Advances in the molecular signaling mechanisms of VEGF/VEGFR2 in fundus neovascularization disease (Review)

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Abstract. Fundus neovascularization disease is a blinding eye disease, and represents an umbrella term for a group of disorders in which VEGF and its receptor VEGFR2 play important roles in promoting neovascularization. Compared with physiological angiogenesis, pathological angiogenesis involves several different regulatory mechanisms, vascular structures and functions, as well as microenvironmental effects. Although the role of VEGF and its receptor in angiogenesis is well documented, research on its major molecular signaling mechanisms is limited. In the present review, a basic overview of the VEGF and VEGFR2 pathways, including their downstream signaling mechanisms and the latest therapeutic advances in the context of fundus neovascularization disease, is provided, and the limitations and future perspectives of current anti-VEGF therapies are discussed. Overall, the purpose of the current review is to provide information on the molecular signaling mechanisms associated with VEGF and VEGFR2 and to perform an in-depth examination of these molecular signaling pathways and their interaction mechanisms. These interaction mechanisms are expected to facilitate the development of more targeted and long-lasting therapeutic regimens and provide novel concepts for the treatment of fundus neovascularization disease.

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1. Introduction

Retinal neovascularization is defined as the abnormal growth of blood vessels from pre-existing small retinal veins under specific disease conditions. Specifically, these newly formed vessels grow out of the pre-existing vascular system (1,2). The retina is nourished by two distinct vascular networks; the inner part of the retina relies primarily on the retinal vascular system of tightly packed endothelial cells (ECs) for nourishment, forming a blood-retinal barrier (BRB) (3). By contrast, the exterior part of the retina relies on a choroid plexus network of highly permeable, fenestrated ECs and few pericytes; here, an external retinal barrier is formed, through which oxygen is transmitted to the photoreceptor cells. At both barriers, lesions can lead to neovascularization and cause severe visual impairment (4,5).

VEGF is widely recognized as a key molecule in the pathogenesis of a variety of ocular diseases, such as proliferative diabetic retinopathy (PDR), retinopathy of prematurity and neovascular age-related macular degeneration (nvAMD); all of these diseases are major causes of visual impairment (6-8), and different ocular diseases associated with VEGF-driven abnormal neovascularization have been reported. The advent of anti-VEGF therapy has revolutionized the therapeutic outlook for fundus neovascularization disease and has improved patient vision (9). Although anti-VEGF therapy has shown clinical feasibility, it still faces challenges, such as short half-life and the need for repeated injections; these challenges increase medical risks and decrease the quality of life of patients to a certain extent. In addition, not all patients benefit from a single anti-VEGF medication, and some problems remain after long-term treatment, such as the possibility of other ocular complications (including hemorrhage and infections) or failure to achieve the desired vision restoration. A low response to VEGF therapy has also been associated with

a reduction in the number of photoreceptor cells. Therefore, anti-VEGF monotherapy can notably suppress fundus neovascularization to some extent but may not be sufficient to restore visual function in the long term (10). Considering the aforementioned issues, the focus of the present review is to explore molecular research advances based on the VEGF/VEGFR2 pathway in the field of fundus neovascularization, with the aim of providing novel treatment concepts in this field.

2. Basics of the VEGF/VEGFR2 pathway

VEGF family members and their biological functions. The VEGF family includes five protein members: VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) (11,12). Among them, the main role of VEGF-A is to induce endothelial cell activation and increase vascular permeability by binding to its corresponding receptors (VEGFR1 and VEGFR2) (13). VEGF-A has several isoforms, such as VEGF111, VEGF121, VEGF145, VEGF165, VEGF189 and VEGF206. For example, VEGF165 was the first isoform to be identified and is considered to be the classic pro-angiogenic VEGF-A subtype (14). VEGF-B has potential for the treatment of coronary artery disease and heart failure. Different from other proangiogenic factors of the VEGF family, in the heart, VEGF-B has the highest expression in cardiomyocytes and is involved in cardiac remodeling after myocardial infarction. Thus, VEGF-B regulates myocardial contraction and metabolism, and VEGF-B exerts cardioprotective effects, protecting cardiomyocytes from ischemia through physiological hypertrophy, Specifically, VEGF-B may activate the downstream akt/mtorc1 pathway to mediate physiological hypertrophy (15). VEGF-C has structural homology with VEGF-D and serves a major role in lymphangiogenesis (16). PLGF can bind to VEGFR1, thereby preventing VEGF from binding to VEGFR2. It is also a factor that promotes abnormal angiogenesis in the retina and subretina (11).

Structure and function of VEGF receptors. VEGFR2 is a tyrosine kinase receptor that consists mainly of an extracellular, a transmembrane and an intracellular structural domain (17). The extracellular domain has seven immunoglobulin homologous structural domain repeats (18). The intracellular domain consists of a kinase structural domain including several tyrosine residues, and VEGF binding to VEGFR2 leads to the phosphorylation of the tyrosine residues and activation of the kinase domain. The key phosphorylation sites include Y1054 and Y1059, whereas the coreceptor neuropilin-1 (NRP-1) is involved in developmental angiogenesis by binding to VEGFR2 (19-21). In addition, phosphorylation of VEGFR2 can activate signaling pathways, such as those promoting endocytosis of the key EC adhesion molecule vascular endothelial (VE)-cadherin, leading to increased vascular permeability (22). The phosphorylation site Y949 of VEGFR2 is targeted to limit vascular permeability in retinopathy through downstream signaling. When the VEGFR2 Y949 signaling pathway is impaired, phosphorylation of the vascular endothelial VE-cadherin Y685 site is reduced. These results indicate that targeting VEGFR2-regulated VE-cadherin phosphorylation may inhibit edema (23). VEGFR1 and VEGFR3 also contain the same three domains as VEGFR2, but their biological functions are different. VEGFR1 can act as a decoy receptor to limit VEGFA/VEGFR2 activity in the physiological environment, while VEGFR3 mainly binds VEGF-C/VEGF-D to regulate the formation of lymphatic vessels (4). A graphical representation of VEGF and its receptors is illustrated in Fig. 1.

Overview of the VEGF/VEGFR2 signaling pathway. VEGF and its receptor VEGFR play a role not only in normal physiological blood vessel growth but also in pathological angiogenesis, tumor growth and metastasis (24,25). VEGF usually regulates angiogenesis by binding to VEGFR1 or VEGFR2. Notably, VEGF mainly binds to VEGFR2, which has more potent proangiogenic activity and higher tyrosine kinase activity than VEGFR1 (26), whereas VEGFR1 is more commonly involved in inflammatory pathways (27).

VEGF binds to VEGFR2 and activates downstream signaling, including the PI3K, Akt, Ras and MAPK pathways, to promote cell proliferation, migration and differentiation (28). Among them, proliferation and migration occur in ECs, thereby regulating vascular patterns by controlling δ-notch signaling (29). VEGF also protects neurons from ischemia via VEGFR2 (30), and VEGFR2 deficiency results in abnormal neuronal angiogenesis (31).

3. Association between VEGF/VEGFR2 and fundus neovascularization disease

Overview of major fundus neovascularization diseases. Retinal neovascularization occurs in a group of ischemic retinopathies resulting from damage to the retinal vasculature, among which DR and retinal vein occlusion (RVO) are the most common. Subretinal or choroidal neovascularization (CNV) occurs in diseases involving the outer retina or Bruch's membrane, and nvAMD is the most common type of such neovascularization (32).

Overview of DR. DR is a tissue-specific neurovascular complication caused by type 1 and type 2 diabetes mellitus. It is estimated that 440 million individuals will have diabetes mellitus by 2030, and DR affects ~29% of diabetic patients (33). The two main categories are the PDR and non-PDR types. The pathophysiological changes in DR include several aspects of neurodegeneration, inflammation and oxidative stress. Current treatments for DR, including anti-VEGF therapy, steroids, laser photocoagulation and vitrectomy, have limitations and side effects such as visual acuity and visual field damage, and new therapeutic strategies need to be explored (34). It has been shown that only laser photocoagulation and anti-VEGF injections are effective treatments in cases of severe retinopathy and that traditional Chinese medicine may be promising in reducing VEGF levels, inflammation, oxidative stress, apoptosis and angiogenesis in DR (35). For example, control of late glycosylation by intraocular injection of genipin may be a strategy to prevent retinopathy (36).

Overview of RVO. RVO is one of the most common retinal vascular diseases (37). Obstruction of the main retinal veins is known as central RVO (CRVO), and obstruction of the smaller veins is known as branch RVO (BRVO). The prevalence rates of CRVO and BRVO are 0.1-0.4 and 0.6-1.2%, respectively, worldwide (38). The pathogenesis of RVO is multifactorial

