

# Understanding Retinopathy of Prematurity: Update on Pathogenesis

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## Key Words

Retinopathy • Nitro-oxidative stress • Trans-arachidonic acids • Succinate • GPR91 • Semaphorin 3A and 3E •  $\omega$ -3 lipids

## Abstract

Retinopathy of prematurity (ROP), an ocular disease characterized by the onset of vascular abnormalities in the developing retina, is the major cause of visual impairment and blindness in premature neonates. ROP is a complex condition in which various factors participate at different stages of the disease leading to microvascular degeneration followed by neovascularization, which in turn predisposes to retinal detachment. Current ablative therapies (cryotherapy and laser photocoagulation) used in the clinic for the treatment of ROP have limitations and patients can still have long-term effects even after successful treatment. New treatment modalities are still emerging. The most promising are the therapies directed against VEGF; more recently the use of preventive dietary supplementation with  $\omega$ -3 polyunsaturated fatty acid may also be promising. Other than pharmacologic and nutritional approaches, cell-based strategies for vascular repair are likely to arise from advances in regenerative medicine using stem cells. In addition to all of these,

a greater understanding of other factors involved in regulating pathologic retinal angiogenesis continues to emerge, suggesting potential targets for therapeutic approaches. This review summarizes an update on the current state of knowledge on ROP from our and other laboratories, with particular focus on the role of nitro-oxidative stress and notably trans-arachidonic acids in microvascular degeneration, semaphorin 3 operating as vasorepulsive molecules in the avascular hypoxic retina and in turn impairing revascularization, succinate and its receptor GPR91 in neuron-mediated retinal neovascularization, and  $\omega$ -3 lipids as modulators of preretinal neovascularization. Copyright © 2011 S. Karger AG, Basel

## Introduction

Retinopathy of prematurity (ROP) has been recognized since 1942 as a blinding disease of premature infants [1]. In more than half a century of intense clinical and laboratory research, great advancements have been

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made in elucidating its pathogenesis and in developing effective therapies for severe stages of ROP. The results of these efforts have yielded cryotherapy and laser photocoagulation, two approaches that consist of destroying the portion of the avascular retina which is the source of growth factors that promote neovascularization. However, these procedures only partially reduce the incidence of blindness in infants with severe late-stage disease, and do not markedly affect visual acuity [2]. Despite the recent demonstration that anti-vascular endothelial growth factor (VEGF) therapy is an effective antiangiogenic therapy for severe ROP [3], long-term defects in visual acuity, disorders in color discrimination and appropriate adaptation to darkness, remain a concern.

The oxygen-induced retinopathy (OIR) animal model of ROP [4, 5] has been very helpful to understand numerous aspects of ROP, including the role of oxidant stress, various growth factors such as VEGF and insulin-like growth factor (IGF) in vascular development, and structure-functional anomalies, as these corresponded to observations in humans. In this review we plan to focus on new and relevant observations regarding nitro-oxidative mediators, notably trans-arachidonic acids (TAAs) in microvascular degeneration, on semaphorin 3A and 3E (Sema3A and 3E) identified as vasorepulsive molecules in the avascular hypoxic retina which impairs revascularization, on succinate and its receptor GPR91 in neuron-mediated retinal neovascularization, and on  $\omega$ -3 lipids that modulate preretinal neovascularization. It should be highlighted that this is not a comprehensive review on ROP pathophysiology, which has recently been reported [6, 7].

### Pathophysiology of ROP

The retinal blood vessel development in humans commences around 16 weeks of gestation in a central-to-peripheral wave at a rate of about 0.1 mm/day [8]. The nasal retina is vascularized by 36 weeks' gestation and the temporal retina around 40 weeks. Because retinas of premature infants are incompletely vascularized, ROP incidence and severity are directly proportional to the degree of prematurity. The development of ROP progresses through two phases. The first phase begins when retinal vascular growth ceases after premature birth. During this time the vessels are particularly vulnerable to injury and may be obliterated by any number of stressors including the amount of oxygen supply, the suppression of vasoproliferation due to decreased VEGF and the scarcity

in cytoprotective factors, notably IGF. Premature infants are exposed to higher oxygen tension after birth compared to that in utero, which leads to a downregulation of the major hypoxia-triggered VEGF, resulting in vasoobliteration of the developing retinal capillaries. This is one of the first events in the development of ROP.

