun and Eichmann 2005; Wilson et al. 2006) (Fig. 1.4d–e). Once the tip cells come in contact with a given cue, they will respond by either advancing, stalling, turning, or retracting depending on which cell surface receptor predominates and the overall intracellular environment of the tip cells (Larrivee et al. 2009). The role of neuronal guidance cues in vascular growth has been comprehensively reviewed (Larrivee et al. 2009; Carmeliet and Tessier-Lavigne 2005; De Smet et al. 2009; Gelfand et al. 2009). Conversely, the stalk cells, stimulated by Dll4/Jagged1/Notch-mediated later cellular inhibition (Hellstrom et al. 2007; Benedito et al.

2009), are located behind tip cells in the wake of the vascular front, and divide in response to VEGF to form a lumen.

1.2.4 Neurovascular Cross Talk in Retinal Vascular Development

The retina is the most metabolically active tissue of the body, per weight. It is also the most external and accessible portion of the central nervous system (CNS). Central neurons such as those that populate the retina require a steady supply of nutrients and oxygen to ensure appropriate neuronal function and sensory transmission. Consequently, nervous and vascular systems must be adequately paired. In recent years, it has become clear that neurons play an important role in instigating, promoting, and steering angiogenesis within nervous tissue and specifically in the retina (Edwards et al. 2011; Fukushima et al. 2011; Kim et al. 2011; Nakamura-Ishizu et al. 2012; Sapieha et al. 2008).

1.2.4.1 Influence of Retinal Ganglion Cells on Vascular Growth

There is a current debate on whether astrocytes or alternatively, retinal neurons such as retinal ganglion cells are primary drivers of retinal developmental vascular growth, since evidence has been provided for both. One possible interpretation to reconcile the contribution of both cell populations during retinal vascular plexus formation suggests astrocytes contribute to trophic support of vessels through providing a template for growth (Fruttiger et al. 1996; Gerhardt et al. 2003; Uemura et al. 2006) as described above, while retinal neurons may drive vascular growth to ensure their own metabolic support (Sapieha 2012). This view is supported by findings in the human fetus during the formation of the outer vascular plexus of the retina at 25–26 weeks of gestation (Hughes et al. 2000). Interestingly, this time frame coincides with the first appearance of visually evoked potentials and thus functional neurons (Dreher and Robinson 1988) and likely reflects an increase in oxygen consumption and metabolic activity from the newly operating neurons and their need for fuel (Cringle et al. 2006). In addition, cell-specific ablation of VEGF, HIF-1α, or HIF-2α in astrocytes has no overt defects on developmental retinal vascularization (Weidemann et al. 2010; Scott et al. 2010), while neuroretinal-specific knockout of HIF-1α substantially perturbs retinal vascular development (Caprara et al. 2011; Nakamura-Ishizu et al. 2012) suggesting that a neuronal cell population, rather than astrocytes, likely provides angiogenic factors during retinal development. One such neuronal cell population is retinal ganglion cells (RGCs). Among retinal neuron cell types, RGCs are the most anatomically coupled with the superficial retinal vascular plexus. A specific role for RGCs in vascular development was established using mouse models of genetic ablation of RGCs (Edwards et al. 2011; Sapieha et al. 2008). In transgenic mice which express a toxin in newly formed RGCs and hence eliminate RGCs as they form (Mu et al. 2005), astrocytic networks remain largely intact, yet these mice are completely devoid of a retinal vascular plexus and show persistent hyaloid vasculature (Sapieha et al. 2008). Similarly, *Math* $5^{-/-}$ mice which lack 95 % of RGCs do not form a functional retinal vascular layer (Edwards et al. 2011).

1.2.4.2 Photoreceptors and Retinal Vascular Growth

In addition to RGCs, photoreceptors may also play a significant role in determining retinal energy demand and hence influence vascular growth. Photoreceptors are the most abundant neuronal cell population in the retina and also require very high rates of oxygenation (Wangsa-Wirawan and Linsenmeier 2003). While the direct impact of photoreceptors on retinal vascular development is unclear given their contemporaneous maturation and migration towards the outer retina during retinal vascular formation, evidence for a role of photoreceptor in vascular maintenance comes directly from clinical observations. Patients suffering from both proliferative diabetic retinopathy and retinitis pigmentosa with progressive photoreceptors loss show considerably less pathological retinal angiogenesis than diabetic patients