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Microvascular complications and diabetic retinopathy: recent advances and future implications

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Abstract

Retinal microvascular alterations have been observed during diabetic retinopathy (DR) due to the retinal susceptibility towards subtle pathological alterations. Therefore, retinal microvascular pathology is essential to understand the nature of retinal degenerations during DR. In this review, the role of retinal microvasculature complications during progression of DR, along with recent efforts to normalize such alterations for better therapeutic outcome, will be underlined. In addition, current therapeutics and future directions for advancement of standard treatment for DR patients will be discussed.

Diabetic retinopathy (DR) is a nonproliferative (intraretinal microvascular changes) or proliferative (growth of abnormal new blood vessels) manifestation of retina and it is the most significant complication of the eye. Retinal microvascular alterations have been observed during DR due to the retinal susceptibility towards subtle pathological alterations [1]. Therefore, retinal microvascular pathology is essential to understand the nature of retinal degenerations during DR. Retinal microvascular dysfunction in diabetes is clinically characterized by microaneurysms, hemorrhages, lipid exudates, macular edema, capillary occlusion, cotton-wool spots and finally neovascularization, and these groups of retinal abnormalities are called as DR [2]. The typical treatment choice of DR neovascularization with laser photocoagulation does not have a significant improvement in visual acuity for a longer period of time. Moreover, various novel pharmacological therapies to target the essential biochemical mechanisms that produce DR are also being assessed in order to reduce the limitations of current treatment options [3]. In this review, the role of retinal **microvasculature** complications during progression of DR along with recent efforts to normalize such alterations significantly affects for better therapeutic outcome will be outlined. Current therapeutics and future directions for advancement of standard treatment for DR patients will also be discussed.

Vascular degeneration in DR

It has been evident that one of the earliest abnormalities observed in DR is the reduction of retinal perfusion due to the constriction of major arteries and arterioles [4,5]. This dampened

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retinal blood supply results in a series of biochemical and metabolic alterations, which further stimulate cellular signaling cascades. The earliest induction of cellular signaling pathway includes activation of several PKC isoforms (e.g., PKC- α , β , γ and δ) among which the PKC δ isoform is preferentially stimulated in DR [6]. This event eventually elevates vascular permeability, blood–retinal barrier damage and loss of endothelial tight junctions [4,7]. Moreover, dysfunctioning of ionic channels located in the retinal arteriolar vascular smooth muscle cells (BK channels), also causes retinal vasoconstriction during early phase of DR. Therefore, BK channel dysfunctioning represents an important mechanism underlying the hypoperfusion in DR [1,8]. In addition to the above alterations, retinal pericytes loss is another characteristic feature of DR causing endothelial cell degeneration, microvascular destabilization and perfusion alterations [4,9,10]. Pericyte loss has been linked to PKC activation and PDGF inhibition [11]. Moreover, development of chronic inflammation eventually causes capillary obstruction and retinal leukostasis due to an overexpression of retinal intercellular adhesion molecule 1 and CD18 [12,13]. Altogether, a retinal perfusion deficit develops and the retinal oxygenation, which ultimately causes progression of retinal hypoxia [1,14]. Furthermore, enhanced expression of VEGF attributed to hypoxia and secretion of various pro-inflammatory cytokines (TNF- α , IL-6 and IL-1) are other major alterations caused during progression of DR [12,13]. In response to the above changes, thickening of the retinal capillary basement membrane occurs due to overexpression of **fibronectin**, collagen IV and laminin, which causes alterations in vascular integrity [15,16]. Furthermore, in hyperglycemic conditions, retinal mitochondria become dysfunctional and levels of superoxide species are overwhelmed, which eventually accelerate cytochrome c release (mitochondria to cytoplasm), Bax translocation (cytoplasm to mitochondria), capillary cell apoptosis and DNA damage [17]. Overall, alterations in pericyte coverage and basement membrane architecture cause vascular degenerations and mitochondrial dysfunctions modulate retinal capillary cell apoptosis in progressive DR (Figure 1). In the following section, the current as well as future therapies for the treatment of DR will be discussed.

