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DIABETIC RETINOPATHY: NEW PHARMACOLOGICAL TARGETS

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SUMMARY

Diabetic retinopathy (DR) is a secondary complication of diabetes mellitus and represents the most common cause of irreversible vision loss in working people of industrialized countries. DR is generally considered a microvascular complication of diabetes, although the inflammatory component plays a key role. The main cause of vision loss in diabetic patients with proliferative diabetic retinopathy is the diabetic macular edema (DME), that is responsible to the retinal detachment. DME is mainly caused by new angiogenesis, which is a hallmark of the advanced stage of DR (proliferative diabetic retinopathy, PDR). Retinal neovascularization is principally driven by pro-angiogenic factors (*e.g.*, VEGF-A, PlGF), inflammatory mediators (TNF-α, interleukins, chemokines) and oxidative stress-related elements. Chronic hyperglycemia is the primary causative factor of DR, however, several points of DR etiopathogenesis are still unclear. Many other factors are involved during the early stages of DR such as the retinal hypoxia, that is a trigger of VEGF release in the back of the eye. Up to now, the pharmacological approaches for DR are intravitreal anti-VEGF agents and corticosteroids. However, some patients can be refractory to anti-VEGF therapy, therefore, efforts must be carried out to discover novel pharmacological targets to handle DR. Hereby, the current literature will be revised about novel potential pharmacological targets, with a focus on PlGF, miRNAs and purinergic P2X7 receptor. Future drug development campaigns on these targets might lead to better clinical outcomes, possibly in the early phase of the disease.

Key words

Diabetic retinopathy; angiogenesis; hypoxia; anti-VEGF; inflammation.

Impact statement

Currently, management and treatment of diabetic retinopathy (DR) are characterized by several unmet medical needs. Particularly, early-stage DR lacks of approved therapeutical intervention, and its pathogenesis is multifactorial. Pharmacological research should focus on novel pharmacological targets, that address pathogenetic factors of DR, such as inflammation, oxidative stress and angiogenesis.

INTRODUCTION

Diabetic retinopathy (DR) represents a major public health concern, and it is the leading cause of vision loss in working age (1). The prevalence of DR among diabetic patients is about 40%, and approximately 5-10% of these individuals have vision threatening conditions (2, 3). Chronic hyperglycemia is the primary causative factor of diabetic retinopathy, however, etiopathogenesis of DR is still unclear (4-7). Ophthalmologists classify diabetic retinopathy mainly into two stages, the non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) (8). The NPDR is characterized by lesions due to chronic hyperglycemia, that can lead to microaneurysms due to instability of

capillary walls. As soon as microaneurysms start leaking, NPDR can develop in macular edema and consequent impaired vision, due to deposition of fluid under the macula. The presence of this fluid, composed by lipids, leads to the formation of yellow deposits, called hard exudates. Moreover, with the progression of the disease, the affected vessels can be obstructed, then leading to impaired retinal perfusion. Retinal ischemia can cause the infarction of the nerve fiber layer, resulting in fluffy and white patches, called cotton wool spots (CWS). Main cause of NPDR to PDR progression is represented by an extensive retinal ischemia (9), which promotes vitreoretinal neovascularization. In fact, the retina is a high oxygen demanding tissue; under ischemia, cells release angiogenic factors, like vascular endothelial growth factor-A (VEGF-A) and placental growth factor (PlGF), which promote neovascularization. These vessels are typically fragile, fenestrated, brittle and leaky. Leaking vessels can cause vitreous hemorrhages, which are associated with gliosis and fibrovascular scar formation. Moreover, contraction of fibrous tissue can result in tractional retinal detachment and sudden loss of vision (10, 11), along with further activation of pro-fibrotic pathways. As soon as extensive vitreous hemorrhage occurs, the PDR patient is considered at high-risk of vision loss due to retinal detachment (4, 12). In case diabetic retinopathy affects the macula, the disease is also termed 'diabetic maculopathy'. Vascular leakage at the macula leads to macula swelling (diabetic macular edema, DME), which is the most common cause of blindness in diabetic patients (13, 14). DME is most prevalent during PDR, following progressive vascular and neural damage (15). Diabetic DME can be classified as 'ischemic' or 'non-ischemic', based on the involvement or preservation of the perifoveal capillary network, respectively (10). Several causative factors contribute to the pathogenesis of DME, including hypoxia and oxidative stress, upregulation of VEGF-A, alteration of the blood-retinal barrier (BRB), retinal vessel leukostasis, pericyte loss, and vascular hyperpermeability (16, 17).