This relatively vascular-depleted retina which becomes increasingly hypoxic by an increased metabolic demand of the developing retina triggers the vasoproliferative or second phase of ROP. In this phase, an overproduction of hormones and growth factors to ensure adequate perfusion to the now hypoxic retina takes place; in particular, VEGF, but also growth hormones, including IGF-1 are produced. These factors influence proteins of the extracellular matrix, such as vitronectin, fibronectin and fibrinogen, to deposit adhesive fibrins and induce growth, differentiation and migration on endothelial cells [9]. The formation of these new vessels is anarchic and excessive resulting in invasion of the vitreous, where in traction on the retina and bleeding can occur. This critical stage of ROP occurs most frequently around 33–34 weeks postconceptionally [10].

ROP is not only a vascular disorder, but is also associated with a number of functional visual deficits. Although peripheral functional visual disorders have been described in ROP, the most pronounced deficits arise from central disorders of the retina, as attested by defective cone and rod functions [11, 12]; similar observations have been made in the OIR model of ROP [13]. A marked involution of the central choroid and choriocapillaris, the exclusive circulatory supply of nutrients and oxygen to the outer retina, was recently reported as an important contributor to central photoreceptor compromise in the OIR/ROP [14]; accordingly, preservation of the choroid conserved photoreceptor integrity. Hence, these observations unveil an unprecedented central choroidal involution in OIR; interestingly, a similar albeit limited observation has been made in human ROP [15].

### Risk Factors of ROP: Increased Oxygenation

Numerous factors participate in the genesis of ROP. Although some studies have reported that exposure to multiple gestation, apnea, race, light, anemia, sepsis, prolonged mechanical ventilation and multiple transfusions are risk factors for ROP [16], the precise individual role of these factors in the development of the disease is not well established. On the other hand, low birth weight and gestational age as well as high postnatal oxygenation (dis-

cussed below) have consistently been shown to increase the risk of ROP.

The intrinsic property of many organs to maintain blood flow constant over a range of perfusion pressures and oxygen tensions to meet metabolic demand of the tissues is termed autoregulation. Preterm infants who suffer complications of prematurity exhibit total absence of autoregulation of ocular blood flow resulting in an exaggerated delivery of potentially toxic oxygen to the retina when they are exposed to oxygen supplementation to overcome respiratory insufficiency [17]. This relative inability of the neonate to control oxygen delivery to the retina is largely due to high perinatal levels of prostaglandins (such as PGD<sub>2</sub> and PGE<sub>2</sub>) and nitric oxide (NO) that profoundly influence vasomotor tone and override the autoregulatory response [18, 19].

The rise in carbon dioxide tension (hypercapnia) is another factor significantly contributing to the disruption of regulation of retinal and choroidal blood flow in infants born prematurely. Both acute and sustained hypercapnias markedly increase retinal and choroidal blood flow in premature infants. The acute rise in ocular blood flow in response to hypercapnia is largely PGE<sub>2</sub>-dependent. During sustained hypercapnia, this increase in PGE<sub>2</sub> induces expression of endothelial NO synthase (eNOS), which releases NO and in turn mediates the delayed carbon dioxide-induced rise in ocular hemodynamics and further curtails ocular blood flow autoregulation [20]. This marked induction in ocular hyperemia is clinically relevant, since hypercapnia has been associated with ROP in humans and in OIR in experimental animals [21].

### Vaso-Obliteration

In response to the ensuing retinal hyperoxia, the particularly vulnerable endothelial cells at the vascular front degenerate, producing vasoconstriction of immature vessels and consequent destruction of the vascular bed. A number of factors participate in the endothelial cytotoxicity that leads to the vaso-obliteration phase: (1) oxygen-dependent suppression of growth factors such as VEGF and erythropoietin through stabilization of hypoxia-inducible factor [22, 23]; (2) decreased expression of the maternal and nutrition-dependent factor IGF-1 [24]; (3) reduced presence of anti-inflammatory factors which suppress production of excessive cytotoxic concentrations of TNF- $\alpha$  in the developed retina [25]; (4) inability of the newborn to autoregulate oxygen delivery [26]; (5) in-

creased generation of reactive oxygen species (ROS) and their peroxidation products, notably thromboxane A<sub>2</sub>, platelet-activating factor (PAF) and lysophosphatidic acid (LPA), along with a reduced concentration of major components of the antioxidant systems in the neonates, including heme oxygenase-1, metallothionein, Cu-Zn superoxide dismutase, catalase, vitamins C and E, and glutathione peroxidase [27]; (6) nitrate stress that also results in an increased production of TAAs that contributes to retinal vascular degeneration [28], and (7) increased vulnerability of the neuroretinal endothelium to cytotoxic agents such as prostanoids and isoprostanes derived by oxidant stress and lipid peroxidation due to high content of polyunsaturated fatty acids in the immature retina [29].