Current therapies

Anti-VEGF therapy

Several molecules have been implicated in neovascular diseases however, VEGF appears to play a central role in the pathogenesis of DR [18–21]. Elevated levels of VEGF have been reported in the ocular fluid in patients with progressive DR as compared with normal eye [22]. The aqueous VEGF levels have demonstrated strong correlation with the severity of retinopathy and these observations were found statistically significant compared with normal eyes [23]. Moreover, reduced retinal and iris neovascularization along with pre- and post-operative vitreous hemorrhage have been observed in many patients in response to VEGF inhibition during ongoing clinical trials [24,25]. These observations clearly suggest the potential role of anti-VEGF therapy in the treatment of DR. An overview of three important anti-VEGF agents currently used in the treatment of DR is presented below.

Bevacizumab—Bevacizumab (AvastinTM, Genentech Inc., CA, USA) is a full-length recombinant humanized antibody (149 kDa), with 93% of its amino acid sequence is derived from human IgG, which binds to all subtypes of VEGF efficiently. It is a US FDA approved anti-VEGF agent used for the treatment of various cancers such as colorectal cancer, non-squamous non-small-cell lung cancer, and advanced breast and brain cancer. However, the off-label use of bevacizumab includes intravitreal administration to successfully treat neovascular age-related macular degeneration [26], macular edema from nonischemic central retinal vein occlusion [27], iris neovascularization [28] and pseudophakic cystoid macular edema [29]. A clinical trial of bevacizumab for the treatment of DR reports

significant improvement in the visual acuity with no ocular or systemic adverse events following intravitreal injection (6.2 µg–1.25 mg) via the pars plana [30]. A complete resolution of neovascularization elsewhere, neovascularization of the disc and iris neovascularization were observed in 59% (20 out of 34 eyes), 73% (19 out of 26 eyes) and 82% (9 out of 11 eyes) patients, respectively. This study suggests that short-term intravitreal injection of bevacizumab is well tolerated and associated with a rapid improvement from retinal and iris neovascularization, which ultimately leads to reduction in progressive DR. Moreover, bevacizumab has demonstrated significant biological activity even with the lowest dose (6.2 µg) tested, and thus, possibilities of systemic side effect associated with high dose (1.25 mg) can be avoided. However, there is a need to conduct elaborated clinical studies to confirm the above conclusion[30]. In another clinical trial, more than 11% reduction in central subfield thickness was observed during the third week in 43% (36 out of 84) bevacizumab-treated and 28% (5 out of 18) laser treated eyes, which was further reduced to 37% (31 out of 84) and 50% (9 out of 18), respectively, during the sixth week of treatment [31]. Besides several drawbacks, including retrospective nature, limited follow-up and small number of patients, the above study demonstrates only short-term efficacy of bevacizumab in patients with progressive DR. However, the long-term results of bevacizumab therapy in DR are still not known due to the lack of appropriate clinical trials. Therefore, caution should be taken until the availability of sufficient reports showing safety, dosing, efficacy and duration of effect following intravitreal bevacizumab in the treatment of progressive DR.

Ranibizumab—Ranibizumab (Lucentis™, Genentech, Inc., CA, USA), unlike bevacizumab, which is a full humanized antibody, is a recombinant humanized monoclonal antibody fragment that is also active against all isoforms of VEGF_A. It has been reported that ranibizumab is more effective in improving visual acuity when used in conjunction with laser photocoagulation therapy for the treatment DR [201]. According to Phase II clinical trials conducted by Genentech for assessing the efficacy and safety of ranibizumab for 24 months, significant and rapid improvement was observed in conditions associated with DR which includes improvement in:

- Patient's visual acuity (VA) (able to read at least 15 additional letters: 18.1% of patients in the sham injections vs 44.8% of patients in the 0.3-mg ranibizumab dose group and 39.2% in the 0.5-mg ranibizumab dose group, respectively);
- Abnormal growth of new blood vessels inside eye (retinal neovascularization: 13.8% of patients in the sham injections vs 0.8% of patients in the 0.3-mg ranibizumab dose group and 4.0% in the 0.5-mg ranibizumab dose group, respectively);
- Bleeding inside the eye (vitreous hemorrhage: 13% of patients in the sham injection vs 3.2% in both of the ranibizumab dose groups).

Moreover, expected ocular side effects such as traumatic cataracts, bacterial eye infection, elevated intra ocular pressure (IOP), and retinal detachment were found negligible or insignificant [202]. Overall, ranibizumab appeared as a potential candidate for the treatment of DR due to its efficacy in improving the visual acuity and lowering the VEGF levels.