THE RETINAL NEUROVASCULAR SYSTEM AS A BASIS TO UNDERSTAND DIABETIC RETINOPATHY

The retina is the innermost light-sensitive tissue of the eye, able to convert light to electrochemical signals, at first through photoreceptors, that transmit electrochemical signals to retinal neuronal circuitry (bipolar, amacrine cells). Neuroretinal electrochemical stimuli are thereafter processed and collected by retinal ganglion cells (RGCs), that transmit signals to the visual cortex by means of the optic nerve, that is constituted by RGCs axons (18, 19). The retina is characterized by a complex vascular system, whose integrity is necessary for the correct retinal function, providing nutrients and oxygen to the inner and outer retina (1). The retinal vascular system, similarly to central nervous system, is characterized by blood-retinal barrier (BRB), which maintains the right *milieu*. The BRB includes the inner and outer components. Inner BRB (iBRB) is characterized by junctions between endothelial cells (ECs) and supporting pericytes and astrocytes; while in the outer BRB (oBRB), junctions are between retinal pigmented epithelial cells (RPEs) (20, 21) (**figure 1**). Diabetes can affect both iBRB and oBRB before and after neovascular events, involving endothelial cells, pericytes (at the capillary level), vascular smooth muscle cells (arteriolar/arterial level), glia, neuronal processes, associated immune cells, and if choroid is affected, also RPEs (22).

Pericytes, endothelial cells and iBRB

Depletion of pericytes is a hallmark of DR. Pericytes wrap capillary walls and share basal lamina with endothelial cells, with which they directly interact through N-cadherin and connexin-43 hemi channels (19, 23, 24). Pericytes wrap around retinal capillaries providing structural support, modulation of endothelial cell function and homeostasis. In the inner BRB, retinal endothelial cells form the physical barriers between vascular lumen and the retina. Retinal endothelial cell-cell junctions include tight-, adherens- and gap-junction, that regulate sev-

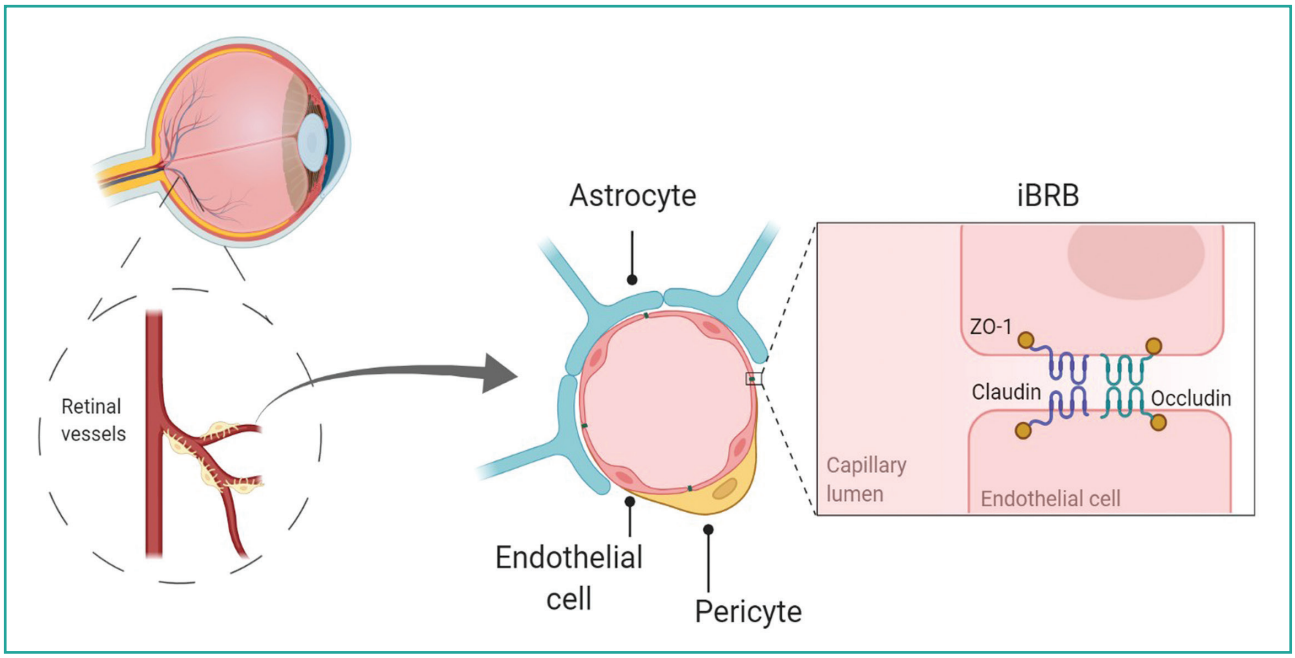

Figure 1. The neovascular unit.
In a healthy retina, vessels are structured by endothelial cells closely associated with pericytes, maintaining the function of the inner blood retina barrier (iBRB). In particular, adjacent endothelial cells are connected by tight junctions and adherens junctions.

eral cell functions such as migration, growth, protection from cell death (necrosis, apoptosis) and damage (inflammation, ischemia) (19, 25).

Retinal glia and neurons

Retinal glial cells, including Müller cells and astrocytes, provide metabolic support to neurons and play a critical role in iBRB homeostasis and integrity (26, 27). Moreover, Müller cells regulate glucose flux between the circulation and retinal neurons and have a role in providing substrates for aerobic metabolism in neurons by gluconeogenesis (28).