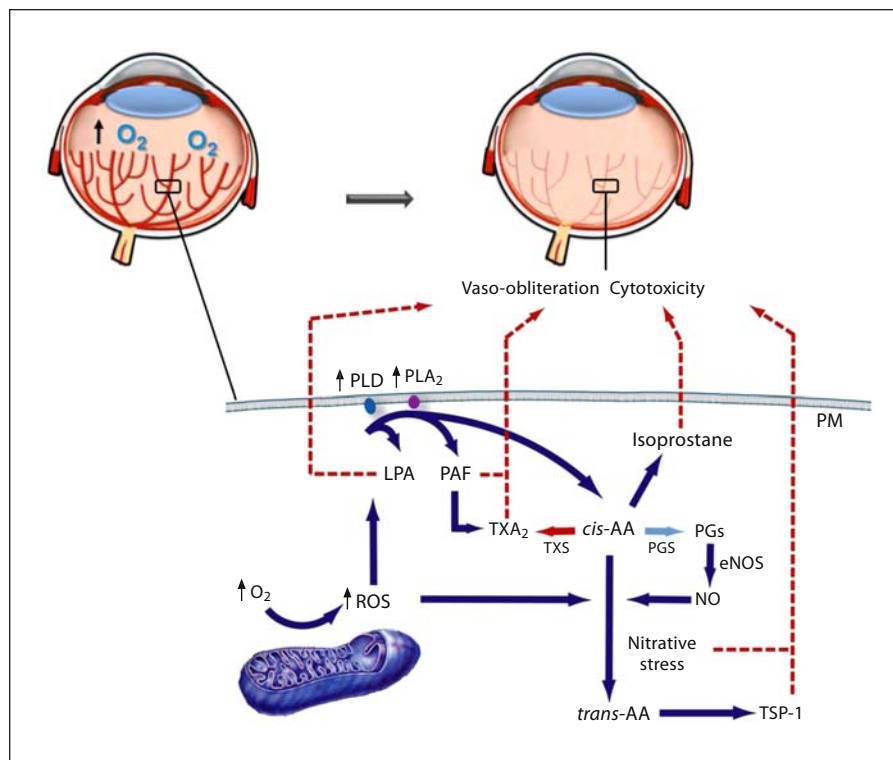
### Nitro-Oxidative Stress, and Lipid Oxidation and Nitration

ROS and oxidative stress are known to contribute to the retinal vaso-obliteration in ROP. NO, a complex molecule that exerts both protective and proangiogenic properties in the eye, can have differential effects in OIR depending on the redox state of the retina. In vivo for example, we showed that eNOS expression and activity is increased when the redox state is shifted toward an oxidative environment [30, 31]. Under these conditions, NO can react with ROS affecting the cell function by a process named nitro-oxidative stress. As a result, generation of nitrites, nitrates and peroxynitrite, and protein tyrosine nitration are enhanced increasing retinal microvascular degeneration [32]. Genetic ablation [33] and pharmacologic inhibition of eNOS [34], as well as the use of antioxidants [35, 36], has been shown to attenuate hyperoxia-induced retinal microvascular degeneration, demonstrating the importance of nitro-oxidative stress in ROP.

Lipid peroxidation of cell membranes secondary to inadequate oxygen tension is pivotal to the pathogenesis of ROP and, together with nitrate stress, among the most toxic consequences of increased ROS. Polyunsaturated fatty acids (PUFAs) of membrane phospholipids are common targets for peroxidation, resulting in loss of cell membrane function and structural integrity. The retina is highly susceptible to lipid peroxidation, as it is composed of lipids with elevated levels of PUFAs such as docosahexaenoic acid (DHA), *cis*-arachidonic acid (AA) and choline phosphoglyceride.

Prostanoids are synthesized from AA by the sequential action of phospholipase A<sub>2</sub> (PLA<sub>2</sub>) and cyclooxygenase (COX) triggered by oxidant stress and peroxidation.

**Fig. 1.** The effects of oxidant stress on premature retinal vasculature. The premature retina is relatively deficient in antioxidants. Consequently, oxidant stress is more likely to induce peroxidation and nitration that is cytotoxic to retinal microvasculature. Downstream mediators of peroxidation, notably the phospholipids PAF and LPA, the nonenzymatically derived prostanoids, isoprostanes, and nitration products (of particular relevance here are the TAAs), are all cytotoxic to retinovascular endothelium, causing vaso-obliteration. PGS = PG synthase; TXS = thromboxane synthase.