Pegaptinib sodium—Pegaptinib sodium (Macugen™ (OSI), Eyetech Pharmaceutical, Inc., NY, USA) is a PEGylated neutralizing RNA aptamer. It binds with high specificity to VEGF₁₆₅ (VEGF isomer), a protein that plays a critical role in angiogenesis and also increases blood vessels permeability/leakage, which are two of the primary pathological processes responsible for the vision loss in progressive DR. Pegaptinib sodium was approved in December 2004 by the FDA for the treatment of all types of neovascular age-

related macular degeneration, regardless of lesion subtype or size. In one of the Phase II clinical trials, where the aim was to evaluate the safety and efficacy of pegaptinib sodium injection for the treatment of diabetic macular edema (DME) in DR patients, it was observed that intravitreal injection of pegaptinib sodium (0.3 mg) caused significant regression of neovascularization and DME in patients with advanced DR [32]. In this study, 169 individuals participated and the subdivision was according to 0.3 mg pegaptinib, (n = 44), 1 mg pegaptinib (n = 42), 3 mg pegaptinib (n = 42) and sham group (n = 41). When all the subgroups of pegaptinib-treated participants were compared with sham for improvement in VA, a significant numbers of patients gained two and three lines of VA. Furthermore, an improvement in VA was also accompanied by improvement in retinal thickness especially with the 0.3-mg pegaptinib group ($75\text{ }\mu\text{m}$ in 49% patients compare to 19% in sham, whereas $100\text{ }\mu\text{m}$ in 42% patients compare to 16% in sham). In all the subgroups of pegaptinib-treated participants, no evidence of cataract formation/ progression or sustained IOP elevation with minor ocular adverse reactions (largely transient, injection procedure related and mild to moderate in character) was observed [32]. In another efficacy study conducted by Eyetech Pharmaceutical Inc. on 1208 patients, it was observed that loss of less than 15 letters of VA between base line and week 54 was approximately 55% in sham group, whereas with 0.3-, 1- and 3-mg dose of pegaptinib were found to be approximately 65–70% [33]. Moreover, VEGF Inhibition Study in Ocular Neovascularization trial pivotal Phase III studies indicated that intravitreal injection of pegaptinib causes significant clinical benefit with selective VEGF blockade and is a safe and efficacious choice for the treatment of progressive DR and age-related macular degeneration [33,34].

Long-acting steroids

Various clinical studies of corticosteroids to treat DR-induced macular edema are under way. A brief summary of steroids used for the treatment of DME is presented below.

Dexamethasone—Dexamethasone (9-fluoro-11 β , 17, 21-trihydroxy-16 α -methylpregna-1, 4-diene-3, 20-dione) is one of the most widely used anti-inflammatory and angiostatic agent. Dexamethasone is able to reduce intracellular as well as extracellular edema, and also suppresses the macrophage, lymphokine, angiogenic growth factors, such as VEGF and other inflammatory mediators. Due to wide applications, dexamethasone appeared as a potential candidate for the treatment of DME. In one clinical trial for evaluating the safety and efficacy of intravitreal dexamethasone implant (OzurdexTM, 0.7 mg) for the treatment of DME, significant (p = 0.004) changes in the retinal thickness was observed in the dexamethasone-treated group compared with placebo at the end of week 26. Moreover, at week 8, 30.4% of patients gained ≥ 10 letters in best-corrected VA. The study concluded that dexamethasone demonstrates statistically and clinically significant improvements in both vision and vascular complications of DME with acceptable safety profile [35]. In another clinical study, 171 eyes with persistent DME of ≥ 90 days were treated with either 350- μg or 750- μg dexamethasone have exhibited significant improvement in visual activity (≥ 10 letters) and reduction in central foveal thickness [36]. However at 180 days, no significant difference in improvement of visual activity or reduction in DME was observed between the treatment and observation group. Moreover, slower elevation of IOP was also reported in both treatment groups.