Immune cells

The development of new blood vessel is also supported by microglia, monocyte-derived tissue macrophages of the central nervous system. Interestingly, retinal microglia is present in the retina before development of vascularization (29). In the adult retina, ramified microglia cells were found in the inner and outer plexiform layers, and are able to produce factors that support neuronal survival. Several types of trauma or insults lead to microglia activation, characterized by amoeboid morphology transition and production of pro-inflammatory cytokines (30).
Nowadays, the improvement of the research methodologies allows us to mimic, *in vitro*, the real complexity of the ocular structures and barriers. Recently, two labs (Wisniewska-Kruk *et al.* and Fresta *et al.*,) set up two different *in vitro* BRB models based on a triple co-culture of retinal cells, the first research's group used bovine cells, the second research's group used human cells. Fresta *et al.* used human retinal pericytes, astrocytes and endothelial cells to mimic the human BRB with the same cellular layer order and the same numerical ratio. This *in vitro* paradigm is useful to study and investigate the molecular mechanism related to DR, and to test new pharmacological molecules (31, 32).

PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

The pathogenesis of DR is complex and involves multiple interlinked mechanisms, in-

cluding metabolic modifications, mitochondrial dysfunction, vascular damage, apoptosis, inflammation, and oxidative stress (33-36). Several pathways have been proposed to better understand microvascular complications during DR along with sustained hyperglycemia: *e.g.*, accumulation of advanced glycation end-products (AGEs), inflammation, activation of protein kinase C and neuronal dysfunction (19, 37). All these pathological modifications lead to increased vascular permeability and capillary depletion, resulting in macular edema and retinal neovascularization.

Hyperglycemia and Retinal Microvasculopathy

One of the earliest abnormalities observed in DR is related to retinal blood vessels, with the constriction of arteries and arterioles and blood flow anomalies (38-40). Vessel abnormalities result in a series of metabolic and biochemical alterations, like: (i) induction of activation of several PKC isoforms (*e.g.*, PKC-α, -β, -δ and –ε; in particular PKCβII isoform (41, 42); (ii) altered function of ionic channels in smooth muscle cells (BK channels) present in the retinal arteriolar vasculature (43-45). As mentioned before, retinal pericytes loss is another hallmark of the early events of DR. Several *in vitro* and *in vivo* studies report that hyperglycaemia leads to pericyte loss (34, 46, 47) or degenerated pericytes, also called "ghost cells". Therefore, pericytes loss leads to endothelial cells degeneration, microvascular destabilization and perfusion alterations with consequent ischemic events due to capillary occlusion (38, 48-50). On this regard, pericyte-like differentiation of human adipose-derived mesenchymal stem cells (hASCs) has been recently proposed as putative therapeutic tool for restoring damaged BRB (51).

Retinal inflammation

Several studies were focused on the role of inflammatory processes in early stages of DR, although, inflammatory mechanisms are still poorly understood. Chronic low-grade inflammation has been detected in different stages of DR, both in diabetic animal models and in patients (52, 53), along with increased systemic and local expression of proinflammatory cytokines (54). In particular, microvascular endothelium, activated by these cytokines and angiogenic growth factors, expresses pro-inflammatory molecules (*e.g.*, IL-1β, IL-6, TNF-α, high-mobility group box-1 (HMGB1) and chemokines (MCP-1), involved in leukocyte recruitment and activation (55-57). Leukocyte-endothelium adhesion, mediated by adhesion molecules, has been implicated in leukostasis during diabetes. Sequential adhesive interactions between endothelial cells and leukocytes, are modulated by adhesion molecules (*e.g.*, ICAM-1) present in the surface of endothelial cells, which interact with the leukocyte counter-receptor CD18 (58, 59). All these inflammatory responses may contribute to neovascularization in the retina during DR, especially under hypoxic conditions. Furthermore, increasing data suggest a crucial role of tool like receptors (TLRs) in the pathogenesis of DR; indeed, TLR4 expression is significantly increased in diabetic retinas, activating the linked inflammatory pathways (60). As regards the biochemical pathways involved in DR, expression of inflammatory cytokines might be mediated by activation of mitogen-activated protein kinases (MAPKs) (61), as well as ERKs, normally involved in several cellular processes (62). ERK pathway can influence NF-κB activation, by the regulation of NF-κB-dependent genes expression, *e.g.* inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) (63).

Retinal hypoxia

The retina is one of the most oxygen and glucose demanding tissue (64). Retinal hypoxia represents an important causative factor for DR development, and plays a central role in progression of NPDR to PDR, due to the release of some soluble mediators such as cytokines, chemokine and growth factors, which promote the growth of extraretinal neovascularization (65). Ocular ischemic events are

considered crucial for promotion of vascular abnormalities, due to endothelial cells adaptation to stress, which upregulate several genes, like VEGF-A (66). Furthermore, VEGF-A and other hypoxia-regulated growth factors, are controlled by hypoxia-inducible factor (HIF) (67). HIF is a heterodimer, HIF-1α (inducible subunit) and HIF-1β (constitutively expressed). Oxygen deprivation, induces HIF-1α to translocate into the nucleus and to bind the hypoxia-response elements (HREs) in DNA, leading to expression of inflammatory and pro-angiogenic genes, promoting inflammation and angiogenesis, respectively (68, 69).