The accumulation of peroxides eventually favors thromboxane (TXA<sub>2</sub>) production over that of prostaglandins. TXA<sub>2</sub> is a potent vasoconstrictor as well as a cytotoxic agent in microvessels [37]. Consistent with these events having a role in ROP, inhibitors of COX and TXA<sub>2</sub> synthase selectively curtail oxygen-induced retinal vaso-obliteration in rodents [37]. In contrast to prostaglandins produced by COX, isoprostanes are formed nonenzymatically in situ from the peroxidation of AA and then released by phospholipases; they exceed the production of prostaglandins under oxidizing conditions. Isoprostanes may contribute to microvascular injury in ROP, as they are indirectly cytotoxic, since they trigger the production of TXA<sub>2</sub> [38] (fig. 1). Nitritive stress results in *cis*- to *trans*-isomerization of AA (TAA), and this was recently shown to contribute to retinal vascular degeneration in a mouse model of ROP [28]. Circulating levels of plasma TAA are increased in oxygen-induced microvascular degeneration and are known to be associated with induction of nitritive stress [39]. More specifically, TAA formation is abrogated in mice treated with NOS inhibitors and in mice deficient in eNOS [34] (fig. 1). The endothelial cytotoxicity induced by TAA results from the formation of the anti-angiogenic and proapoptotic thrombospondin-1 (TSP-1) [34].

Other lipids generated during peroxidation are important proinflammatory mediators, including LPA and PAF. Choline phosphoglycerides are the precursors of PAF. Constitutive levels of PAF are maintained by the de novo pathway to ensure normal housekeeping cellular function. Otherwise, PAF is abundantly generated under oxidative stress by the remodeling pathway and calcium-dependent activation of cytosolic PLA<sub>2</sub>. In ROP, AAs are cleaved off from membrane phospholipids (by cPLA<sub>2</sub>) to yield lysophospholipids, which in turn can be acetylated and converted to PAF [40]. Both the vasomotor and cytotoxic effects of PAF in the premature infant are mediated to a large extent by TXA<sub>2</sub> [41, 42]. Thus, PAF, which is generated concomitantly with TXA<sub>2</sub> during oxidant stress and amplifies formation of TXA<sub>2</sub>, contributes to retinal vaso-obliteration in ischemic retinopathies (fig. 1). Along the same lines, LPA is also released from lysophosphatidylcholine by the action of lysophospholipase D and can partake in retinal inflammation leading to microvascular cytotoxicity [43].

#### *Insulin-Like Growth Factor-1*

Other factors present in utero that partake in normal fetal development are deficient in preterm infants at risk for ROP. IGF-1 is a polypeptide protein hormone essential

for fetal development at all stages of pregnancy [44]. Plasma levels of IGF-1 rise with gestational age, particularly during the third trimester of pregnancy [45]. Premature infants suffer a decrement in the serum levels of IGF-1 due to loss of placenta and sufficient nutrient supply in utero [46].

The association between low serum levels of IGF-1 and ROP was explored in the elegant works of Smith and colleagues [47, 48] who demonstrated that a deficiency in growth hormone and downstream IGF-1 caused retardation in retinal vessel growth akin to patterns noted in premature babies with ROP. Interestingly, in human subjects, low serum levels of IGF-1 directly correlate with the severity of ROP [49], and IGF-1-binding protein IGFB3 was also found to be decreased in premature infants contributing with retinal vessels depletion [50].

IGF-1 modulates vessel survival by controlling the VEGF-induced activation of Akt and ERK1/2 mitogen-activated protein kinase – essential for endothelial cell proliferation [24, 48]. In this regard, IGF-1 appears to act as a permissive factor for VEGF-dependent endothelial growth and survival such that VEGF alone would be insufficient to induce the exaggerated angiogenesis associated with ROP and other proliferative retinopathies. To this respect, clinical trials are being undertaken to address the merits of treatment with IGF-1 at an early stage to prevent the vaso-obliteration in the premature infants. So far, these preliminary studies have shown that infusion of fresh frozen plasma, which is a source of exogenous IGF-1 [51], or the intravenous administration of recombinant protein complex of rhIGF-1 and rhIGFBP-3 in premature infants increase serum IGF-I levels to the levels normally found in utero at the corresponding gestational age [52].

Experimental animals, notably mice that received exogenous recombinant human IGF-I, exhibited higher IGF-I levels with weight gain, matured faster and developed less OIR [53]. These findings in mice are the first to support the notion that higher availability of (endogenous or exogenous) IGF-I reduces OIR risk, and support the notion that ROP may be preventable.