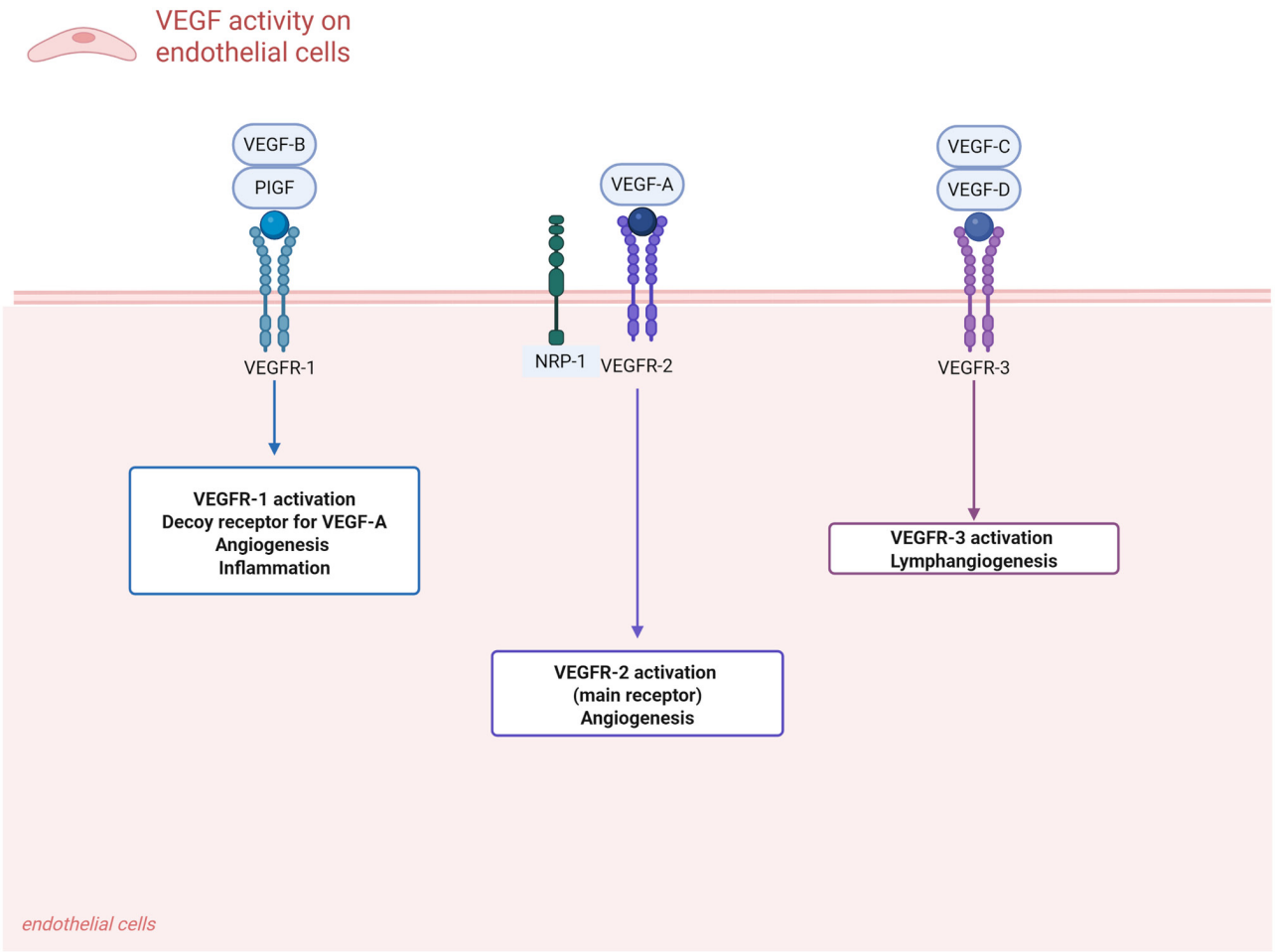

Figure 1. Schematic representation of VEGF signaling and receptor interactions. VEGF family members, including VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF, mediate key angiogenic, inflammatory and lymphangiogenic processes through binding to their cognate receptors VEGFR-1, VEGFR-2 and VEGFR-3. Notably, the coreceptor NRP-1 forms functional complexes with VEGFR-2, amplifying downstream signal transduction during pathological angiogenesis. PlGF, placental growth factor; NRP-1, neuropilin-1.

and involves complex interactions between multiple vascular and inflammatory mediators. VEGF is a potent mediator of vascular permeability and inflammation and plays a central role in the pathogenesis of RVO; additionally, several cytokines, including IL-6, IL-8 and C-C motif chemokine 2 (CCL2) (39,40), have been reported to be involved.

nvAMD. nvAMD is an important cause of vision loss in elderly individuals, the worldwide prevalence of the disease is 8.7% (41) and it mainly affects the deeper retinal layers of the macula and the surrounding choroidal system. nvAMD pathogenesis is closely related to age, oxidative stress and lipid metabolism. The molecular pathways of macular neovascularization include angiogenesis and arteriogenesis. For example, platelet-derived growth factor (PDGF), angiopoietin (Ang)-1 and Ang-2 play crucial roles in regulating angiogenesis and influencing vascular growth, maturation and stabilization, and VEGF is an important factor in the development of CNV and retinal leakage (42,43).

VEGF/VEGFR2 expression and its role in neovascular disease

DR. Key pathological processes in DR include impaired capillary perfusion and subsequent tissue ischemia; these processes can lead to retinal microinfarcts and collateral vessel formation, in which VEGF-A promotes neovascularization by activating VEGFR2 (44,45). Unlike physiological processes where vascular growth is balanced by anti-angiogenic factors, in the pathological process of DR, pathological vascular structural disturbances caused by pericyte loss are associated with high VEGF expression (46). VEGFR2 is highly expressed in diabetic microvessels; therefore, it may be used as a biomarker for the early diagnosis of DR. For example, a recent study revealed that the application of VEGFR2 nanoprobes for *in vivo* detection may have translational potential in the early diagnosis of DR (47).

nvAMD. nvAMD is characterized by CNV, in which the VEGF/VEGFR2 pathway is involved. Choroidal ECs secrete CCL2, which attracts macrophages to CNV lesions and promotes macrophage M1 polarization; additionally, M1-type macrophages/microglia secrete IL-6 and TNF-α, which synergize with VEGF to promote pathological angiogenesis and contribute to CNV development (48,49). Although intravitreal injection of anti-VEGF drugs has become the first-line treatment for nvAMD, this treatment has a number of drawbacks, including the need for repeated injections, poor or no response in some patients and complications such as retinal fibrosis (41)

Therefore, in addition to the traditional VEGF/VEGFR2 pathway-targeted therapy, VEGF-C and VEGF-D signaling also plays key roles. Hypoxia-induced retinal VEGF-C expression induces pathological retinal neovascularization to a similar extent as VEGF-A, and VEGF-D has been shown to promote angiogenesis in corneal angiogenesis models (50).

4. Advances in molecular mechanisms

Role of VEGF/VEGFR2 in pathological angiogenesis. Neovascularization begins with stimulation by hypoxia-inducible factor (HIF). Under normoxia, HIF-1α is unstable and rapidly degraded, whereas under hypoxic conditions, HIF-1α dimerizes with HIF-1β, which translocates to the nucleus and activates transcription of the target gene VEGF, leading to the production of VEGF by stromal cells; VEGF then diffuses into pre-existing blood vessels before binding to VEGFR2 (51). The binding of VEGF to VEGFR2 results in disruption of the basement membrane of ECs, which are transformed into tip cells with high migratory potential. When tip cells form, the activation of VEGFR2 receptors follows VEGF gradients or other proangiogenic stimuli to direct neovascularization (52,53).

Gene regulatory mechanisms associated with VEGF/VEGFR2 ERK/MAPK pathways. The ERK/MAPK mechanistic signaling pathway involves a variety of kinases, and ERK1/2 are members of the classical MAPK cascade (54). This pathway is associated with a variety of diseases, including several types of cancer and cardiovascular disease (55,56). Moreover, the ERK/MAPK pathway is among the main targets of VEGFR2 activation. The binding of VEGF-A to VEGFR2 phosphorylates phospholipase C-γ (PLC-γ) and activates the ERK/MAPK pathway; subsequently, adipose mesenchymal stem cells differentiate into ECs to promote neointima formation (13,57).

Fucoxanthin may play an important role in the prevention of neovascularization. Fucoxanthin interacts with VEGF, thereby impairing the ability of VEGF to activate VEGFR2 and its associated downstream signaling, namely the phosphorylation of MEK and ERK; thus, fucoxanthin acts as an anti-angiogenic agent (58). For specific fundus neovascularization diseases, such as nvAMD, the search for new therapeutic targets is particularly important, since anti-VEGF treatment still results in poor end-stage visual acuity. Zhou *et al* (59) reported that blockade of the VEGF signaling pathway in human retinal pigment epithelial (RPE) cells increased IL-8 secretion through the MEK/ERK1/2 axis, whereas overactivation of the VEGF pathway decreased IL-8 production. IL-8 expression is upregulated in hRPE cells after VEGF signaling inhibition, which is associated with nvAMD. Therefore, IL-8 could serve as an alternative therapeutic target for nvAMD (59). A recent study revealed that elevated levels of YKL-40 and VEGF and activation of the ERK pathway were observed in the neural retina and RPE/choroidal tissues of laser-induced CNV mice. Moreover, after intravitreal injection of the anti-YKL-40 antibody, the levels of YKL-40 and phosphorylated proteins in the ERK pathway decreased; these results indicated that the anti-YKL-40 antibody inhibited the activation of the ERK pathway. These results have indicated that YKL-40 could be a new target for the diagnosis and treatment of CNV (60). The association between the ERK/MAPK pathway and VEGFR2 activation indicates an unprecedented role for this receptor in response to stimulation by various mechanical factors. Specifically, ERK activation is related to the phosphorylation of VEGFR-2 at Y1175 and Y1214 (61). Although the MAPK pathway is prevalent in numerous diseases, the specificity of VEGFR2 as a mechanoreceptor could provide unique targets and concepts for the angiogenic process.

c-Src pathway. c-Src is a cytoplasmic tyrosine kinase associated with cell or endosomal membranes, which also plays a crucial role in regulating VEGF signaling. c-Src is similarly associated with a variety of diseases, including cancer and cognitive disorders (62,63). c-Src directly interacts with VEGFR2 in response to VEGF stimulation, thereby acting as a mechanotransduction protein downstream of VEGFR-2 signaling (64). c-Src serves a role in several cellular processes, including adhesion, motility, proliferation and differentiation (65). In addition, c-Src activity is regulated, at least in part, by mechanical factors such as shear stress and matrix adhesion. c-Src lies upstream of other effectors, such as the ERK pathway, and has been shown to phosphorylate tyrosine residues on VEGFR2, which further activates both VEGFR2 and c-Src by creating a positive feedback loop (66). A previous study suggested that in the Akimba model of diabetic retinopathy, the inhibition of c-Src family kinases with highly specific inhibitors could be an attractive therapeutic intervention for retinal vasculopathy (67). Another study further demonstrated the potential relationship between VEGFR2 and c-Src. This study indicated that PLC-γ is induced downstream of VEGFR2 phosphorylation at Y1173 (pY1173). The Y1173/PLC-γ/endothelial nitric oxide synthase (eNOS)/c-Src pathway was examined in both the healthy and tumor vasculature of VEGFR2Y1173F/+ mice; this pathway was associated with reduced PLC-γ and eNOS activation and suppressed vascular leakage. The PLC-γ pathway downstream of VEGFR2 py1173 can couple VEGFR2 to c-Src. High PLC-γ expression is associated with angiogenic activity and poor prognosis (68). Overall, c-Src acts as a mechanotransduction factor to regulate VEGFR2 activation, which subsequently affects angiogenesis and could be a target for future studies.

PI3K/Akt pathway. The activation of the PI3K/Akt pathway stimulates EC proliferation, mainly by recruiting Akt to the cell membrane and inhibiting apoptosis; however, the activation of PLC-γ leads to the modulation of the intracellular calcium concentration or the production of eNOS through the activation of nuclear factor of activated T cells. This ultimately increases vascular permeability and affects EC proliferation and survival through the aforementioned processes (69,70). The molecular chaperone heat shock protein 90 (HSP90) is a promising molecular target, and HSP90 inhibitors have been indicated to induce anti-angiogenic effects by affecting the PI3K/Akt/eNOS signaling pathway in ECs and downregulating VEGFR2 expression (71). In another study, a peptide (VGB3) mimicked the interaction between VEGF-B and VEGFR1, and binding to VEGFR2 abolished the PI3K/AKT/mTOR pathway and inhibited the proliferation and tube formation of human umbilical vein ECs (HUVECs) (72). Thus, inhibition of the PI3K/Akt axis by inhibitors of VEGFR2 could reduce angiogenesis, and these inhibitors are expected to be applied to further models of neovascular disease in the future.