Retinal angiogenesis

Angiogenesis is a crucial mechanism in physiological vascular development and during pathological conditions. Angiogenesis is related to ECs that, stimulated by some angiogenic factors, generate new blood vessels (70). Indeed, this process is characterized by the angiogenic growth factors, which activate the receptors present on resident ECs; then, endothelial cells begin to release specific enzymes such as matrix metalloproteinases (MMPs) which degrade the basement membrane, leading ECs to leave the original vessel wall. After that, endothelial cells start to proliferate into the surrounding matrix, thanks to the adhesion molecules (**figure 2**).

The main regulators of angiogenesis are the vascular endothelial growth factors (VEGF-A, VEGF-B, VEGF-C, and VEGF-D) and the placental growth factor (PlGF) (71-76). VEGFs can bind to three tyrosine kinase receptors:

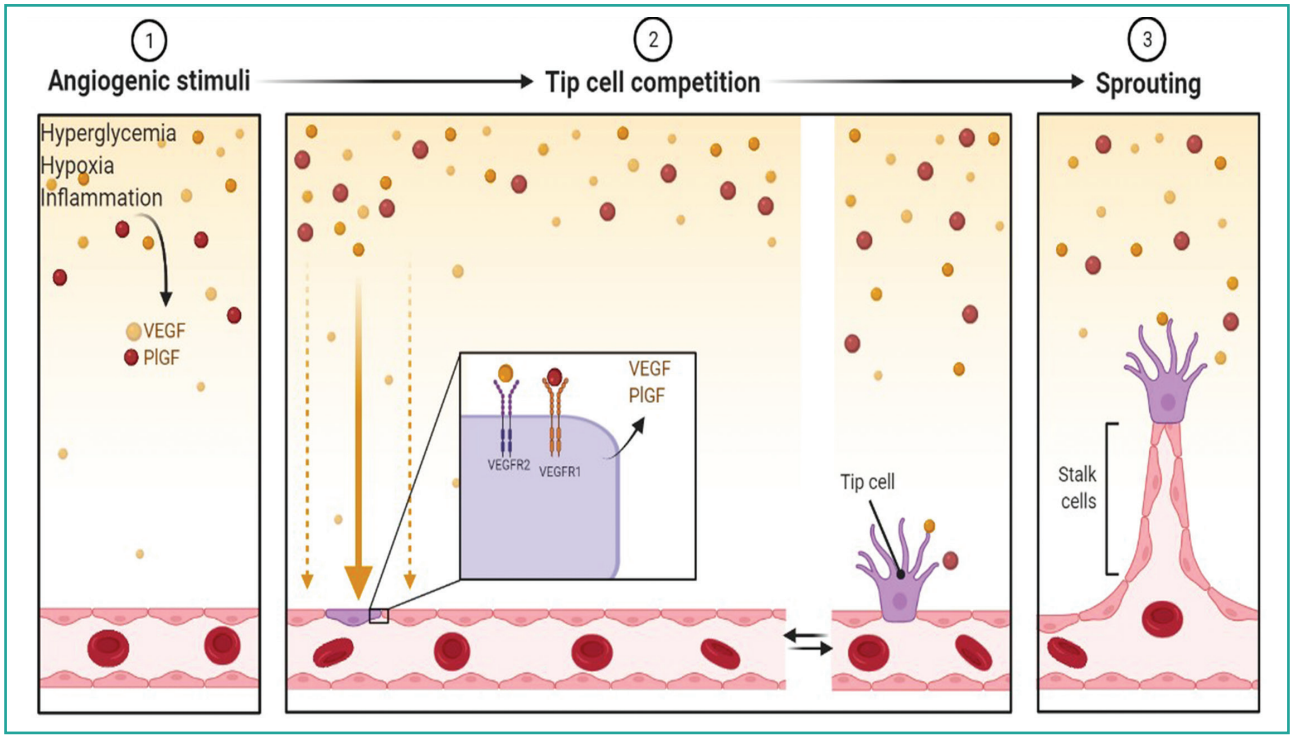

Figure 2. Retinal angiogenesis in diabetic retinopathy.
Angiogenic factors (*i.e.*, VEGF-A and PlGF) stimulate angiogenesis in tissues. VEGF-A/PlGF bind to VEGFR-2/VEGFR-1 on the surface of endothelial cells (ECs), triggering competition between neighboring cells as they differentiate. In normal intact retinal vessels, blood flow is regular and the vascular configuration is stable. However, in diabetic retinopathy, this process is strongly exacerbated by several phenomena, such as hyperglycemia, hypoxia and inflammation. The expression of angiogenic factors is increased and the growth of new blood vessels is uncontrolled. The neovascularization is typical of the later stages of diabetic retinopathy, with the formation of new unstable vessels. This leads to vascular damage, loss of endothelial tight junctions, pericytes detachment and basement membrane thickening (iBRB breakdown).