### *Semaphorins*

Anatomical studies since the fifteenth century have shown that vascular and neural networks are architecturally paralleled and interact physically. Nevertheless, only in the last decade has it come to light that blood vessels and nerves are guided through the body by the same families of cues. One of the most versatile examples of these families is the class 3 semaphorins (Sema3), first identified as glycoproteins that negatively mediate neuronal

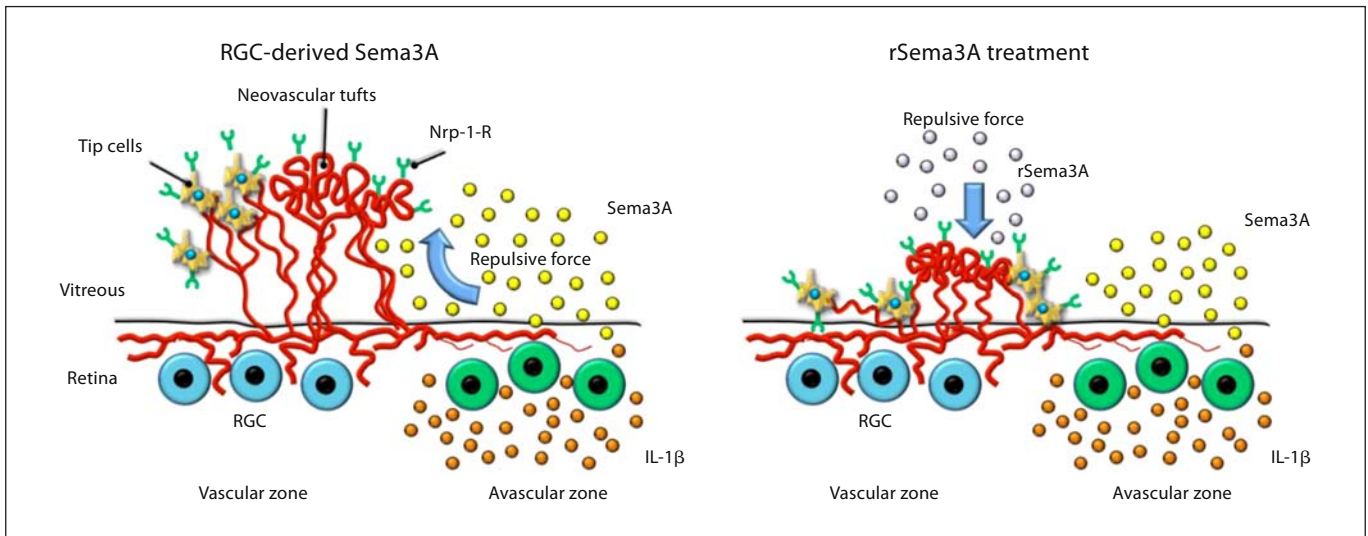
guidance by binding to neuropilin and repelling neurons away from the site production [54]. Sema3 are now regarded as key regulators of cellular processes such as endothelial and tumor cell survival, proliferation, apoptosis, and migration [55]. Considered potent inhibitors of tumor growth and vessel density, Sema3 acts primarily by inhibiting the cell motility and migration of endothelial cells by inducing the collapse of the actin cytoskeleton via neuropilins and plexins. Semaphorins such as Sema3A and Sema3E bind, respectively, to neuropilin and plexin-D1.

The role of semaphorins in the pathophysiology of ROP was recently investigated. Using knockdown of the retinal ganglion cell Sema3A, our laboratory demonstrated for the first time that in response to hypoxia, retinal neurons secrete neuronal guidance cue Sema3A, which acts as a chemical barrier to repulse new vessels and in turn prevent revascularization of hypoxic retinal tissue (fig. 2) [56]. An important regulator of Sema3A generation was found to be the proinflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ). In a somewhat complementary paradigm, Sema3E arising from neurons was also found to suppress anarchic extraretinal vascular outgrowth without affecting retinal vascular regeneration in the OIR model [57]. Together, these observations uncover major roles for semaphorins in governing the guidance of retinal vascular development.