Triamcinolone acetonide—Triamcinolone acetonide is a synthetic steroid used for the treatment of DME to downregulate the expression of VEGF- and Flk-1 [37]. Clinical trial for evaluating the efficacy and safety of intravitreal triamcinolone acetonide (4 mg) for refractory diffuse DME shows significant (p = 0.005) reduction in central macular thickness compared with placebo. This study concluded that single intravitreal injection of triamcinolone acetonide (4 mg) efficiently decreases macular thickening for short term due

to diffuse DME [38]. In a recently conducted clinical trial, 840 eyes of 693 subjects with DME were randomized to bolus intravitreal triamcinolone (1 or 4 mg) or focal or grid laser treatment [39,40]. Treatment with bolus intravitreal triamcinolone did not demonstrate any long-term benefits over focal or grid laser treatment as an outcome of high triamcinolone exposure to the eyes. Bolus delivery of intravitreal triamcinolone has demonstrated elevation of IOP and higher risk of cataract formation, which was not observed in laser treatment groups. In order to reduce the risk associated with IOP and cataract, a formulation with sustained delivery of steroids need to be developed.

PKC inhibitors

Protein kinases are important proteins to regulate a wide variety of signaling pathways in humans. It was reported that animals overexpressing PKC- in vascular tissues eventually develop retinal blood vessels abnormalities similar to those observed in DR patients [41]. To date the following two PKC inhibitors have demonstrated clinical potential for the treatment of DR.

LY333531—LY333531 (Ruboxistaurin mesylate, Eli Lilly, IN, USA) was found to ameliorate the vascular complications of diabetes, which includes abnormalities in retinal blood flow, neovascularization and VEGF-mediated effects on permeability in animal models [42]. During the animal study it was found that LY333531 provides competitive reversible inhibition of PKC- 1 and - 2, with value of approximately 5 nM, which was an IC₅₀ significantly lower than other PKC inhibitors [42]. Moreover, the results from PKC-inhibitor demonstrated that drug reduces the incidence of visual loss by 40% and increases the visual improvement (two-fold) in patients with moderately severe to severe nonproliferative DR. Overall, LY333531-based animal studies have demonstrated improved vascular complications, retinal blood flow abnormalities and its ability to reach the human retina in bio-effective concentration. This has led to the discovery of the first oral pharmacologic agent to reduce the visual loss in diabetic patients over an extended period [43,44]. Recently published results of Phase III clinical trials carried out with 32-mg/day oral dose of ruboxistaurin reported significant reduction in sustained moderate visual loss relative to the placebo group [45]. Results from similar Phase III clinical trials of ruboxistaurin combined with previous studies have further confirmed significance of ruboxistaurin in reduction of vision loss, most likely due to its effects on macular edema [46].

PKC 412—Another PKC inhibitor, PKC 412, has demonstrated its potential to become a good option for the treatment of DR induced macular edema. A Phase II clinical studies for evaluating efficacy and safety of PKC 412 on 141 patients shows significant improvement in retinal thickness (assessed using optical coherence tomography) and visual acuity at the dose of 100 mg/day and 150 mg/day following oral administration for 3 months [47].

Anti-oxidant therapy

High glucose content is one of the major risk factors that has been directly correlated with the development of various vascular dysfunctions in DR. A higher production of reactive oxygen species (ROS) [48] and inflammatory markers [49,50] have been shown to be associated with the high glucose concentration [51]. Recent reports have suggested that high glucose-generated ROS-mediated oxidative stress may play a major role in the progression of DR and- therefore, clinical use of antioxidants may have a great potential in the treatment of DR [51]. Kowluru *et al.* have described that several diabetes-induced retinal abnormalities, which are postulated in the development of retinopathy, are influenced by oxidative stress, and are considered to be interrelated [51].

Lipoic acid—Lipoic acid (LA), a disulfide derivative of octanoic acid, is able to alter the redox status of cells and interact with thiols and other antioxidants [52]. In addition to its antioxidant properties, LA improves the glucose uptake, a mechanism similar to insulin-stimulated glucose uptake [52]. During DR, in addition to elevated super-oxide levels, retina becomes dysfunctional due to increased pro-apoptotic protein and cytochrome c release from the mitochondria. This type of phenomenon usually leads to the increased retinal capillary cell apoptosis [53]. LA has been demonstrated to reduce:

- Retinal capillary cell apoptosis;
- Diabetic-induced nitrotyrosine elevation;
- Mitochondrial overproduction of ROS;
- Retinal mitochondrial and cytosolic ratios of NAD⁺ to NADH in rat DR model.

Moreover, LA has not only demonstrated its ability to reduce the VEGF levels but it also preserves the pericyte coverage of retinal capillaries, which may provide additional endothelial protection in rat DR model [54–56].