VEGFR-1 (Flt-1), -2 (KDR), and -3 (Flt-4) (77). VEGFR-1 (78) and VEGFR-2 (79–81) are the main receptors involved in angiogenesis. VEGFR-2 (also known as Flk1) is expressed on endothelial cells. Binding to VEGFR-1 (also known as Flt1) leads to the activation of quiescent endothelial cells and promote vascular permeability (82-85). VEGF-A is significantly increased in ocular tissues from patients with diabetes (86). All the mechanisms linked to the progression of DR, are responsible of the overproduction of VEGF-A, including hypoxic events. Besides stimulation of endothelial cell growth, VEGF-A can also promote the disassembly of junctions between endothelial cells, leading to vascular permeability (BRB breakdown).

Fibrosis

Angiogenesis and subsequent fibrotic events occur with progression of PDR. Fibrosis can cause the formation of fibrovascular epiretinal membranes, which lead to retinal complications such as tractional retinal detachment and, at last, vision loss (87-89). Fibrosis is a complex reparative process that is activated to restore damaged tissue, by means of remodelling extracellular matrix (ECM). Cell proliferation, ECM deposition and neovascularization are key mechanisms during PDR, usually stimulated by pathological conditions like hypoxia or inflammation, promoting formation of fibrotic tissue (90, 91). Along with microglia and astrocytes, Muller cells in response to retinal injury, participate to fibrotic events, through production of inflammatory and angiogenetic mediators (92, 93). Fibrosis can also be promoted by retinal hypoxia, leading to a consequent overproduction of VEGF-A (94-96). Several growth factors play a role in fibrosis, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and the pro-fibrotic connective tissue growth factor (CTGF) (97-99). Precisely, increased levels of CTGF were found in the vitreous of patient with PDR (100, 101) and it has been supposed that CTGF could be a downstream mediator of TGF-β, the main regulator of pro-fibrotic effects.

PHARMACOLOGICAL TREATMENT OF DR

Currently, only PDR can be pharmacologically treated, and no approved treatments are available for NPDR. As mentioned above, the hallmarks of this disease are the abnormal vessel growth in retinal area, up-regulation of inflammatory factors, and the breakdown of the blood-retinal barrier (**figure 3 A**). Clinical history of PDR has been revolutionized with anti-angiogenic treatments, that are invasive and expansive. Along with the anti-VEGF agents (34), anti-inflammatory drugs are also used (102). Steroids are potent drugs to quench inflammation and reduce edema, fibrin deposition, capillary hyperpermeability and phagocytic migration typical of the inflammatory response (103-105). Furthermore, they also counteract the action of VEGF-A (106). Three corticosteroids are actually approved to handle diabetic macular edema (DME): dexamethasone (DEX), fluocinolone acetonide (FA) and triamcinolone acetonide (TA). The limitation of these drugs is related to the side effects such as cataract and rise in intraocular pressure (102).

Anti-VEGF therapy

The anti-VEGF therapies have revolutionized the treatment of DR. These medications, such as ranibizumab (Lucentis, Genentech) and aflibercept (Eylea, Regeneron), called vascular endothelial growth factor inhibitors (anti-VEGF), have a consolidate history in terms of efficacy and safety for the treatment of DME.
Ranibizumab is a 48 kDa antigen-binding fragment (Fab) of a humanized monoclonal antibody with high affinity for VEGF-A (**figure 3 B**) (107); it binds with high affinity all the VEGF-A isoforms (such as VEGF-A_{165}, VEGF-A_{110} and VEGF-A_{121}) reducing the activation of VEGFR-1 and VEGFR-2 receptors. The small size of this fragment enhances its diffusion from the vitreous to the retina and the choroid, improving