### **Neovascularization: Novel Mediators**

#### *Succinate and Its Receptor, GPR91*

As a result of vaso-obliteration, the retina attempts to reinstate adequate levels of oxygen and nutrients to the hypoxic/ischemic tissue; in this process, it orchestrates neovascularization. Factors that partake in this neovascularization include VEGF, fibroblast growth factor, hepatocyte growth factor, platelet-derived growth factor and erythropoietin [58]. We recently explored the mechanisms involved in regulating expression of angiogenic factors by exploring the role of major intermediary energy metabolites, notably the Krebs cycle intermediate, succinate. In this respect, it was shown that succinate levels, which increase during hypoxic conditions, induce a vaso-proliferation response in the retina by activating its G protein-coupled receptor GPR91 [59]. This receptor, expressed particularly in highly vascularized tissues [60], was also abundantly expressed in retinal ganglion cells [59]. GPR91 exerted control in the expression of numerous major angiogenic factors, such as VEGF and angiopoietin 1 and 2, and suppressed antiangiogenic thrombo-



**Fig. 2.** Role of Sema3A as vasorepulsive molecules in the retina. Ischemic neurons in the avascular zones produce Sema3A secondary to inflammatory stress (IL-1 $\beta$ ). Pathologic neovascular tufts are enriched in neuropilin-1 receptor (Nrp-1-R). Retinal

ganglion cell (RGC)-derived Sema3A impedes revascularization and repels neovessels away from the avascular neural retina towards the vitreous (left), whereas intravitreal rSema3A (injected) prevents preretinal invasion of pathological neovessels (right).

spondin-1. Genetic disruption in GPR91 expression or in retinal ganglion cell development interfered with retinal angiogenesis during development as well as in the OIR model of ROP (fig. 3). These unprecedented observations indicated that hypoxia-driven metabolic changes in neurons contribute in attempting to restore vascular supply in the OIR model by elevating the formation of intermediate metabolites such as succinate. These novel findings provide innovative therapeutic avenues aimed at restoring sufficient vascular supply after neural ischemic insult.

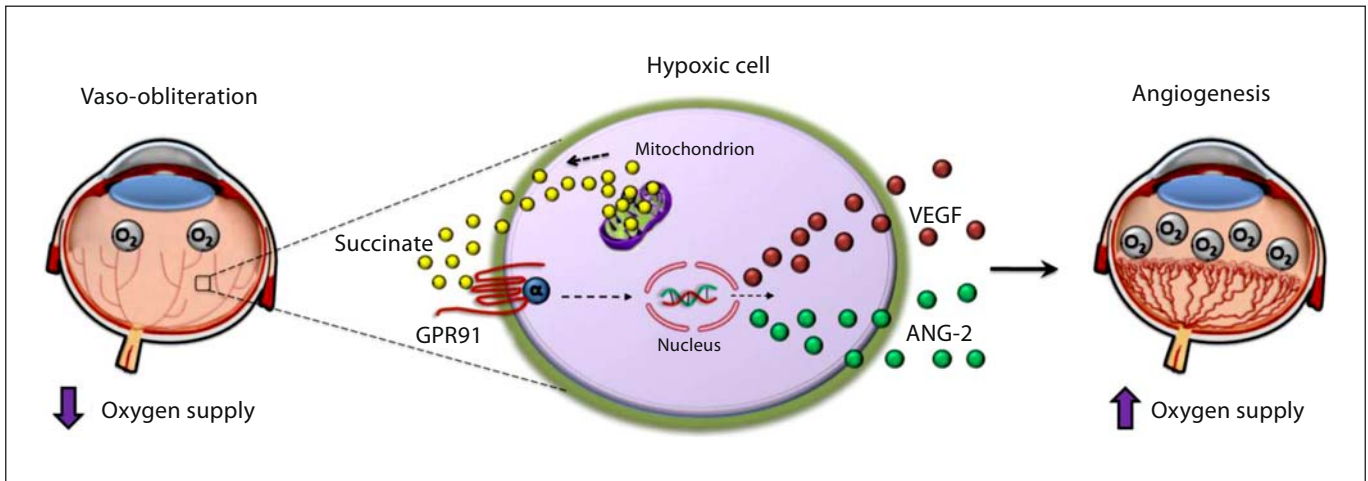
#### *Inflammatory Mediators*

Inflammation is a complex, highly regulated sequence of events that can be provoked by a variety of stimuli. It is the primary process through which tissues, including the eye, repair damage and defend themselves against abnormal conditions. However, excess inflammation is pathological and can result in tissue destruction, leading to visual dysfunction and blindness. The role of inflammation in ROP has been poorly investigated; however, some evidence in humans point to its contribution in ROP. For instance, Sood et al. [61] found that in patients who later developed ROP, there were higher systemic levels of IL-6 and C-reactive protein, and lower concentrations of neurotrophin-4 and IL-17, compared to controls on postnatal days 0–3; whereas on postnatal days 7–21 there were higher levels of IL-18 compared to controls.