Calcium dobesilate—Calcium dobesilate (CaD), 2,5-dihydroxybenzene sulfonate, has been widely prescribed in the treatment of DR and exerts its pharmacological effect via improving the capillary permeability [57,58]. In a clinical study conducted by the DX-retinopathy study group on total of 194 patients (69 on CaD and 68 on placebo), significant difference was observed between CaD versus placebo ($p = 0.002$) treated patients (2-g daily for 24 months) [59]. The results clearly suggest that CaD significantly lowered the permeability of retinal capillary and shows significant beneficial effect in controlling hemorrhages, capillary leakage and progression of DR.

Current therapies: summary & limitations

Although anti-VEGF therapies have shown promising therapeutic benefits in DR, such as regression of retinal neovascularization, and has also overcome the need of surgical intervention. The major limitation of anti-VEGF therapy is recurrence of proliferation upon treatment discontinuation. Moreover, retinal detachment and systemic ‘off-target’ effects are another critical issues associated with anti-VEGF therapies. The use of long-acting steroids has also emerged as a potential therapy targeting the inflammatory cascade of DR. However, adverse events such as elevated intraocular pressure and increased incidence of cataracts are major limitation of corticosteroids.

Detailed understanding of microvascular dysfunctioning in DR should help to develop advanced therapies which must display therapeutic effects (neovascularization reduction) without interrupting normal vasculature. Several promising preclinical advances and innovative targets may offer exciting opportunity to medicinal chemists for developing ‘next-generation’ medicine that will considerably improve the current standards of care and therapeutic outcomes.

Future therapies

Microtubule depolymerizing vascular-disrupting agents

Vasculature targeting is a promising approach for treating DR. In DR, newly formed vessels proliferate very rapidly resulting in frail, thin vessels with minimal smooth muscle reinforcement. Vascular-disrupting agents (VDAs) can selectively target highly proliferative vasculature relative to normal vessels due to the fundamental structure divergences. *Cis*-combretastatin A4 phosphate (fosbretabulin, CA4-P), an inactive prodrug converts to combretastatin A4 (Figure 2) in presence of endogenous nonspecific phosphatases [60].

Fosbretabulin binds to the colchicine-binding site on α -tubulin subunits and hampers microtubule polymerization in highly proliferative endothelial cells [61,62]. In mice model, CA4-P has demonstrated successful regression of choroidal as well as retinal neovascularization. Phase II clinical trials are in progress to evaluate safety and efficacy of CA4-P in patients with polypoidal choroidal vasculopathy [203] and choroidal neovascularization (CNV) [204]. Newly synthesized, colchicine site targeted drug C9 (Figure 3) exhibited excellent anti-angiogenic and vasculature disrupting properties due to down-regulation of Raf-MEK-ERK signaling molecules [63]. Recently developed Ac-EEED peptide interrupts interaction of actin with actin-binding protein. Ac-EEED fusion peptide is likely to interfere with mitogenic, adhesion and differentiation signals of the cells. In addition, one report has suggested that Ac-EEED-induced cytoskeletal alterations in pericytes in the CNV model would compromise survival, migration and adhesion of growing vascular smooth muscle cells and pericytes [64]. Endogenous iso-actin specific control protein, α -actinin-4 have been studied as a target for cytoskeletal remodeling. Overexpression of α -actinin-4 in capillary endothelial cells inhibited cell migration during *in vitro* wound healing study. Moreover, it has restrained capillary formation and demonstrated collapse of performed endothelial tubes during *in vitro* angiogenesis assay [65]. Microtubule depolymerizing VDAs also demonstrate anti-angiogenic activities with endothelial cells responsive to these drugs [66]. The idea of co-administration of a VDA along with other anti-angiogenic agents may become an attractive alternative for non-cancerous pathologies distinguished by excessive angiogenesis, such as DR. However, early-phase clinical trials of VDAs have revealed cardiovascular toxicity profiles, therefore, careful recognition of cardiovascular risk factors is necessary [67].