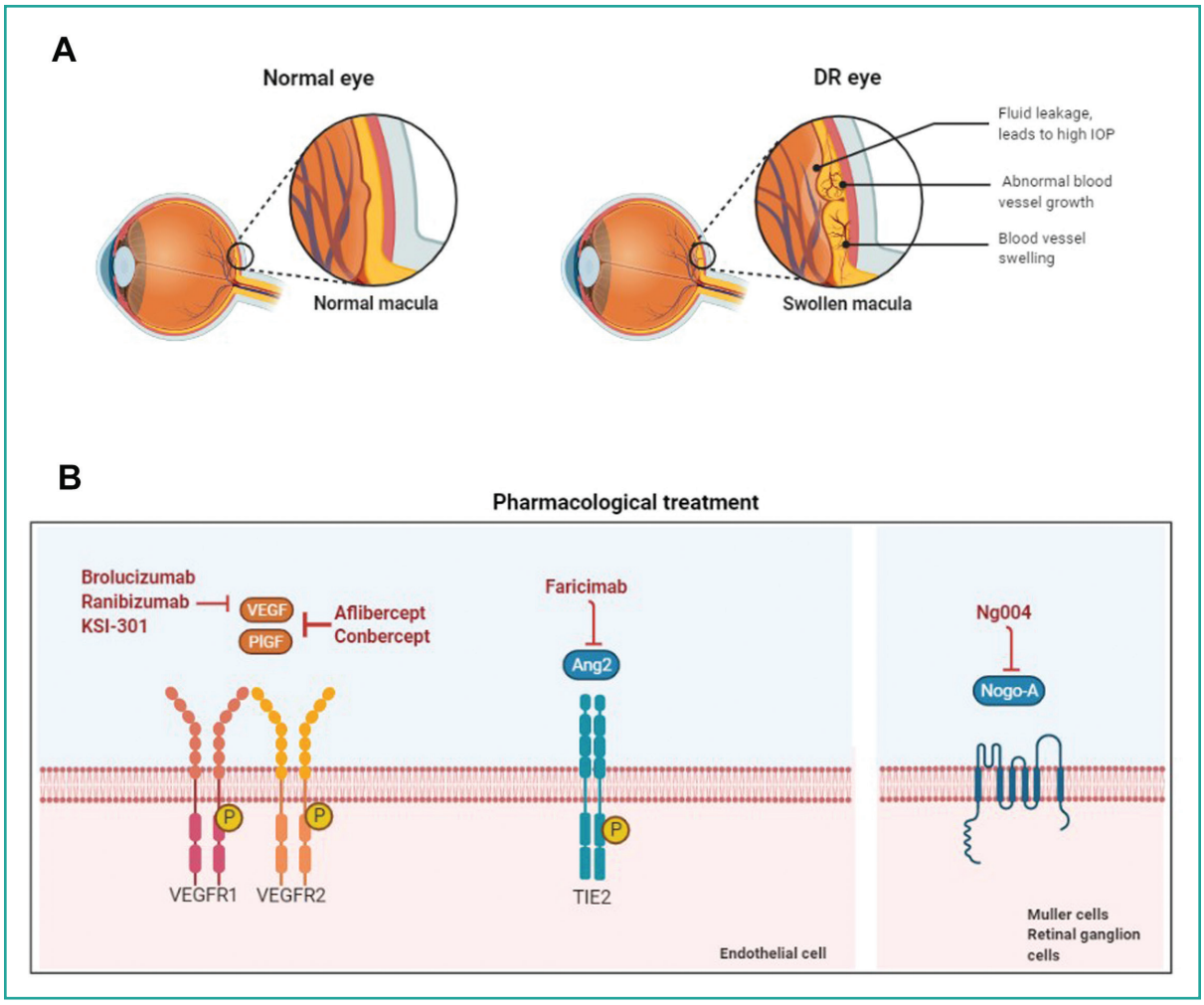

Figure 3. Diabetic retinopathy clinical hallmarks and treatments. **(A)** Diabetic retinopathy is the leading cause of vision loss in diabetic patients and is characterized by abnormal vessel growth in retinal area, inflammation, breakdown of the blood-retinal barrier and fluid accumulation. **(B)** Therapeutic strategies targeting different signalling pathways involved in the pathogenesis of proliferative DR and diabetic macular edema.

the pharmacokinetic profile, compared to bevacizumab (108).

Aflibercept has been approved by Food and Drug Administration (FDA) in 2011 for the treatment of age-related macular degeneration (AMD), for impaired vision due to secondary macular edema, caused by retinal vein occlusion (Branch RVO or central RVO) and for the treatment of visual impairment due to myopic choroidal neovascularization (CNV). Recently, aflibercept was also approved for the treatment of diabetic macular edema. Aflibercept (VEGF-trap) is a fusion protein (115 kDa) bearing two binding domains of VEGF receptors (**figure 3 B**) (109). Moreover, aflibercept's binding affinity to VEGF-A_{165} is almost 100-fold greater than ranibizumab and bevacizumab (110-112), and is the only anti-VEGF agents that binds PlGF, although with lower affinity (38.9 nM dissociation constant – KD), compared to VEGF-A (0.49 nM dissociation constant – KD) (113).

Conbercept (Lumitin) is a 141 kDa recombinant fusion protein composed of the second Ig domain of VEGFR-1 and the third and fourth Ig domains of VEGFR-2, fused to the constant region (Fc) of human IgG1 (**figure 3 B**). Considering the increasing need for less frequent intravitreal injections of anti- VEGF, conbercept

has designed to improve dose regimens and compliance. Similarly to aflibercept, conbercept has multiple targets (114).
In 2021 FDA approved the faricimab (Roche) with the following indications: wet AMD and DME. This antibody targets two different pathways involved in progression of these retinal diseases: angiopoietin-2 (Ang-2) and vascular endothelial growth factor-A (VEGF-A) (**figure 3 B**). Faricimab showed positive results across four phase III studies in AMD and DME. Faricimab clinical trials proved a non-inferiority efficacy evidence, compared to aflibercept (115, 116).
Brolucizumab (Beovu, Novartis), recently approved in US and EU, is a humanized single-chain antibody fragment (scFv) targeting three major isoforms of VEGF-A (*e.g.*, $VEGF_{110}$, $VEGF_{121}$, and $VEGF_{165}$) (**figure 3 B**). Compared with other VEGF-A inhibitors, brolucizumab is smaller (26 kDa). In 2021 Novartis announced the positive results of the Phase III KESTREL study. This study assessed safety and efficacy of 6 mg brolucizumab in patients with DME.
Although most of patients show beneficial effect by the approved anti-VEGF agents, a significant percentage of people are poor responders. To overcome this unmet medical need, NovaGo Therapeutics is developing a first-in-class fully human antibody therapy with a novel mechanism of action (NG004) targeting the protein Nogo-A. This latter is endogenously expressed by Müller cells and retinal ganglion cells, and it represents one of the most up-regulated protein during DR (117, 118). It has been demonstrated that the block of this protein could lead to the reduction of angiogenesis and inflammation, as already demonstrated in an *in vivo* model of retinal injury (excitotoxicity-induced neuroinflammation) (119).
As regards the safety profile of intravitreal anti-VEGF agents some adverse reactions such as endophthalmitis, intraocular inflammation, intraocular pressure elevation and ocular hemorrhage are sometimes associated with the treatment (120-122). Besides that, VEGF protein has a physiological role in the retina, so the prolonged period of treatment with these compounds could be deleterious (123). Moreover, these agents have a short half-life, and the widely treatment schedule is the treat-and-extend regimen with several injections for several months. For these reasons new agents and innovative delivery systems are under investigation.