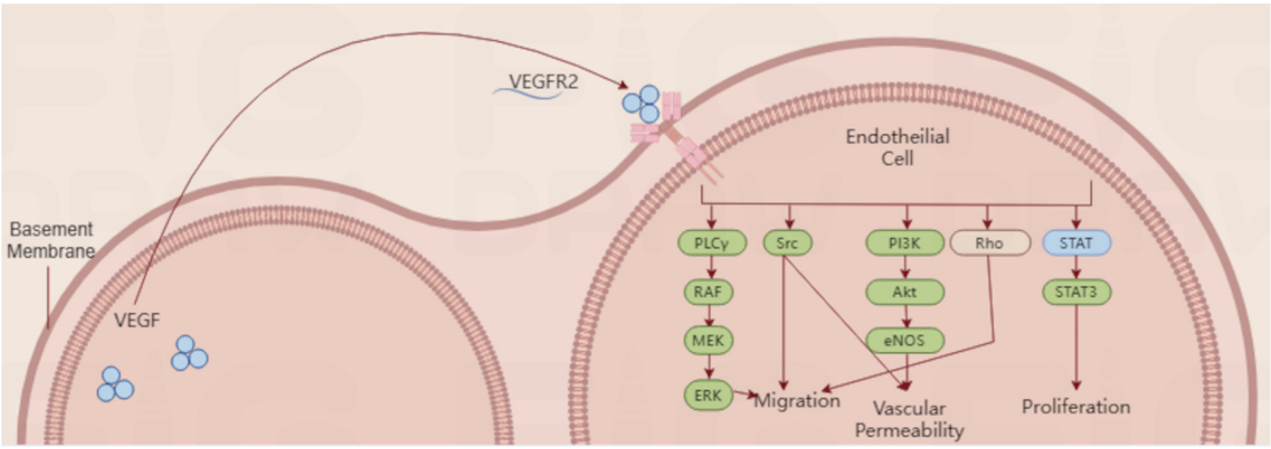

Figure 2. VEGFR-2 signaling pathway and downstream mediators. The VEGFR-2 receptor is activated after binding with VEGF ligands and initiates downstream signaling pathways, including those in the MAPK/ERK, c-Src, PI3K/Akt, Rho and STAT3 pathways. The cellular characteristic affected by each pathway is indicated by arrows. PLC-γ, phospholipase C-γ; eNOS, endothelial nitric oxide synthase.

Rho GTPase family pathway. The Rho family of small GTPases are molecular switches capable of converting extrinsic stimuli into cytoskeletal rearrangements. In vascular ECs, Cdc42, Rac1 and RhoA control cell migration and cell-cell junction formation downstream of angiogenic and inflammatory cytokines. For example, in cell migration, Cdc42 leads to filopodia formation by promoting actin linear extension. Rac1 promotes lamellipodia formation through the WAVE2 complex and arp2/3. RhoA regulates vascularization and permeability by promoting actinomyosin contraction and serves an important role in EC migration and vascular stabilization (73,74).

During angiogenesis, VEGF attracts ECs, whereas semaphorin 3E (Sema3E) repels them. The small GTPase RhoJ plays a multifaceted role in this EC-directed migration. In the GTP-bound state, RhoJ interacts with the cytoplasmic structural domain of plexin D1. RhoJ released from plexin D1 induces cell contraction upon stimulation with Sema3E; this further mediates the Sema3E-induced binding of plexin D1 to VEGFR2, transphosphorylation of VEGFR2 at Y1214 and activation of p38, leading to reverse EC migration. Upon stimulation with VEGF-A, RhoJ promotes the formation of an all-receptor complex consisting of VEGFR2, plexin D1 and NRP-1, which prevents the degradation of internalized VEGFR2 and maintains intracellular signaling. Upon conversion to the GDP-bound state, RhoJ is diverted from plexin D1 to VEGFR2, which terminates VEGFR2 signaling. As confirmed in an oxygen induced retinopathy mouse model, RhoJ deficiency in the ECs of ischemic retina effectively inhibits abnormal angiogenesis (75).

In addition, an increase in RhoA activity can potentially cause anti-angiogenic effects, as shown by Hauke *et al* (76). Hauke *et al* (76) reported that, compared with dominant-negative RhoA, active and wild-type RhoA markedly inhibited EC proliferation, migration, tube formation and vascular sprouting *in vitro*. In addition, active RhoA reduced HUVEC-associated angiogenesis *in vivo*. These results indicated that RhoA itself may have an anti-angiogenic effect rather than being dependent on the RhoA/Rho-associated coiled-coil kinase axis for its action. In another study, Katari *et al* (77) focused on the Rho/Yes-associated protein (YAP)/VEGFR2 mechanotransductional pathway regulating the transient receptor potential cation channel subfamily V member 4 (TRPV4) activity in the tumor microenvironment, and identified endothelial TRPV4 as a new alternative therapeutic target for tumor angiogenesis; however, its role in fundus neovascularization disease is unknown, and more studies are needed to confirm its role in vascular normalization.

Based on the above studies, Rho GTPases could be considered potential targets for the treatment of abnormal angiogenesis and hyperpermeability in retinal vascular diseases.

STAT pathway. STAT family members are activated downstream of VEGFR2 and are particularly important for EC survival and proliferation. Ramshekar *et al* (78) explored retinal endothelial STAT3 as a downstream effector of VEGF-triggered signaling and examined its ability to promote the development of vascularization in the vitreous body and delay the expansion of intraretinal vascularization. The study showed that subretinal delivery of lentiviral vectors driven by a Cdh5 promoter-expressing short hairpin RNA targeting STAT3 reduced vitreous vascularization but did not prolong intraretinal vascularization; these results indicated that VEGF-triggered activation of STAT3 in the retinal endothelium was needed for intravitreal vasculature formation, but not for intraretinal vascularization. In addition, Yu *et al* (79) identified apatinib as a potential target for alleviating neovascularization and fibrosis in nvAMD; its mechanism of action was related to the inhibition of VEGFR2 activation, which prevented angiogenesis and fibrosis through the downregulation of STAT3 phosphorylation. In summary, the STAT pathway plays a key role in inhibiting neovascularization, and the discovery of new clinical inhibitors can aid in the treatment of retinal and choroidal vascular diseases. The downstream mediators of the VEGFR2 signaling pathway are illustrated in Fig. 2.

5. Current treatment strategies

Anti-VEGF therapy: Drugs and their mechanisms of action. Anti-VEGF therapy plays a crucial role in blocking

pathological angiogenesis. However, a large body of clinical data indicates that VEGF-targeted therapies may be limited by decreased visual acuity after repeated administration, with a loss of efficacy after the initial response (80). Patients with disease recurrence may develop resistance to anti-VEGF therapy through unknown mechanisms. Therefore, a single anti-VEGF therapy does not adequately prevent intraocular neovascularization, and other new molecular pathways need to be targeted to further improve or effectively complement the anti-VEGF effect. The following is an overview of the main current therapeutic agents.

Bevacizumab. Bevacizumab (Avastin®) is a humanized anti-VEGF antibody developed by Genentech, Inc., which inhibits VEGF signaling by binding to VEGF-A, VEGF-C and VEGF-D and preventing their interaction with VEGFR2 (81). Bevacizumab has been reported to be effective in treating nvAMD when it is administered intravitreally. Bevacizumab is also less costly than other drugs, such as ranibizumab, pegaptanib and aflibercept, and is effective in the long-term treatment of nvAMD (82,83). However, patients whose eyes were treated with aflibercept were almost three times more likely to stop treatment after 1 year than patients whose eyes were treated with bevacizumab (43 vs. 15%). These results reveal the superiority of aflibercept over bevacizumab and have important clinical implications for treatment selection in patients with nvAMD (84). Therefore, more prospective studies focusing on the comparative impact and efficacy of anti-VEGF therapeutics on neovascularization diseases are needed.

Despite some of the clinical benefits of anti-VEGF therapy, monthly injections may lead to numerous ocular complications, including hemorrhage, infection and endophthalmitis. Reddy *et al* (85) determined the efficacy of mesenchymal stem cell-derived small extracellular vesicles (MSC-sEVs) loaded with the anti-VEGF drug bevacizumab by measuring the efficacy of this drug in a rat model, and the results indicated that bevacizumab-loaded MSC-sEVs reduced the frequency of intravitreal injections needed to treat DR. The reduction in the frequency of injections may reduce ocular complications and improve patient compliance, providing the possibility of improved patient treatment.

Ranibizumab. Ranibizumab (sold under the brand name Lucentis® and developed by Genentech, Inc./Novartis AG) is a recombinant humanized monoclonal fragment antigen-binding (Fab) region that targets VEGF. Like bevacizumab, ranibizumab prevents the activation of VEGFR1 and VEGFR2 by binding with high affinity to all VEGF isoforms, thereby inhibiting angiogenesis (86).

For different diseases, ranibizumab and aflibercept have different effects. Because the differences between the effects of aflibercept and ranibizumab on the choroid of patients with BRVO-macular edema (ME) are not known, Kishishita *et al* (87) included 36 patients with BRVO-ME who were treated with intravitreal injections of aflibercept or ranibizumab and observed changes in the choroidal thickness of the subcentral concave area for a follow-up period of 12 months or longer. The results revealed that the effects of ranibizumab and aflibercept on choroidal thickness in patients with BRVO-ME were the same. However, in another study, ranibizumab provided a complementary solution to the poor outcome of aflibercept treatment. In patients with nvAMD who were >50 years old and exhibited an inadequate response to aflibercept, treatment with ranibizumab for 6 months improved visual acuity (88). To investigate the advantages and disadvantages of the injection modalities reported in clinical trials, a 2-year study by Debourdeau *et al* (89) examined 3,313 eyes with nvAMD; 1,243 eyes were categorized as 'pro re nata' (PRN) and 2,070 eyes were initiated on the 'treat-and-extend' (T&E) program. The PRN protocol applies to regular monthly visits after the lesion has become quiescent, while the T&E protocol adds treatment intervals after stabilization of the lesion to maintain therapeutic stability. After treatment with ranibizumab and aflibercept, eyes treated with the T&E program had better visual acuity (VA) outcomes compared with eyes treated with PRN.

Owing to the high cost of current anti-VEGF treatments, patients with nvAMD require frequent intravitreal injections for optimal visualization, which may lead to undertreatment for patients with a high financial burden. As a result, the ranibizumab port delivery system (PDS) was developed, and Eichenbaum *et al* (90) proposed surgical implantation of this refillable device in the vitreous cavity, which would enable the sustained release of ranibizumab. Although ranibizumab is a widely used anti-VEGF drug, the risk of adverse events and safety as well as the efficacy of PDS need to be further investigated. A study by Lowater *et al* (91) revealed that patients receiving PDS had a high incidence of adverse events, including 25% experiencing vitreous hemorrhage and 20% experiencing hyphemia. Future studies include further improving the therapeutic safety of PDS. An ongoing clinical trial (92) is further exploring the potential of PDS with ranibizumab. Carlà *et al* (92) reported that the level of clinical efficacy (the incidence of adverse reactions was determined according to the examination of the eyes of the patients) of ranibizumab given continuously with PDS was comparable to the efficacy of the IVI treatment in nvAMD. However, a high incidence of adverse effects remains; vitreous hemorrhage occurred in 68% of patients and endophthalmitis occurred in 18% of patients. Therefore, future studies are needed to better define the long-term efficacy of PDS and improve patients' vision.