Pro-angiogenic factors

PDGF—Intraocular delivery of anti-VEGF agents only partially inhibits retinal neovascularization, which indicates significant role of other growth factors in the development of retinal neovascularization. Reports suggest that PDGF- β is a potent chemo-attractant of retinal pigment epithelial cells and its effect is further synergized in presence of fibronectin [68]. Overexpression of PDGF- β in retina causes intense retinal detachment and proliferative retinopathy, similar to an advanced stage of DR [69]. PDGF- β is one key molecule involved in recruitment, survival and proliferation of pericytes [9,70,71]. Recently published results demonstrated that partially selective kinase inhibitor, PKC412 (Figure 4) inhibited phosphorylation by PDGF and VEGF receptors and it also inhibited iso-forms of PKC [72]. These inhibitions exhibited effect. However, in a complete abolition of retinal neovascularization. However, no significant reduction in retinal neovascularization was observed when CGP57148 and CGP53716, selective inhibitors of phosphorylation by the PDGF receptor, were utilized [72]. Various kinase inhibitors have been investigated for the simultaneous inhibition of both VEGF and PDGF receptors for the treatment of ocular neovascularization [73,74]. A PEGylated aptamer, E10030 selectively inhibits PDGF- β and stimulates stripping of pericytes from the endothelial cells [75]. It improves sensitivity of mature vasculature for anti-VEGF treatment. Therefore, simultaneous administration of anti-VEGF agents along with E10030 may inhibit CNV. In fact, in Phase I clinical trials, 90% of the subjects have observed CNV regression when treated with ranibizumab and E10030, relative to 10% of the patients treated with ranibizumab alone [75,76]. A Phase II clinical study is currently under progress [205].

IGF-1—IGF-1 has been substantially evaluated for its role in the development of retinal neovascularization in DR [77,78]. Overexpression of IGF-1 in transgenic mice demonstrated similar alterations as human DR, such as thickening of capillary basement membrane and loss of pericytes [79]. A specific IGF-1 antagonist has significantly suppressed retinal neovascularization in animal models [77]. Reports suggest that IGF-1 upregulates

expression of HIF-1, a transactivator of VEGF gene. HIF-1 not only contributes in the overexpression of VEGF but also upregulates other promoter gene, such as Ang-2. Ang-2 participates in angiogenesis by enhancing sensitivity of retinal capillaries towards the VEGF [80]. According to this pathway, IGF-1 exhibits its proangiogenic activity via VEGF, therefore, co-administration of IGF-1 antagonist along with anti-VEGF agent may not offer recently published report, involvement of IGF-1 in DR via a different pathway has been discussed. IGF-1 enhances Akt/GSK-3 signaling cAMP responsive transcription factor CREB phosphorylation, which alters expression of ECM/adhesion molecules resulting in retinal capillary endothelial dysfunction, blood–retinal-barrier breakdown and vessel leakage in DR [81]. Therefore, treatment of DR by co-administration of VEGF and IGF-1 antagonists could be beneficial.

FGF-2—Folkman and coworkers have identified FGF-2 with the molecular weight of 14.8 kDa, which has not only stimulated *in vitro* endothelial cell proliferation, but has also enhanced angiogenesis *in vivo* [82,83]. FGF-2 stimulates angiogenesis by activating endothelial cell process, such as cell migration, proteolytic enzyme secretion and tube formation.[84–86]. However, the role of FGF-2 in retinal neovascularization is debatable. FGF-2 deficient mice did not show any significant changes in the area of retinal vasculature relative to the wild type mice. Similarly, no significant difference in the levels of neovascularization was observed when retinas of both mice models were made ischemic. Transgenic mice (rho/FGF-2) with overexpression of FGF-2 in photoreceptor cells did not show retinal neovascularization, which indicates that FGF-2 expression is not sufficient or necessary for the development of neovascularization [87,88]. However, investigators demonstrated that ruptured Bruch's membrane or photoreceptor cells or both in transgenic mice exhibited significantly higher area of CNV relative to the wild-type mice [89]. These results suggested that only in the case of cell injuries, FGF-2 stimulates neovascularization but not in the normal conditions.