NOVEL MOLECULAR TARGETS

Placental Growth Factor (PlGF)

PlGF has been implicated in pathological angiogenesis, especially in retinal disorders, although its function is less well understood (85), compared to VEGF-A. Oppositely to VEGF-A, PlGF is not required during physiological angiogenesis but plays a role only during pathological conditions (82, 83, 112, 124-128). Secreted PlGF specifically acts through VEGFR-1. Furthermore, it has been showed that VEGF-A and PlGF can form heterodimers (129) which can bind both VEGFR-1 and VEGFR-2, stimulating endothelial cells migration and vasorelaxation via the nitric oxide pathway (130, 131). Moreover, it has been found that VEGF/PlGF heterodimer can lead to activation of a positive feedback and overproduction of VEGF-A, which binds also the VEGFR-2. Therefore, PlGF may stimulate angiogenesis directly through VEGFR-1 but also indirectly through VEGFR-2 (83, 128). PlGF acts also through neuropilin receptor 1 (NRP1) (124, 127, 132), that is expressed in angiogenic vessels (133, 134). As well as VEGF-A, PlGF is expressed by endothelial cells in hypoxic environment (135-137). The PlGF overexpression is driven by HIF-1α, which is able to recognize a hypoxia responsive element (HRE) located in the second intron of PlGF gene (136). One interesting recent evidence demonstrated that aflibercept and a specific anti-PlGF antibody exert anti-inflammatory effects in the diabetic retina. Specifically, aflibercept and anti-PlGF antibody protected retinal endothelial cells (HRECs) and primary mouse retinal pigmented epithelial

cells (mRPEs) from cell damage induced by high glucose levels, blocking the activation of the ERK pathway with the subsequent suppression of TNF-α release (113).

miRNAs

There is an increasing interest on microRNAs as putative biomarkers for the progression of DR (138). Platania *et al.*, demonstrated that small set of miRNAs were dysregulated in serum and retina of diabetic mice. These miRNAs were also dysregulated in serum of patients with diabetic retinopathy. In the *in-vivo* study, these miRNAs modulated not only VEGF-A expression (up-regulation) but also the neurotrophic factor BDNF (down-regulated) (139). Moreover, Santovito *et al.*, reported that DR is associated with higher circulating levels of miR-25-3p and miR-320b and lower levels of miR-495-3p, in patients with type 2 diabetes and diabetic retinopathy (140). Interestingly, it has been demonstrated a specific association between miRNAs expression and hypoxic microenvironment; in fact, retinal hypoxia led to the upregulation of six miRNAs (miR-20a-5p, miR-20b-5p, miR-27a-3p, miR-27b-3p, miR-206-3p, miR-381-3p) in human retinal endothelial cells. These miRNAs, are capable to interfere with the expression of genes belonging to the TGF-β pathway at post-transcriptional level. In fact, the dysregulation of these miRNAs has driven and promoted angiogenesis and fibrosis, through the modulation of VEGF-A, TGF-β and HIF-1α, in retinal endothelial cells (135, 141-144). Moreover, Shao *et al.* identified miR-136 and miR-374 dysregulation as hallmark of proliferative DR (145). The putative involvement of miRNAs in the pathogenesis of DR is also linked to the direct activation of the inflammatory pathway through the TLR-4, as well demonstrated in several *in vitro* and *in vivo* models of DR; in fact, different miRNAs are associated with the regulation of TLR-4 expression during diabetic retinopathy (146-148). Currently, evidence about post-transcriptional regulation of PlGF expression by miRNAs has not been retrieved. A high-throughput screening of miR-