Aflibercept. Aflibercept was developed by Regeneron Pharmaceuticals, Inc (93). It is a recombinant fusion protein that fuses the extracellular immunoglobulin-like (Ig) structural domain 2 of VEGFR1 and the extracellular Ig structural domain 3 of VEGFR2 to the fragment crystallizable (Fc) portion of human IgG1; this process enables stronger binding of aflibercept to VEGF-A and VEGF-B than the previously used ranibizumab or bevacizumab (94). Aflibercept was approved for the treatment of AMD in 2011 and has subsequently been used to treat certain DRs (95).

For the treatment of AMD, Cao *et al* (84) examined 122 eyes of 106 patients with nvAMD who received injections of aflibercept for 3 consecutive months (n=70) or bevacizumab (n=52), followed by a treatment-extension interval regimen. The results revealed that patients whose eyes were treated with aflibercept were three times more likely to discontinue treatment after 1 year compared with those whose eyes were treated with bevacizumab (43 vs. 15%). These observations revealed that aflibercept was superior to bevacizumab and had important clinical implications for patient treatment selection.

Table I. Comparison of different anti-VEGF drugs.

Name	Target	Initial treatment frequency	Maintenance treatment frequency	Adverse events
Bevacizumab	VEGF-A	Weekly (first 4 injections)	Extended intervals (4-8 weeks)	9% intraocular pressure elevation; 4% vitreous hemorrhage
Ranibizumab	VEGFR2	Monthly (extended after 3 injections)	T&E	11% intraocular pressure elevation; 3% vitreous hemorrhage
Aflibercept	VEGF-A; VEGF-B; PlGF	Monthly (3 injections)	T&E	14% intraocular pressure elevation; 3% conjunctivitis; 2% vitreous hemorrhage
Faricimab	VEGF-A; Ang-2	Every 4 weeks (first 4 injections)	Every 16 weeks	3% conjunctivitis; 3.9% RPE RAP

The percentages refer to the rates of patients with adverse events out of the total number of patients analyzed in each study. Ang-2, angiopoietin-2; T&E, treat-and-extend; PlGF, placental growth factor; RPE RAP, retinal pigment epithelial retinal angiomatous proliferation.

In another study, Kucukevcilioglu *et al* (96) focused on a multicenter comparison of the 24-month efficacy of ranibizumab, aflibercept and the ranibizumab-aflibercept switch. Their results indicated that visual outcomes, including VA and central macular thickness, were similar in non-switchers (aflibercept and ranibizumab groups) and switchers (from ranibizumab to aflibercept) after 2 years of follow-up; moreover, patients that received aflibercept required fewer injections, office visits or additional treatments and the treatment was beneficial for patients whose families were not financially stable. To investigate the effects of different anti-VEGF drugs on AMD, Kanadani *et al* (97) utilized the T&E protocol in an observational study of 131 patients with exudative nvAMD and compared four anti-VEGF drugs: Ranibizumab, aflibercept, bevacizumab and aflibercept. The study revealed that intravitreal aflibercept administration resulted in better visual and anatomical improvements in the T&E protocol, with notably fewer injections, compared with the other drugs tested. However, large multicenter randomized clinical trials with longer follow-up periods are needed to assess whether this therapeutic route is effective and whether it provides better results in the treatment of retinal vascular diseases.

Faricimab. The Ang/Tie pathway plays an important role in maintaining vascular stability and synergizing with VEGF. However, under pathological conditions, Ang-2 and VEGF-A synergistically can cause vascular leakage and neoangiogenesis. In addition, Ang-2 can increase vascular permeability by activating FAK phosphorylation via β1 integrins, inducing cytoskeletal remodeling and pericyte loss (98). Faricimab is a bispecific anti-VEGF/Ang-2 antibody and exhibits improved vascular stability and reduced retinal inflammation compared with single anti-VEGF therapy (99). To evaluate the safety and efficacy of faricimab, Khanani *et al* (100) conducted a phase III clinical trial for the treatment of nvAMD, and concluded that a fixed dosing regimen every 16 weeks was required in the first year based on the patients' disease activity, while this was incorporated into the second year in a personalized treatment interval (PTI) format. The PTI approach aims to reduce patient burden by customizing treatment intervals to meet the needs of individual patients. Moreover, Heier *et al* (101) demonstrated that extended treatment intervals with faricimab (every 16 weeks) reduced the patient burden of treatment compared with aflibercept, which inhibited the VEGF pathway alone and was given every 8 weeks, and provided visual benefit for patients with nvAMD and diabetic macular edema. To assess ocular anatomical and functional outcomes in patients with nvAMD treated with vitreous faricimab, Pandit *et al* (102) examined the central fovea thickness (CFT), maximal fiber-vascular pigment epithelial detachment (fvPED) and Snellen visual acuity and compared them with those of patients who were previously switched to faricimab after other anti-VEGF treatments. The proportion of hydrops in the retina before conversion was 36.7%, which decreased to 24.8% after conversion. The proportion of eyes with subretinal fluid was 53.2% before conversion and decreased to 26.6% after conversion. The results revealed that vitreous faricimab improved anatomical outcomes compared with those of patients with previously treated nvAMD while maintaining visual acuity in the short term. Moreover, Szigiato *et al* (103) similarly observed the short-term efficacy of faricimab and evaluated the CFT, fvPED, subretinal fluid and intraretinal fluid of patients; their results indicated a notable reduction in CFT and fvPED and stabilization of visual acuity, as well as a better visual outcome, occurred in patients that switched to faricimab after previous anti-VEGF therapy. However, long-term clinical studies are needed to confirm the long-term efficacy of faricimab. A comparison of different anti-VEGF drugs is presented in Table I (104-106).

Research progress in VEGFR2-targeted therapy. In gene editing technology, the creation of dominant-negative VEGFR2 using the multifunctional prime editing system could block aberrant retinal angiogenesis in a mouse model of oxygen-induced retinopathy (107). Additionally, advances have been made in the development of molecular inhibitors that target VEGFR2. CLK inhibitors (MU1210 and T3-CLK) have been shown to reduce the mRNA and protein expression, as well as the downstream signaling of VEGFR2. This was partly due to the reduced activity of the WNT/β-catenin pathway, since the activation of this pathway induced VEGFR2 expression, whereas the knockdown of β-catenin

blocked VEGFR2 expression. Notably, no alternative splicing of VEGFR2 was detected. Therefore, C81 may be a promising compound for the treatment of diseases that depend on angiogenesis and inflammation, as they impair both processes (108). Furthermore, Tang *et al* (109) reported that acrizanib, which is a small-molecule inhibitor of VEGFR2, acts by differentially inhibiting multiple phosphorylation sites of VEGFR2 in ECs, namely Y951, Y996, Y1059, Y1175 and Y1214. Among them, the highest (Y1214) and lowest (Y996) inhibition differed by ~2.5-fold. This has an important impact on physiological angiogenesis and pathological neovascularization. In summary, inhibitors developed on the basis of VEGFR2 may attenuate angiogenesis and provide better treatment outcomes.

Inhibiting neoangiogenesis by interfering with the VEGF and Ang-2/Tie2-related pathways is a novel concept. Using *in vitro* and *in vivo* experiments, Lei *et al* (110) demonstrated that 5α-hydroxycostic acid (isolated from the natural plant *Viburnum vulgare*) had a therapeutic effect on neovascularization in a rat CNV model by inhibiting cell proliferation and angiogenesis. These results indicated that 5α-hydroxycostic acid may inhibit neovascularization by interfering with the Ang-2/Tie2-related pathway and may be a candidate for the treatment of CNV. In addition, Liu *et al* (111) suggested that intravitreal injection of borsub, which is a reversible proteasome inhibitor, could ameliorate CNV by antagonizing the VEGF-A/VEGFR2 and PDGF-D/PDGF receptor-β pathways. These results indicate that the development of multitarget therapies may hold potential in addressing the shortcomings of inadequate single-target therapies.

Therapeutic approaches involving gene editing are also being developed. Zeng *et al* (112) depleted VEGFA using a novel CRISPR/Cas9 system, which inhibited the proliferation, migration and tube formation of HUVECs *in vitro* and ultimately inhibited corneal neovascularization *in vivo*. In addition, Huang *et al* (113) performed recombinant adeno-associated virus (AAV) serotype 1-mediated CRISPR/Cas9 editing of the genomic VEGFR2 locus, which suppressed angiogenesis in mouse models of oxygen-induced retinopathy and laser-induced CNV. These results indicate that gene editing may be used in the future for the treatment of fundus diseases.

6. Challenges and future research directions

Limitations of the current study

High cost. The drugs bevacizumab, ranibizumab and aflibercept are expensive and require frequent intraocular injections; therefore, they can be a heavy burden for economically disadvantaged families (114). Challenges also remain in the selection of specific anti-VEGF drugs. From a practical point of view, ophthalmologists usually choose a specific anti-VEGF drug because it improves vision and reduces the cost or number of injections. However, numerous challenges remain in the treatment of patients with nvAMD because certain anti-VEGF drugs are limited to disease-specific reimbursement (115).

Poor visual outcomes. While it has been demonstrated that continued injections are needed to achieve better vision, the financial situation of the patient's family and compliance are key factors, and financial difficulties or a lack of compliance may lead to inadequate treatment and increased medical risk. Both can lead to suboptimal vision outcomes (116). Subretinal fibrosis is an end-stage sequela of nvAMD and can lead to permanent central vision loss in patients with nvAMD. Even when adequate anti-VEGF therapy is given, ~1/3 of patients still develop irreversible visual impairment due to subretinal fibrosis (117). Moreover, geographic atrophy secondary to AMD is a progressive, irreversible loss of vision involving photoreceptors, RPE cells and choroidal capillaries, and its progression may be more rapid in patients receiving anti-VEGF therapy (118). In addition, normal retinal structures may be affected by certain treatments. For example, Zhuang *et al* (119) further explored the short-term changes in the normal retinal vasculature after anti-VEGF treatment in patients with nvAMD; their results indicated that anti-VEGF treatment transiently affected the relatively normal retinal vasculature, which could lead to edema of the nerve fibers on the nasal side of the optic disc. Therefore, anti-VEGF therapy may affect both healthy and diseased tissues of the fundus, thereby affecting the regression of patients' vision.

Mechanisms related to drug resistance: *Changes in VEGF isoform expression and epigenetic regulation*. The VEGF isoform VEGF165 is either in a diffusible state (one half) or bound to heparan sulfate proteoglycan on the cell surface (the other half), whereas VEGF121 lacks a heparin-binding domain and diffuses freely in tissues (120). Serine/arginine protein kinase 2 binds and activates its downstream target serine/arginine splicing factor 1 (SRSF1), which increases splicing and expression of VEGFA165 (121). Another study showed that 30 kPa extracellular matrix rigidity inhibited the expression and nuclear accumulation of YAP to regulate the expression of SRSF1 via runt-related transcription factor 2, which subsequently inhibited the expression and secretion of VEGF165 in tumor cells (122).

In epigenetic regulation, the DNA methylation-regulated VEGFR signaling pathway may be involved in the development of diabetic cardiovascular disease. VEGF-B hypomethylation may be a potential biomarker for early intervention in patients with this disease. The expression of DNA methyltransferases, such as DNA methyltransferase (DNMT)1, DNMT3a and DNMT3b, may serve as potential biomarkers of the anti-VEGF diabetic ME response (123).