In another study, Sato et al. [62] analyzed the vitreous levels of 27 cytokines in eyes with ROP and found higher levels of several cytokines in eyes with ROP, including IL-6, IL-7, IL-10, IL-15, eotaxin, fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon- $\gamma$ -inducible protein-10 and (mostly) VEGF. Studies in our laboratory showed that COX-2, an early gene product of inflammation, contributes to preretinal neovascularization in ischemic retinopathies through generation of PGE<sub>2</sub> which in turn acts on its EP<sub>3</sub> receptor [63, 64].

#### *$\omega$ -3 Lipids*

$\omega$ -3 lipids [notably DHA and eicosapentaenoic acid (EPA)] have been found to exert a number of beneficial biological properties such as diminished triglycerides, cytoprotection including of neural tissue, decreased oxidant stress and (most noteworthy) decreased inflammation [65, 66]. The retina contains the highest concentration of DHA of all tissues [67]. Premature newborns are relatively deficient in  $\omega$ -3 lipids, and supplementation with DHA and EPA has been found to improve visual acuity [68]. The efficacy of  $\omega$ -3 lipids on ROP/OIR has recently been reported. In mouse pups of dams fed  $\omega$ -3 supplements starting on postnatal day 1, decreased oxygen-induced vaso-obliteration and neovascularization were observed [25]. The supplementation with  $\omega$ -3



**Fig. 3.** The proangiogenic effects of the metabolite succinate in ROP. Hyperoxia induces microvascular degeneration in the retina with consequent decrement in the oxygen supply that stimulates the production and accumulation of succinate. Through its actions on its receptor, GPR91, which is present on retinal ganglion cells, succinate induces production of proangiogenic factors, par-

ticularly VEGF and angiopoietin 2 (ANG-2), which trigger neovascular formation. This new vessel growth attempts to restore oxygen supply to the hypoxic retina; however, in ROP this compensatory vascular expansion is exaggerated and penetrates the vitreous.

lipids led to increased formation of cytoprotective and anti-inflammatory metabolites, notably neuroprotectins and resolvins which exerted their effects in part by suppressing TNF- $\alpha$ . The same group of investigators also uncovered that 5-lipoxygenase metabolites of DHA, specifically 4-hydroxy-DHA, mediate the antivasoproliferative effects of DHA independently of VEGF formation, but dependent of PPAR- $\gamma$  activation [69]. In line with these animal observations,  $\omega$ -3 supplementation to preterm infants was found to reduce the risk of severe ROP [70]. Hence, supplementation with DHA/EPA appears to improve visual acuity and protect against the development of ROP [71]; however, the latter needs to be confirmed.