Extracellular matrix molecules

In last decade, participation of extracellular matrix molecules (ECM) in a process of angiogenesis has been widely studied. ECM molecules, such as fibronectin, directly bind to the endothelial cell transmembrane receptors (integrins), via a specific binding sequence (Arg-Gly-Asp) and modulate the behavior of cells [90]. Upregulation of various integrin receptors on neovascularization participating endothelial cells, such as $\alpha 5 \beta 1$, $\alpha v \beta 3$ and $\alpha v \beta 5$, have been reported and their contribution in the ocular neovascularization have also been confirmed [91–93]. Intense staining signals for $\alpha v \beta 3$ in the retinas of patients with DR were observed relative to no detectable signals in normal human retinas. Intraperitoneal or periocular delivery of $\alpha v \beta 3$ selective antagonist exhibited significant inhibition of retinal neovascularization in the murine model [93]. Systemic delivery of integrin receptor $\alpha 5 \beta 1$ antagonist demonstrated suppression and regression of CNV [91]. JSM6427 exhibited selective affinity towards $\alpha 5 \beta 1$ in monkey and rabbit animal models and showed significant reduction in CNV[94]. Volociximab, a monoclonal antibody designed to bind with $\alpha 5 \beta 1$ showed significant reduction in CNV [95]. Phase I clinical trials for the safety and tolerability of JSM6427 and volociximab are currently underway [206, 207]. Reports suggest that ECM molecules may bind with soluble factors and proteolytic enzymes can degrade these complexes to reactivate ECM molecules [96–98]. Degradation products of ECM also possess anti-angiogenic activity. Various collagen fragments, such as restin [99], endostatin [100], canstatin [101] and arresten [102] with anti-angiogenic activity have been results indicate that selective integrins antagonists may prove a beneficial adjunct to the main stream treatment of the DR.

Other endogenous neovascularization inhibitors

In addition of anti-angiogenic ECM molecules, several other endogenous anti-angiogenic molecules, such as PEDF [103–106], angiostatin [107], vasohibin [108] and **thrombospondin** [109,110] have been identified. In Phase I clinical trials, intravitreal delivery of adenoviral vector expressing human PEDF (AdPEDF.11) exhibited an impressive safety profile along with anti-angiogenic activity [111]. Vasohibin is an endogenous negative feedback regulator protein, which inhibits angiogenesis in an autocrine manner. In endothelial cells VEGF and FGF-2 upregulates KIAA1036 mRNA responsible for synthesis of vasohibin [108]. Vasohibin has significantly inhibited VEGF and FGF-2 stimulated angiogenesis in animal model. Elevated levels of VEGF in ischemic retinopathy also demonstrated higher levels of vasohibin mRNA [112]. Expression of vasohibin mRNA was reduced with the blocking of VEGF mRNA using VEGF siRNA, which helped to understand that upregulation of vasohibin mRNA was due to higher levels of VEGF and not because of some other features of retinopathy. Overexpression of vasohibin caused neovascularization reduction and opposite scenario (knockdown of vasohibin) has showed enhanced vascularization. The administration of exogenous, vasohibin-1 has strongly reduced angiogenesis without any adverse effects [113–115]. Using vector systems several other proteins with choroidal and/or retinal neovascularization inhibitory effects have been evaluated including soluble VEGF receptor-1 (Flt-1) [116–118], soluble Tie2 [119], tissue inhibitor of metalloproteinases-3 [120] and endostatin [121,122].

Treatments targeting mitochondrial dysfunction

In hyperglycemic conditions, retinal mitochondria has become dysfunctional and levels of super-oxide species are overwhelmed, which eventually accelerate apoptosis of capillary cells. Twofold higher levels of superoxide were observed in retinal mitochondria of wild-type diabetic mice relative to nondiabetic mice [123]. Reduction in levels of superoxide reduces leakage of cytochrome c and Bax in cytosol, which eventually prevents apoptosis in both pericytes and endothelial cells [124]. Mice overexpressing mitochondrial super-oxide dismutase (MnSOD) demonstrated significant inhibition of diabetes induced mitochondrial superoxide. MnSOD mimic agent, MnTBAP (Figure 5) abolished mitochondrial dysfunction and inhibited further development of DR [125]. Role of MMP2 in the development of DR has also been investigated. The administration of MMP2 siRNA showed significant inhibition of glucose induced MMP2. In addition, leakage of mitochondrial cytochrome c in to cytosol was also reduced [126]. Several other, MnSOD mimetic agents, such as tempol [127] and M40403 (imisopasem manganese) (Figure 6) [128], have also been studied in a rat animal model. Increased peroxynitrite via breaking of DNA single strand has led to the activation of poly (ADP ribose) polymerase, which plays a crucial role in the development of DR [129–131]. Administration of a potent PARA inhibitor, PJ-34 (Figure 7), in diabetic rats, has significantly abolished diabetes induced retinal microvascular cell death and progression of early lesions of DR [132]. Treatment of diabetic rats with FP-15 (a potent peroxynitrite decomposition compound) (Figure 8) demonstrated reduction of leukocyte entrapment in retinal microcirculation during the early diabetic period [130].