Urgent issues, potential research opportunities and the application of new technologies. Since anti-VEGF therapy is limited by the low response rates of single drugs, drugs targeting multiple neoangiogenic pathways are required. The application of existing anti-VEGF drugs should be optimized and prognostic biomarkers should be evaluated to identify those that predict effective responses. Biomarkers need to be prospectively validated in independent randomized trials; however, novel drug discovery and the development of multitarget therapeutics are also important. In this context, the bis-antibody-based drug RO-101 is an ideal candidate for the treatment of retinal diseases. In preclinical models, RO-101 has shown similar or greater effects than current anti-VEGF treatments, including neointimal growth abrogation with comparable or longer half-lives. In addition, RO-101 has shown strong binding affinity for VEGF-A and Ang-2, and is biocompatible with retinal tissue in animal studies, indicating potential compatibility for use in humans (124). Therefore, further studies are expected to validate its effectiveness and value in

the clinic. In addition, a new technology has been developed in which carbon nanodonut (CND) with a donut-like structure is synthesized using sodium alginate (SA) and 1,8-diaminooctane (DAO) as raw materials. In human umbilical vein endothelial cells, CND can reduce the levels of reactive oxygen species and proinflammatory cytokines (such as IL-6 and IL-1β) five-fold compared with bevacizumab. In addition, CND has a strong affinity for VEGFA165, with a dissociation constant of $2.2x10^{-14}$ M; this value is >1,600 times stronger than that of the commercial drug bevacizumab (Avastin®). Therefore, SA/DAO-CNDs have the potential to be used as drugs for the treatment of various angiogenesis-related ocular diseases (125). Patient compliance and financial pressure are equally challenging. The high cost of anti-VEGF therapy and the need for multiple injections have led to decreased compliance and financial stress for several patients. However, investigating sustained-release materials that can prolong the duration of drug action, minimize the number of intravitreal injections, and improve patient compliance is a viable solution to this problem and can lead to significant therapeutic benefits. Two extended-release materials are currently undergoing clinical trials (NCT03677934 and NCT03953079), including PDS and GB-102. PDS mainly targets VEGF-A, while Gb-102 is a tyrosine kinase inhibitor that targets VEGF-A and PDGF. Their role is to reduce the burden of frequent intravitreal injections on patients and doctors. Although basic research indicates that extended-release drugs have strong therapeutic applications, the preparation of drug carriers with good compatibility with drugs, long release times and prolonged duration of action is still a major challenge at present (126,127).

Gene therapy applied directly to the eye has also shown potential for long-lasting drug applications. In one study, an AAV2 vector was used to fuse a truncated form of soluble VEGFR2 to the FC portion of human IgG1. AAV2-mediated gene delivery of sVEGFR-2-Fc was effective in inhibiting laser-induced CNV in mice and was considered a promising tool for developing therapeutic tools for retina-related diseases and preventing neovascularization (128). In addition, genes encoding anti-VEGF Fab proteins have shown potential in the treatment of retinal diseases. RGX-314 uses a novel recombinant AAV8 vector to induce the production of anti-VEGF Fab proteins. Various phase II and III studies are currently underway to evaluate the efficacy of RGX-314 in patients with nvAMD (129,130). In a study, RGX-314 (currently known as ABBV-RGX-314) expression consistently inhibited VEGF-A and was safe and effective in treating patients with nvAMD (131). Another type of gene therapy involves ixoberogene soroparvovec (ixo-vec), formerly known as ADVM-022, which is administered by intravitreal injection. This technology utilizes AAV.7m8 as a vector to generate an aflibercept-carrying transgene for the treatment of nvAMD, and the phase II LUNA trial assessing the safety and efficacy of ixo-vec is currently in progress (132). Finally, 4D-150, which contains transgenes that target aflibercept and VEGF-C, is currently planned to enter a phase III clinical trial for nvAMD (133). However, gene therapy targets, routes of administration and potential safety issues require further validation.

Understanding the structural biology of VEGFR2 has markedly advanced drug design. As previously described, the complete VEGFR2 receptor comprises an extracellular region, a transmembrane domain and an intracellular kinase domain. Insights from ligand-binding domain and coreceptor complex structures, such as the VEGF-VEGFR2 complex and VEGFR1/VEGFR2 extracellular fusion region, have guided the development of therapeutic agents such as ranibizumab and aflibercept, which are now widely used to treat AMD. Furthermore, by targeting the catalytic structural domain, 'DFG-out' (type II) VEGFR2 inhibitors effectively suppress VEGFR2 phosphorylation, reducing neoangiogenesis and overcoming drug resistance (134). Future research directions include capturing the transient states of VEGFR2 structural activation to design conformation-specific inhibitors, integrating genomic, proteomic and structural data for precision-targeted therapies and developing nanomedicines capable of penetrating the BRB based on structural insights. Thus, structural biology serves not only as a fundamental tool for elucidating VEGFR2 function but also as a cornerstone for overcoming current therapeutic limitations and advancing next-generation ophthalmic treatment.

Single-cell RNA sequencing (scRNA-seq) technology offers novel insights into the cellular heterogeneity and molecular pathways of retinal diseases (135). Gene expression profiles vary across distinct retinal cell types and disease microenvironments. In AMD, which predominantly affects the macula, pathological alterations are most pronounced in macular neuroglial cells, microglia and astrocytes during disease progression. Single-cell sequencing may reveal gene expression changes in different cell types (136). Transcriptomic analysis of retinal samples from patients with DR at various stages revealed dysregulated mechanisms, including the Hippo signaling pathway and gap junction formation (137). scRNA-seq enables the identification of disease-specific cellular subpopulations and facilitates the prognosis of anti-VEGF therapeutic efficacy through cell type-specific gene expression signatures, thereby guiding personalized treatment strategies. Future directions include integrating single-cell assay for transposase-accessible chromatin with high-throughput-seq (to delineate epigenetic regulation) with the phosphoproteomic profiling of VEGFR2 activation to dissect drug resistance mechanisms, as well as establishing comprehensive ophthalmic single-cell databases to inform individualized clinical decision-making.

7. Conclusion

Anti-VEGF drugs for the treatment of neovascularization fundus diseases focus mainly on clinical efficacy, injection regimens and safety. However, problems, such as the use of single therapeutic targets and low response rates, remain; additionally, the high treatment cost and economic pressure for the patients are the root causes of decreased patient compliance. Therefore, future studies should focus on the development of novel anti-VEGF drugs with multiple targets and drug delivery systems, non-invasive drug delivery, gene therapy and artificial intelligence technology, aiming to address current therapeutic limitations, such as reducing the number of injections, increasing patient compliance and improving the cure rate (138). Non-invasive drug delivery and gene therapy can be used to solve the problems of anti-VEGF treatment complications and low response rates. Although the current cost of gene

therapy is high, with the continuous progress of science and technology, this is expected to decrease. The economic burden on patients may be reduced in the future through national policies, health insurance payment systems, commercial insurance and the possibility of patients themselves jointly bearing medical costs. Artificial intelligence technology could be used to screen patients, treatment outcomes can be predicted using targeted biomarkers and diagnostic accuracy may be improved.

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Authors' contributions

HSL analyzed the literature and wrote the original draft. XGH designed the study and critically revised the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Swift MR and Weinstein BM: Arterial-venous specification during development. Circ Res 104: 576-588, 2009.
2. Vishwakarma S and Kaur I: Molecular mediators and regulators of retinal angiogenesis. Semin Ophthalmol 38: 124-133, 2023.
3. Díaz-Coránguez M, Ramos C and Antonetti DA: The inner blood-retinal barrier: Cellular basis and development. Vision Res 139: 123-137, 2017.
4. Uemura A, Fruttiger M, D'Amore PA, De Falco S, Joussen AM, Sennlaub F, Brunck LR, Johnson KT, Lambrou GN, Rittenhouse KD and Langmann T: VEGFR1 signaling in retinal angiogenesis and microinflammation. Prog Retin Eye Res 84: 100954, 2021.
5. Selvam S, Kumar T and Fruttiger M: Retinal vasculature development in health and disease. Prog Retin Eye Res 63: 1-19, 2018.
6. Hu WH, Zhang XY, Leung KW, Duan R, Dong TT, Qin QW and Tsim KW: Resveratrol, an inhibitor binding to VEGF, restores the pathology of abnormal angiogenesis in retinopathy of prematurity (ROP) in mice: Application by intravitreal and topical instillation. Int J Mol Sci 23: 6455, 2022.
7. Yan J, Deng J, Cheng F, Zhang T, Deng Y, Cai Y and Cong W: Thioredoxin-interacting protein inhibited vascular endothelial cell-induced HREC angiogenesis treatment of diabetic retinopathy. Appl Biochem Biotechnol 195: 1268-1283, 2023.
8. Heloterä H and Kaarniranta K: A linkage between angiogenesis and inflammation in neovascular age-related macular degeneration. Cells 11: 3453, 2022.
9. Bakri SJ, Thorne JE, Ho AC, Ehlers JP, Schoenberger SD, Yeh S and Kim SJ: Safety and efficacy of anti-vascular endothelial growth factor therapies for neovascular age-related macular degeneration: A report by the american academy of ophthalmology. Ophthalmology 126: 55-63, 2019.
10. Xu X, Han N, Zhao F, Fan R, Guo Q, Han X, Liu Y and Luo G: Inefficacy of anti-VEGF therapy reflected in VEGF-mediated photoreceptor degeneration. Mol Ther Nucleic Acids 35: 102176, 2024.
11. Apte RS, Chen DS and Ferrara NL: VEGF in signaling and disease: Beyond discovery and development. Cell 176: 1248-1264, 2019.
12. Shaw P, Dwivedi SKD, Bhattacharya R, Mukherjee P and Rao G: VEGF signaling: Role in angiogenesis and beyond. Biochim Biophys Acta Rev Cancer 1879: 189079, 2024.
13. Olsson AK, Dimberg A, Kreuger J and Claesson-Welsh L: VEGF receptor signalling - in control of vascular function. Nat Rev Mol Cell Biol 7: 359-371, 2006.
14. Peach CJ, Mignone VW, Arruda MA, Alcobia DC, Hill SJ, Kilpatrick LE and Woolard J: Molecular pharmacology of VEGF-A isoforms: Binding and signalling at VEGFR2. Int J Mol Sci 19: 1264, 2018.
15. Mallick R and Ylä-Herttuala S: Therapeutic potential of VEGF-B in coronary heart disease and heart failure: Dream or vision? Cells 11: 4134, 2022.
16. Wada H, Suzuki M, Matsuda M, Ajiro Y, Shinozaki T, Sakagami S, Yonezawa K, Shimizu M, Funada J, Takenaka T, *et al*: Distinct characteristics of VEGF-D and VEGF-C to predict mortality in patients with suspected or known coronary artery disease. J Am Heart Assoc 9: e015761, 2020.
17. Sarabipour S, Ballmer-Hofer K and Hristova K: VEGFR-2 conformational switch in response to ligand binding. Elife 5: e13876, 2016.
18. Simons M, Gordon E and Claesson-Welsh L: Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol 17: 611-625, 2016.
19. Koch S and Claesson-Welsh L: Signal transduction by vascular endothelial growth factor receptors. Cold Spring Harb Perspect Med 2: a006502, 2012.
20. Kendall RL, Rutledge RZ, Mao X, Tebben AJ, Hungate RW and Thomas KA: Vascular endothelial growth factor receptor KDR tyrosine kinase activity is increased by autophosphorylation of two activation loop tyrosine residues. J Biol Chem 274: 6453-6460, 1999.
21. Gelfand MV, Hagan N, Tata A, Oh WJ, Lacoste B, Kang KT, Kopycinska J, Bischoff J, Wang JH and Gu C: Neuropilin-1 functions as a VEGFR2 co-receptor to guide developmental angiogenesis independent of ligand binding. Elife 3: e03720, 2014.
22. Gavard J and Gutkind JS: VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol 8: 1223-1234, 2008.
23. Smith RO, Ninchoji T, Gordon E, André H, Dejana E, Vestweber D, Kvanta A and Claesson-Welsh L: Vascular permeability in retinopathy is regulated by VEGFR2 Y949 signaling to VE-cadherin. Elife 9: e54056, 2020.
24. Shibuya M: VEGF-VEGFR signals in health and disease. Biomol Ther (Seoul) 22: 1-9, 2014.
25. Mabeta P and Steenkamp V: The VEGF/VEGFR axis revisited: Implications for cancer therapy. Int J Mol Sci 23: 15585, 2022.
26. Melincovici CS, Boşca AB, Şuşman S, Mărginean M, Mihu C, Istrate M, Moldovan IM, Roman AL and Mihu CM: Vascular endothelial growth factor (VEGF)-key factor in normal and pathological angiogenesis. Rom J Morphol Embryol 59: 455-467, 2018.
27. Shibuya M: VEGF-VEGFR system as a target for suppressing inflammation and other diseases. Endocr Metab Immune Disord Drug Targets 15: 135-144, 2015.
28. Patel SA, Nilsson MB, Le X, Cascone T, Jain RK and Heymach JV: Molecular mechanisms and future implications of VEGF/VEGFR in cancer therapy. Clin Cancer Res 29: 30-39, 2023.
29. Shibuya M and Claesson-Welsh L: Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res 312: 549-560, 2006.
30. Jin KL, Mao XO and Greenberg DA: Vascular endothelial growth factor: Direct neuroprotective effect in in vitro ischemia. Proc Natl Acad Sci USA 97: 10242-10247, 2000.