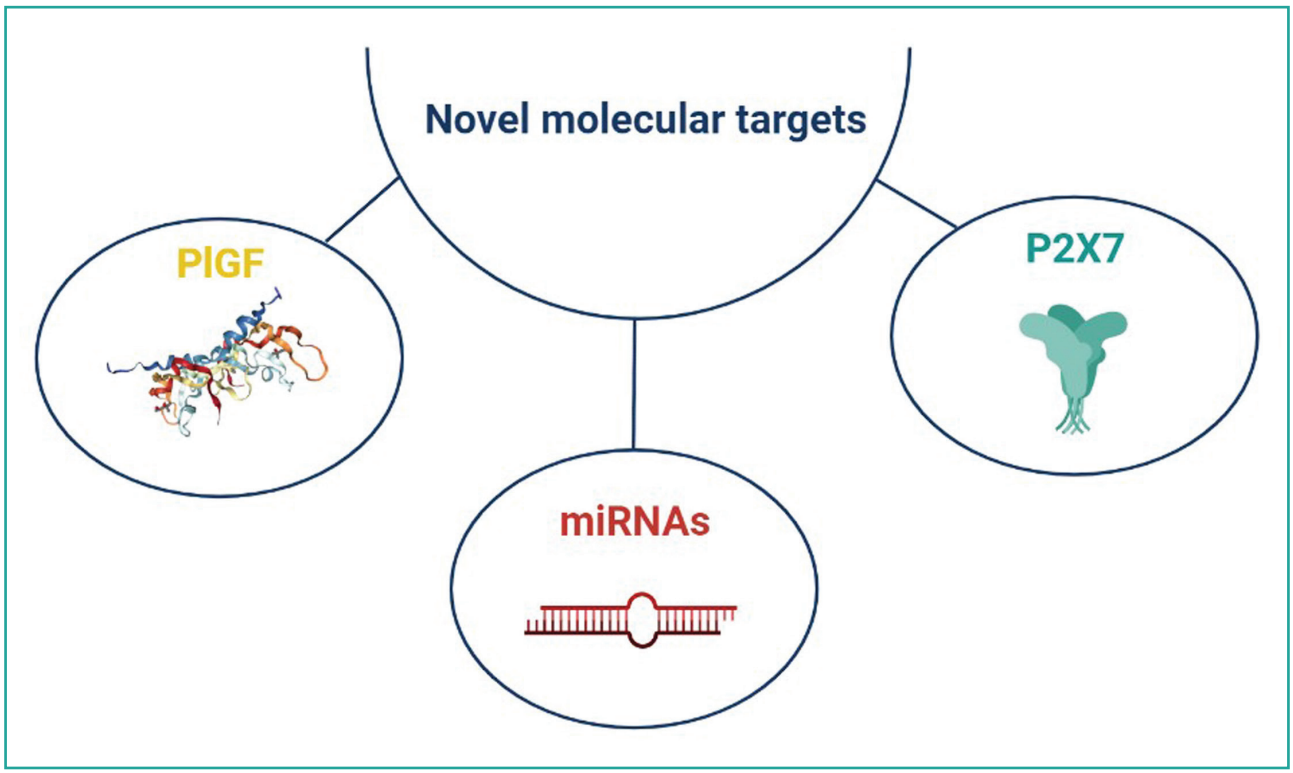

Figure 4. Novel molecular targets for the treatment of diabetic retinopathy.

NAs potentially related to PlGF would address this issue.

P2X7 receptor

In the last decade an important link between DR and purinergic receptor has been demonstrated, considering P2X7 receptor as a putative pharmacological target in this retinal disease (149-151). P2X7R is a member of the family of purinoceptors, ligand-gated membrane ion channels activated by extracellular ATP. This receptor is widely distributed in all retinal layers and also in retinal microvasculature. P2X7R stimulation promotes a wide range of cellular responses, ranging from proliferation to cell death, from cytokines release to reactive oxygen species (ROS) production. The early up-regulation and activation of P2X7R has been related to several types of retinal diseases, and its antagonism revealed benefits against inflammation, oxidative stress and angiogenesis, both *in vitro* and *in vivo* studies (152-156). In particular, the selective antagonist of this receptor (JNJ47965567) has shown anti-inflammatory effect, through the decreased activation of inflammasome and IL-1β production, in several pathological conditions. Moreover, P2X7R inhibition up-regulated the expression of junction proteins in the iBRB, which is compromised in early DR (156, 157). Furthermore, it was found a significant activation of P2X7R during retinal hypoxia, and the P2X7R blockade, by selective P2X7R antagonists A740003 and AZ10606120, inhibited the HIF-1α and VEGF-A retinal overexpression (151, 158).

CONCLUSIONS

Increasing evidence suggest that retinal neurodegeneration and inflammation are implicated in the pathogenesis of diabetic retinopathy. Several recent studies were carried out to explore new pharmacological targets, potentially able to counteract retinal neurodegeneration and inflammation. The present review summarizes new hints and puzzle pieces about the etiopathogenesis of DR, addressing several hypotheses and trying to identify and validate novel and promising pathways implicated in this pathology. Currently, the first-line pharmacological therapy for PDR and DME is represented by intravitreal injection of anti-VEGF agents and corticosteroids, respectively. However, the current proliferative diabetic retinopathy pharmacotherapy is characterized by frequent, invasive and expensive treatments, that have a significant impact on health system. There are several unmet medical needs in the management of DR that stimulate the pharmacological research to develop novel pharmacological targets and drugs to counteract the early phases of diabetic retinopathy.

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ETHICS

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Conflict of interests

The authors declare that they have no conflict of interests.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Availability of data and materials

N/A

Ethical approval

N/A

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