Nonselective treatments

Polyamines are very important for the growth of proliferating cells. Polyamine analogues have been studied in the treatment of diseases with excessive proliferation, such as DR. Polyamine antagonists interfere with the biosynthesis and metabolism of polyamine and also cause induction of cell apoptosis. Intravitreal or periocular injections of various polyamine analogues, such as CGC-11144 and CGC-11150 exhibited significant reduction in the area of established CNV. In addition, apoptosis in the CNV lesions were also observed with no effects on normal retinal cells or blood vessels [133]. Single periocular injection of

CGC-11047 and CGC-11093 exhibited anti-angiogenic effect for 2–3 week and inhibited retinal or choroidal neovascularization in animal model [134].

Future perspective

DR is a complex ocular complication due to its multifactorial nature. The major treatment challenge is to develop an efficient therapy that can target multiple pathways involved in the pathogenesis of DR. Retinal vascular degeneration and mitochondrial dysfunctions are major attributes of DR. Normalization of vascular degeneration and restoration of mitochondrial function is a promising therapeutic approach for the management of disease progression. Continued research in this field can undoubtedly offer new insights into DR prevention and treatment.

Key Terms

Microvasculature	Composed of blood vessels containing endothelial cells lining the blood vasculature and perivascular cells tightly covering basement membrane of endothelial cells. Overall, these cells are embedded within protein-rich extracellular matrix and interstitial fluid
Fibronectin	High-molecular-weight extracellular matrix glycoprotein engaged in various cellular processes, including embryogenesis, cell migration/adhesion blood clotting and tissue repair
Thrombospondin	Subunit of a disulfide-homotrimeric adhesive glycoprotein, which mediates cell-to-matrix and cell-to-cell interactions. Its putative functions include modulation of cell migration, adhesion, proliferation and angiogenesis

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of interest

of considerable interest

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Executive summary

Retinal vascular degeneration & mitochondrial dysfunctions are major attributes of diabetic retinopathy

- Retinal perfusion and oxygenation deficits along with alterations in pericyte coverage and basement membrane architecture cause vascular degenerations. Moreover, mitochondrial dysfunctions caused by oxidative stress and mitochondrial DNA damage modulate the retinal capillary cell apoptosis in progressive diabetic retinopathy.

Current treatment for diabetic retinopathy mostly rely on anti-VEGF agents, long-acting steroids, PKC inhibitors & antioxidant agents

- Bevacizumab, ranibizumab and pegaptinib sodium are important anti-VEGF agents currently used in the treatment of diabetic retinopathy. All three agents have shown potential to improve the visual acuity by lowering the VEGF levels.
- Long-acting steroids (dexamethasone and triamcinolone acetonide) and PKC inhibitors (ruboxistaurin mesylate and PKC 412) have clinically demonstrated their potential to improve various vascular complications, such as retinal thickness and blood flow abnormalities.
- Antioxidant lipoic acid is a potential agent to alter the redox status of the retinal cells along with improving mitochondrial abnormalities.

Normalization of retinal microvascular degeneration & restoration of mitochondrial function is a promising therapeutic approach for the management of retinopathy progression

- The major treatment challenge is to develop an efficient therapeutic regimen targeting multiple pathways involved in the pathogenesis of diabetic retinopathy.
- Vascular-disrupting agents can selectively target highly proliferative vasculature relative to normal vessels due to the fundamental structure divergences. The idea of co-administration of a vascular-disrupting agent along with a pro-angiogenic growth factor inhibitor or anti-angiogenic agent may become an attractive alternative for retinopathy.
- Superoxide dismutase and mimic agents have demonstrated significant inhibition of diabetes induced mitochondrial superoxide levels.
- Continued research for evaluating potential of microvasculature and mitochondria targeting agents can undoubtedly offer new insights for treating diabetic retinopathy.

Figure 1.
Microvascular and mitochondrial dysfunctions in diabetic retinopathy.

Figure 2.
Cis-combretastatin A4.

Figure 3.
C9 or 2H-indol-2-one, 1, 3-dihydro-6-methoxy-1-methyl-3-[(3,4, 5-trimethoxyphenyl) methylene]-, (3E)- (9CI).

Figure 4.
PKC412 (midostaurin).

Figure 5.
MnTBAP.

Figure 6.
M40403 (imisopasem manganese).

Figure 7.
PJ-34.

Figure 8.
FP-15.