31. Okabe K, Kobayashi S, Yamada T, Kurihara T, Tai-Nagara I, Miyamoto T, Mukouyama YS, Sato TN, Suda T, Ema M and Kubota Y: Neurons limit angiogenesis by titrating VEGF in retina. Cell 159: 584-596, 2014.
32. Campochiaro PA: Molecular pathogenesis of retinal and choroidal vascular diseases. Prog Retin Eye Res 49: 67-81, 2015.
33. Abu Serhan H, Taha MJJ, Abuawwad MT, Abdelaal A, Irshaidat S, Abu Serhan L, Abu Salim QF, Awamleh N, Abdelazeem B and Elnahry AG: Safety and efficacy of brolucizumab in the treatment of diabetic macular edema and diabetic retinopathy: A systematic review and meta-analysis. Semin Ophthalmol 39: 251-260, 2024.
34. Wang Z, Zhang N, Lin P, Xing Y and Yang N: Recent advances in the treatment and delivery system of diabetic retinopathy. Front Endocrinol (Lausanne) 15: 1347864, 2024.
35. Kaur A, Kumar R and Sharma A: Diabetic retinopathy leading to blindness-a review. Curr Diabetes Rev 20: e240124225997, 2024.
36. Zhou YM, Cao YH, Guo J and Cen LS: Potential prospects of Chinese medicine application in diabetic retinopathy. World J Diabetes 15: 2010-2014, 2024.
37. Baseline and early natural history report. The central vein occlusion study. Arch Ophthalmol 111: 1087-1095, 1993.
38. Vitiello L, Lixi F, Coppola A, Abbinante G, Gagliardi V, Salerno G, De Pascale I, Pellegrino A and Giannaccare G: Intravitreal dexamethasone implant switch after anti-VEGF treatment in patients affected by retinal vein occlusion: A review of the literature. J Clin Med 13: 5006, 2024.
39. Minaker SA, Mason RH, Bamakrid M, Lee Y and Muni RH: Changes in aqueous and vitreous inflammatory cytokine levels in retinal vein occlusion: A systematic review and meta-analysis. J Vitreoretin Dis 4: 36-64, 2019.
40. Wang B, Zhang X, Chen H, Koh A, Zhao C and Chen Y: A review of intraocular biomolecules in retinal vein occlusion: Toward potential biomarkers for companion diagnostics. Front Pharmacol 13: 859951, 2022.
41. Finocchio L, Zeppieri M, Gabai A, Toneatto G, Spadea L and Salati C: Recent developments in gene therapy for neovascular age-related macular degeneration: A review. Biomedicines 11: 3221, 2023.
42. Cheng S, Zhang S, Huang M, Liu Y, Zou X, Chen X and Zhang Z: Treatment of neovascular age-related macular degeneration with anti-vascular endothelial growth factor drugs: progress from mechanisms to clinical applications. Front Med (Lausanne) 11: 1411278, 2024.
43. Fragiotta S, Bassis L, Abdolrahimzadeh B, Marino A, Sepe M and Abdolrahimzadeh S: Exploring current molecular targets in the treatment of neovascular age-related macular degeneration toward the perspective of long-term agents. Int J Mol Sci 25: 4433, 2024.
44. Nakao S, Zandi S, Hata Y, Kawahara S, Arita R, Schering A, Sun D, Melhorn MI, Ito Y, Lara-Castillo N, *et al*: Blood vessel endothelial VEGFR-2 delays lymphangiogenesis: An endogenous trapping mechanism links lymph- and angiogenesis. Blood 117: 1081-1090, 2011.
45. Duh EJ, Sun JK and Stitt AW: Diabetic retinopathy: Current understanding, mechanisms, and treatment strategies. JCI Insight 2: e93751, 2017.
46. Mrugacz M, Bryl A and Zorena K: Retinal Vascular endothelial cell dysfunction and neuroretinal degeneration in diabetic patients. J Clin Med 10: 458, 2021.
47. Zhang YL, Pirmardan ER, Jiang H, Barakat A and Hafezi-Moghadam A: VEGFR-2 adhesive nanoprobes reveal early diabetic retinopathy in vivo. Biosens Bioelectron 237: 115476, 2023.
48. Liu X, Guo A, Tu Y, Li W, Li L, Liu W, Ju Y, Zhou Y, Sang A and Zhu M: Fruquintinib inhibits VEGF/VEGFR2 axis of choroidal endothelial cells and M1-type macrophages to protect against mouse laser-induced choroidal neovascularization. Cell Death Dis 11: 1016, 2020.
49. Xu J, Tu Y, Wang Y, Xu X, Sun X, Xie L, Zhao Q, Guo Y, Gu Y, Du J, *et al*: Prodrug of epigallocatechin-3-gallate alleviates choroidal neovascularization via down-regulating HIF-1α/VEGF/VEGFR2 pathway and M1 type macrophage/microglia polarization. Biomed Pharmacother 121: 109606, 2020.
50. Leitch IM, Gerometta M, Eichenbaum D, Finger RP, Steinle NC and Baldwin ME: Vascular endothelial growth factor C and D signaling pathways as potential targets for the treatment of neovascular age-related macular degeneration: A narrative review. Ophthalmol Ther 13: 1857-1875, 2024.
51. Cui K, Liu J, Huang L, Qin B, Yang X, Li L, Liu Y, Gu J, Wu W, Yu Y and Sang A: Andrographolide attenuates choroidal neovascularization by inhibiting the HIF-1α/VEGF signaling pathway. Biochem Biophys Res Commun 530: 60-66, 2020.
52. Carmeliet P and Jain RK: Molecular mechanisms and clinical applications of angiogenesis. Nature 473: 298-307, 2011.
53. Wang X, Bove AM, Simone G and Ma B: Molecular Bases of VEGFR-2-Mediated physiological function and pathological role. Front Cell Dev Biol 8: 599281, 2020.
54. Gomes E and Rockwell P: p38 MAPK as a negative regulator of VEGF/VEGFR2 signaling pathway in serum deprived human SK-N-SH neuroblastoma cells. Neurosci Lett 431: 95-100, 2008.
55. Hendrikse CSE, Theelen PMM, van der Ploeg P, Westgeest HM, Boere IA, Thijs AMJ, Ottevanger PB, van de Stolpe A, Lambrechts S, Bekkers RLM and Piek JMJ: The potential of RAS/RAF/MEK/ERK (MAPK) signaling pathway inhibitors in ovarian cancer: A systematic review and meta-analysis. Gynecol Oncol 171: 83-94,2023.
56. Gallo S, Vitacolonna A, Bonzano A, Comoglio P and Crepaldi T: ERK: A key player in the pathophysiology of cardiac hypertrophy. Int J Mol Sci 20: 2164, 2019.
57. Almalki SG and Agrawal DK: ERK signaling is required for VEGF-A/VEGFR2-induced differentiation of porcine adipose-derived mesenchymal stem cells into endothelial cells. Stem Cell Res Ther 8: 113, 2017.
58. Guo GX, Qiu YH, Liu Y, Yu LL, Zhang X, Tsim KW, Qin QW and Hu WH: Fucoxanthin attenuates angiogenesis by blocking the VEGFR2-mediated signaling pathway through binding the vascular endothelial growth factor. J Agric Food Chem 72: 21610-21623, 2024.
59. Zhou LB, Zhou YQ and Zhang XY: Blocking VEGF signaling augments interleukin-8 secretion via MEK/ERK/1/2 axis in human retinal pigment epithelial cells. Int J Ophthalmol 13: 1039-1045, 2020.
60. Bin Y, Liu YY, Jiang SQ and Peng H: Elevated YKL-40 serum levels may contribute to wet age-related macular degeneration via the ERK1/2 pathway. FEBS Open Bio 11: 2933-2942, 2021.
61. Miller B and Sewell-Loftin MK: Mechanoregulation of vascular endothelial growth factor receptor 2 in angiogenesis. Front Cardiovasc Med 8: 804934, 2021.
62. Jin H, Ko YS, Yun SP, Park SW and Kim HJ: P2Y(2)R-mediated transactivation of VEGFR2 through Src phosphorylation is associated with ESM-1 overexpression in radiotherapy-resistant-triple negative breast cancer cells. Int J Oncol 62: 73, 2023.
63. Gao X, Chen J, Yin G, Liu Y, Gu Z, Sun R, Sun X, Jiao X, Wang L, Wang N, *et al*: Hyperforin ameliorates neuroinflammation and white matter lesions by regulating microglial VEGFR(2)/SRC pathway in vascular cognitive impairment mice. CNS Neurosci Ther 30: e14666, 2024.
64. Chen TT, Dong JL, Zhou HY, Deng X, Li R, Chen N, Luo M, Li Y, Wu J and Wang L: Glycation of fibronectin inhibits VEGF-induced angiogenesis by uncoupling VEGF receptor-2-c-Src crosstalk. J Cell Mol Med 24: 9154-9164, 2020.
65. Moysenovich AM, Tatarskiy VV, Yastrebova MA, Bessonov IV, Arkhipova AY, Kolosov AS, Davydova LI, Khamidullina AI, Bogush VG, Debabov VG, *et al*: Akt and Src mediate the photocrosslinked fibroin-induced neural differentiation. Neuroreport 31: 770-775, 2020.
66. Rezzola S, Di Somma M, Corsini M, Leali D, Ravelli C, Polli VAB, Grillo E, Presta M and Mitola S: VEGFR2 activation mediates the pro-angiogenic activity of BMP4. Angiogenesis 22: 521-533, 2019.
67. Sergeys J, Van Hove I, Hu TT, Temps C, Carragher NO, Unciti-Broceta A, Feyen JHM, Moons L and Porcu M: The retinal tyrosine kinome of diabetic Akimba mice highlights potential for specific Src family kinase inhibition in retinal vascular disease. Exp Eye Res 197: 108108, 2020.
68. Sjöberg E, Melssen M, Richards M, Ding Y, Chanoca C, Chen D, Nwadozi E, Pal S, Love DT, Ninchoji T, *et al*: Endothelial VEGFR2-PLCγ signaling regulates vascular permeability and antitumor immunity through eNOS/Src. J Clin Invest 133: e161366, 2023.
69. Yang L, Guan H, He J, Zeng L, Yuan Z, Xu M, Zhang W, Wu X and Guan J: VEGF increases the proliferative capacity and eNOS/NO levels of endothelial progenitor cells through the calcineurin/NFAT signalling pathway. Cell Biol Int 36: 21-27, 2012.

70. Huang TF, Wang SW, Lai YW, Liu SC, Chen YJ, Hsueh TM, Lin CC, Lin CH and Chung CH: 4-Acetylantroquinonol B suppresses prostate cancer growth and angiogenesis via a VEGF/PI3K/ERK/mTOR-dependent signaling pathway in subcutaneous xenograft and in vivo angiogenesis models. Int J Mol Sci 23: 1446, 2022.
71. Qi S, Deng S, Lian Z and Yu K: Novel drugs with high efficacy against tumor angiogenesis. Int J Mol Sci 23: 6934, 2022.
72. Namjoo M, Ghafouri H, Assareh E, Aref AR, Mostafavi E, Hamrahi Mohsen A, Balalaie S, Broussy S and Asghari SM: A VEGFB-Based peptidomimetic inhibits VEGFR2-Mediated PI3K/Akt/mTOR and PLCγ/ERK signaling and elicits apoptotic, antiangiogenic, and antitumor activities. Pharmaceuticals (Basel) 16: 906, 2023.
73. Uemura A and Fukushima Y: Rho GTPases in retinal vascular diseases. Int J Mol Sci 22: 3684, 2021.
74. Claesson-Welsh L and Welsh M: VEGFA and tumour angiogenesis. J Intern Med 273: 114-127, 2013.
75. Fukushima Y, Nishiyama K, Kataoka H, Fruttiger M, Fukuhara S, Nishida K, Mochizuki N, Kurihara H, Nishikawa SI and Uemura A: RhoJ integrates attractive and repulsive cues in directional migration of endothelial cells. EMBO J 39: e102930, 2020.
76. Hauke M, Eckenstaler R, Ripperger A, Ender A, Braun H and Benndorf RA: Active RhoA exerts an inhibitory effect on the homeostasis and angiogenic capacity of human endothelial cells. J Am Heart Assoc 11: e025119, 2022.
77. Katari V, Dalal K, Adapala RK, Guarino BD, Kondapalli N, Paruchuri S and Thodeti CK: A TRP to pathological angiogenesis and vascular normalization. Compr Physiol 14: 5389-5406, 2024.
78. Ramshekar A, Bretz CA and Hartnett ME: RNA-Seq Provides Insights into VEGF-Induced signaling in human retinal microvascular endothelial cells: Implications in retinopathy of prematurity. Int J Mol Sci 23: 7354, 2022.
79. Yu E, Kim H, Park H, Hong JH, Jin J, Song Y, Woo JM, Min JK and Yun J: Targeting the VEGFR2 signaling pathway for angiogenesis and fibrosis regulation in neovascular age-related macular degeneration. Sci Rep 14: 25682, 2024.
80. Hara C, Wakabayashi T, Fukushima Y, Sayanagi K, Kawasaki R, Sato S, Sakaguchi H and Nishida K: Tachyphylaxis during treatment of exudative age-related macular degeneration with aflibercept. Graefes Arch Clin Exp Ophthalmol 257: 2559-2569, 2019.
81. Ribatti D, Solimando AG and Pezzella F: The Anti-VEGF(R) drug discovery legacy: Improving attrition rates by breaking the vicious cycle of angiogenesis in cancer. Cancers (Basel) 13: 3433, 2021.
82. Estarreja J, Mendes P, Silva C, Camacho P and Mateus V: The efficacy, safety, and efficiency of the off-label use of bevacizumab in patients diagnosed with age-related macular degeneration: Protocol for a systematic review and meta-analysis. JMIR Res 12: e38658, 2023.
83. Siktberg J, Kim SJ, Sternberg P Jr and Patel S: Effectiveness of bevacizumab step therapy for neovascular age-related macular degeneration. EYE (Lond) 37: 1844-1849, 2023.
84. Cao X, Sanchez JC, Patel TP, Yang ZY, Guo CY, Malik D, Sopeyin A, Montaner S and Sodhi A: Aflibercept more effectively weans patients with neovascular age-related macular degeneration off therapy with bevacizumab. J Clin Invest 133: e159125, 2023.
85. Reddy SK, Ballal AR, Shailaja S, Seetharam RN, Raghu CH, Sankhe R, Pai K, Tender T, Mathew M and Aroor A, *et al*: Small extracellular vesicle-loaded bevacizumab reduces the frequency of intravitreal injection required for diabetic retinopathy. Theranostics 13: 2241-2255, 2023.
86. Fong AH and Lai TY: Long-term effectiveness of ranibizumab for age-related macular degeneration and diabetic macular edema. Clin Interv Aging 8: 467-482, 2013.
87. Kishishita S, Sakanishi Y, Morita S, Matsuzawa M, Usui-Ouchi A and Ebihara N: Effects of intravitreal injection of ranibizumab and aflibercept for branch retinal vein occlusion on the choroid: A retrospective study. BMC Ophthalmol 22: 458, 2022.
88. Rouvas A, Datseris I, Androudi S, Tsilimbaris M, Kabanarou SA, Pharmakakis N, Koutsandrea C, Charonis A, Kousidou O and Pantelopoulou G: A real-world, multicenter, 6-month prospective study in greece of the effectiveness and safety of ranibizumab in patients with age-related macular degeneration who have inadequately responded to aflibercept: The 'ELEVATE' study. Clin Ophthalmol 16: 2579-2593, 2022.
89. Debourdeau E, Beylerian H, Nguyen V, Barthelmes D, Gillies M, Gabrielle PH, Vujosevic S, Otoole L, Puzo M, Creuzot-Garcher C, *et al*: Treat-and-Extend Versus Pro re nata regimens of ranibizumab and aflibercept in neovascular age-related macular degeneration: A comparative study from routine clinical practice. Ophthalmol Ther 13: 2343-2355, 2024.
90. Eichenbaum DA, Ahmed A and Hiya F: Ranibizumab port delivery system: A clinical perspective. BMJ Open Ophthalmol 7: e001104, 2022.
91. Lowater SJ, Grauslund J, Subhi Y and Vergmann AS: Clinical trials and future outlooks of the port delivery system with ranibizumab: A narrative review. Ophthalmol Ther 13: 51-69, 2023.
92. Carlà MM, Savastano MC, Boselli F, Giannuzzi F and Rizzo S: Ranibizumab port delivery system in neovascular age-related macular degeneration: Where do we stand? Overview of pharmacokinetics, clinical results, and future directions. Pharmaceutics 16: 314, 2024.
93. Sharma T, Dhingra R, Singh S, Sharma S, Tomar P, Malhotra M and Bhardwaj TR: Aflibercept: A novel VEGF targeted agent to explore the future perspectives of anti-angiogenic therapy for the treatment of multiple tumors. Mini Rev Med Chem 13: 530-540, 2013.
94. Baybora H: Perifoveal retinal thickness changes after intravitreal aflibercept injection for choroidal neovascularization in age-related macular degeneration. Photodiagnosis Photodyn Ther 46: 104028, 2024.
95. Heier JS, Brown DM, Chong V, Korobelnik JF, Kaiser PK, Nguyen QD, Kirchhof B, Ho A, Ogura Y, Yancopoulos GD, *et al*: Intravitreal aflibercept (VEGF trap-eye) in wet age-related macular degeneration. Ophthalmology 119: 2537-2548, 2012.
96. Kucukevcilioglu M, Yesiltas YS, Durukan AH, Unlu N, Onen M, Alp MN, Kalayci D, Acar MA, Sekeroglu MA, Citirik M, *et al*: Real life multicenter comparison of 24-month outcomes of anti-VEGF therapy in diabetic macular Edema in Turkey: Ranibizumab vs. aflibercept vs. ranibizumab-aflibercept switch. Medicina (Kaunas) 59: 263, 2023.
97. Kanadani T, Rabelo N, Takahashi D, Magalhaes L and Farah M: Comparison of antiangiogenic agents (ranibizumab, aflibercept, bevacizumab and ziv-aflibercept) in the therapeutic response to the exudative form of age-related macular degeneration according to the treat-and-extend protocol-true head-to-head study. Int J Retina Vitreous 10: 13, 2024.
98. Joussen AM, Ricci F, Paris LP, Korn C, Quezada-Ruiz C and Zarbin M: Angiopoietin/Tie2 signalling and its role in retinal and choroidal vascular diseases: A review of preclinical data. Eye (Lond) 35: 1305-1316, 2021.
99. Foxton RH, Uhles S, Grüner S, Revelant F and Ullmer C: Efficacy of simultaneous VEGF-A/ANG-2 neutralization in suppressing spontaneous choroidal neovascularization. EMBO Mol Med 11: e10204, 2019.
100. Khanani AM, Guymer RH, Basu K, Boston H, Heier JS, Korobelnik JF, Kotecha A, Lin H, Silverman D, Swaminathan B, *et al*: TENAYA and LUCERNE: Rationale and design for the phase 3 clinical trials of faricimab for neovascular age-related macular degeneration. Ophthalmol Sci 1: 100076, 2021.
101. Heier JS, Khanani AM, Quezada Ruiz C, Basu K, Ferrone PJ, Brittain C, Figueroa MS, Lin H, Holz FG, Patel V, *et al*: Efficacy, durability, and safety of intravitreal faricimab up to every 16 weeks for neovascular age-related macular degeneration (TENAYA and LUCERNE): Two randomised, double-masked, phase 3, non-inferiority trials. Lancet 399: 729-740, 2022.
102. Pandit SA, Momenaei B, Wakabayashi T, Mansour HA, Vemula S, Durrani AF, Pashaee B, Kazan AS, Ho AC, Klufas M, *et al*: Clinical outcomes of faricimab in patients with previously treated neovascular age-related macular degeneration. Ophthalmol Retina 8: 360-366, 2024.
103. Szigiato A, Mohan N, Talcott KE, Mammo DA, Babiuch AS, Kaiser PK, Ehlers JP, Rachitskaya A, Yuan A, Srivastava SK and Sharma S: Short-term outcomes of faricimab in patients with neovascular age-related macular degeneration on prior anti-VEGF Therapy. Ophthalmol Retina 8: 10-17, 2024.
104. Grimaldi G, Cancian G, Rizzato A, Casanova A, Perruchoud-Ader K, Clerici M, Consigli A and Menghini M: Intravitreal faricimab for neovascular age-related macular degeneration previously treated with traditional anti-VEGF compounds: A real-world prospective study. Graefes Arch Clin Exp Ophthalmol 262: 1151-1159, 2024.

105. Mori R, Honda S, Gomi F, Tsujikawa A, Koizumi H, Ochi H, Ohsawa S and Okada AA; TENAYA and LUCERNE Investigators: Efficacy, durability, and safety of faricimab up to every 16 weeks in patients with neovascular age-related macular degeneration: 1-year results from the Japan subgroup of the phase 3 TENAYA trial. Jpn J Ophthalmol 67: 301-310, 2023.
106. Diabetic Retinopathy Clinical Research Network; Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, Arnold-Bush B, Baker CW, Bressler NM, *et al*: Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. N Engl J Med 372: 1193-1203, 2015.
107. Huang X, Wu W, Qi H, Yan X, Dong L, Yang Y, Zhang Q, Ma G, Zhang G and Lei H: Exploitation of enhanced prime editing for blocking aberrant angiogenesis. J Adv Res: Jul 10, 2024 (Epub ahead of print).
108. Zech TJ, Wolf A, Hector M, Bischoff-Kont I, Krishnathas GM, Kuntschar S, Schmid T, Bracher F, Langmann T and Fürst R: 2-Desaza-annomontine (C81) impedes angiogenesis through reduced VEGFR2 expression derived from inhibition of CDC2-like kinases. Angiogenesis 27: 245-272, 2024.
109. Tang X, Cui K, Wu P, Hu A, Fan M, Lu X, Yang F, Lin J, Yu S, Xu Y and Liang X: Acrizanib as a novel therapeutic agent for fundus neovascularization via inhibitory phosphorylation of VEGFR2. Transl Vis Sci Technol 13: 1, 2024.
110. Lei W, Xu H, Yao H, Li L, Wang M, Zhou X and Liu X: 5α-Hydroxycostic acid inhibits choroidal neovascularization in rats through a dual signalling pathway mediated by VEGF and angiopoietin 2. Mol Med 29: 151, 2023.
111. Liu Y, Feng M, Cai J, Li S, Dai X, Shan G and Wu S: Repurposing bortezomib for choroidal neovascularization treatment via antagonizing VEGF-A and PDGF-D mediated signaling. Exp Eye Res 204: 108446, 2021.
112. Zeng Z, Li S, Ye X, Wang Y, Wang Q, Chen Z, Wang Z, Zhang J, Wang Q, Chen L, *et al*: Genome editing VEGFA prevents corneal neovascularization in vivo. Adv Sci (Weinh) 11: e2401710, 2024.
113. Huang X, Zhou G, Wu W, Duan Y, Ma G, Song J, Xiao R, Vandenberghe L, Zhang F, D'Amore PA and Lei H: Genome editing abrogates angiogenesis in vivo. Nat Commun 8: 112, 2017.
114. Toutounchian S, Ahmadbeigi N and Mansouri V: Retinal and choroidal neovascularization antivascular endothelial growth factor treatments: The role of gene therapy. J Ocul Pharmacol Ther 38: 529-548, 2022.
115. Yiu G, Gulati S, Higgins V, Coak E, Mascia D, Kim E, Spicer G and Tabano D: Factors involved in anti-VEGF treatment decisions for neovascular age-related macular degeneration: Insights from real-world clinical practice. Clin Ophthalmol 18: 1679-1690, 2024.
116. Wykoff CC, Garmo V, Tabano D, Menezes A, Kim E, Fevrier HB, LaPrise A and Leng T: Impact of Anti-VEGF treatment and patient characteristics on vision outcomes in neovascular age-related macular degeneration: Up to 6-year analysis of the AAO IRIS® Registry. Ophthalmol Sci 4: 100421, 2023.
117. Liu D, Zhang C, Zhang J, Xu GT and Zhang J: Molecular pathogenesis of subretinal fibrosis in neovascular AMD focusing on epithelial-mesenchymal transformation of retinal pigment epithelium. Neurobiol Dis 185: 106250, 2023.
118. Fleckenstein M, Mitchell P, Freund KB, Sadda S, Holz FG, Brittain C, Henry EC and Ferrara D: The progression of geographic atrophy secondary to age-related macular degeneration. Ophthalmology 125: 369-390, 2018.
119. Zhuang X, Su Y, Li M, Zhang L, Mi L, Ji Y, Deng F, Xiao O, Zhang X and Zhou L, *et al*: A prospective observation of influence of anti-VEGF on optic disc vasculature in nAMD patients. Photodiagnosis Photodyn Ther 45: 103863, 2024.
120. Pérez-Gutiérrez L and Ferrara N: Biology and therapeutic targeting of vascular endothelial growth factor A. Nat Rev Mol Cell Biol 24: 816-834, 2023.
121. Wu Y, Wang J, Zhao J, Su Y, Li X, Chen Z, Wu X, Huang S, He X and Liang L: LTR retrotransposon-derived LncRNA LINC01446 promotes hepatocellular carcinoma progression and angiogenesis by regulating the SRPK2/SRSF1/VEGF axis. Cancer Lett 598: 217088, 2024.
122. Bao M, Chen Y, Liu JT, Bao H, Wang WB, Qi YX and Lv F: Extracellular matrix stiffness controls VEGF(165) secretion and neuroblastoma angiogenesis via the YAP/RUNX2/SRSF1 axis. Angiogenesis 25: 71-86, 2022.
123. Camacho P, Ribeiro E, Pereira B, Varandas T, Nascimento J, Henriques J, Dutra-Medeiros M, Delgadinho M, Oliveira K, Silva C and Brito M: DNA methyltransferase expression (DNMT1, DNMT3a and DNMT3b) as a potential biomarker for anti-VEGF diabetic macular edema response. Eur J Ophthalmol 33: 2267-2274, 2023.
124. Xu L, Prentice JR, Velez-Montoya R, Sinha A, Barakat MR, Gupta A, Lowenthal R, Khanani AM, Kaiser PK, Heier JS, *et al*: Bispecific VEGF-A and Angiopoietin-2 Antagonist RO-101 preclinical efficacy in model of neovascular eye disease. Ophthalmol Sci 4: 100467, 2024.
125. Jian HJ, Anand A, Lai JY, Huang CC, Ma DH, Lai CC and Chang HT: Ultrahigh-Efficacy VEGF neutralization using carbonized nanodonuts: Implications for intraocular anti-angiogenic therapy. Adv Healthc Mater 13: e2302881, 2024.
126. Xu M, Fan R, Fan X, Shao Y and Li X: Progress and challenges of Anti-VEGF agents and their sustained-release strategies for retinal angiogenesis. Drug Des Devel Ther 16: 3241-3262, 2022.
127. Zhou C, Lei F, Sharma J, Hui PC, Wolkow N, Dohlman CH, Vavvas DG, Chodosh J and Paschalis EI: Sustained inhibition of VEGF and TNF-α achieves multi-ocular protection and prevents formation of blood vessels after severe ocular trauma. Pharmaceutics 15: 2059, 2023.
128. Puranen J, Koponen S, Nieminen T, Kanerva I, Kokki E, Toivanen P, Urtti A, Ylä-Herttuala S and Ruponen M: Antiangiogenic AAV2 gene therapy with a truncated form of soluble VEGFR-2 reduces the growth of choroidal neovascularization in mice after intravitreal injection. Exp Eye Res 224: 109237, 2022.
129. Desideri LF, Vaccaro S, Vagge A, Nicolò M, Scorcia V, Traverso CE and Giannaccare G: AAV8 gene therapy encoding anti-VEGF Fab Treatment of wet age-related macular degeneration Treatment of diabetic retinopathy. Drugs Future 47: 737-741, 2022.
130. Papaioannou C: Advancements in the treatment of age-related macular degeneration: A comprehensive review. Postgrad Med J 100: 445-450, 2024.
131. Campochiaro PA, Avery R, Brown DM, Heier JS, Ho AC, Huddleston SM, Jaffe GJ, Khanani AM, Pakola S, Pieramici DJ, *et al*: Gene therapy for neovascular age-related macular degeneration by subretinal delivery of RGX-314: A phase 1/2a dose-escalation study. Lancet 403: 1563-1573, 2024.
132. Poulsen K, Hanna K, Nieves J, Nguyen N, Sharma P, Grishanin R, Corbau R and Kiss S: Nonclinical study of ixo-vec gene therapy for nAMD supports efficacy for a human dose of 6E10 vg/eye and staggered dosing of fellow eyes. Mol Ther Methods Clin Dev 33: 101430, 2025.
133. Shirian JD, Shukla P and Singh RP: Exploring new horizons in neovascular age-related macular degeneration: Novel mechanisms of action and future therapeutic avenues. Eye (Lond) 39: 40-44, 2025.
134. Modi SJ and Kulkarni VM: Exploration of structural requirements for the inhibition of VEGFR-2 tyrosine kinase: Binding site analysis of type II, 'DFG-out' inhibitors. J Biomol Struct Dyn 40: 5712-5727, 2022.
135. Zong Y, Xiao S, Lei D and Li H: Discoveries in retina physiology and disease biology using single-cell RNA sequencing. Front Biosci (Landmark Ed) 28: 247, 2023.
136. Lyu Y, Zauhar R, Dana N, Strang CE, Hu J, Wang K, Liu S, Pan N, Gamlin P, Kimble JA, *et al*: Implication of specific retinal cell-type involvement and gene expression changes in AMD progression using integrative analysis of single-cell and bulk RNA-seq profiling. Sci Rep 11: 15612, 2021.
137. Becker K, Klein H, Simon E, Viollet C, Haslinger C, Leparc G, Schultheis C, Chong V, Kuehn MH, Fernandez-Albert F and Bakker RA: In-depth transcriptomic analysis of human retina reveals molecular mechanisms underlying diabetic retinopathy. Sci Rep 11: 10494, 2021.
138. Deng J and Qin YH: Advancements and emerging trends in ophthalmic anti-VEGF therapy: A bibliometric analysis. Int Ophthalmol 44: 368, 2024.