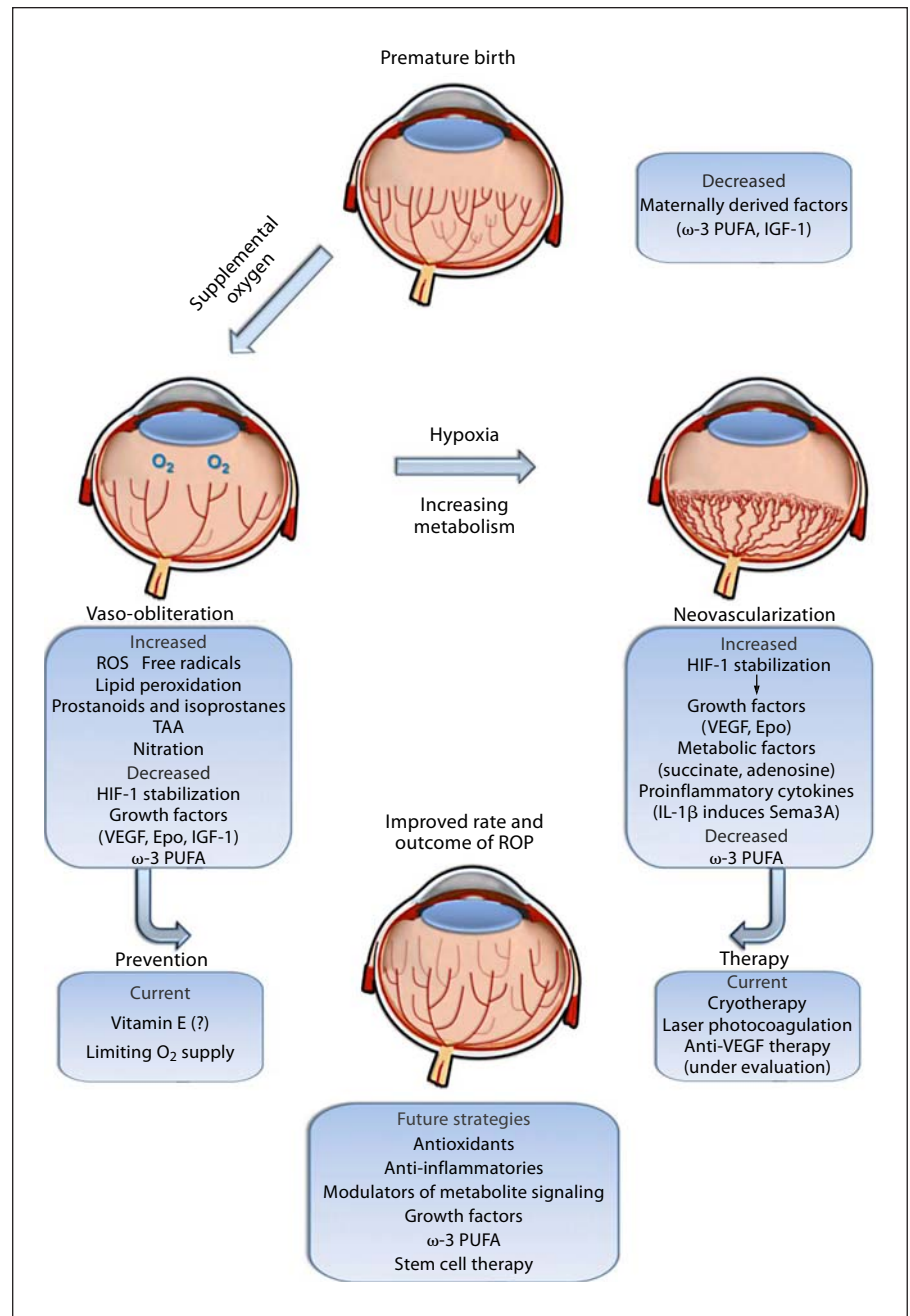
### Therapies and Prevention of ROP

#### Therapies

Ablative therapies including cryotherapy and laser photocoagulation are the procedures most commonly used in the clinic for the treatment of ROP (fig. 4). These treatments destroy tissue in the avascular retina including those that generate VEGF which promotes aberrant preretinal neovascularization. Despite this form of therapy, visual acuity remains largely unaffected and, as expected, peripheral vision is inevitably lost [2].

#### Anti-VEGF Therapy

A promising strategy to counter ROP is the use of the anti-VEGF therapy (fig. 4). Bevacizumab, a VEGF-specific neutralizing antibody is currently used for treatment of several diseases including diabetic retinopathy and macular degeneration. Until recently, bevacizumab was used for ROP in the absence of randomized trials [72]; timing (stage/zone of ROP), dose (0.4–12.5 mg intravitreal) and frequency of administration of bevacizumab, as well as cotreatment with photocoagulation, varied tremendously among reports. Nonetheless, there were generally favorable outcomes (controlled progressive neovascularization) in most reports. A prospective randomized double-blind trial of bevacizumab was recently reported and revealed that the recurrence rate of preretinal neovascularization was significantly greater in laser-treated patients than bevacizumab-treated ones exhibiting proliferative retinopathy in the central zone 1 [3]. Moreover, revascularization of the peripheral retina occurred as expected in a normal subject, while conventional laser therapy led to destruction of the peripheral retina. Despite these promising beneficial effects of bevacizumab in the treatment of ROP, additional studies are needed to determine the optimum dosages, timing of administration, frequency and evaluation of possible collateral effects, especially for neurodevelopment.



**Fig. 4.** Summary of the current concepts in ROP. At birth, premature infants are deficient in several maternally derived factors such as  $\omega$ -3 PUFAs and IGF-1 that are essential for healthy blood vessel development. Moreover, when the premature are mechanically ventilated to overcome pulmonary insufficiencies, the excessive supplemental oxygen contributes to retinal vascular obliteration due to oxidant stress and suppression of oxygen-regulated pro-angiogenic factors such as VEGF and erythropoietin (Epo). Following the initial phase of vascular dropout, a second phase of compensatory and destructive neovascularization results and is driven by hypoxia-induced angiogenic factors, such as VEGF and metabolite factors (succinate). Vasorepulsive molecules (Sema3A) also operate, avoiding the revascularization to the avascular zones. Current therapeutic interventions rely on invasive procedures such as laser photocoagulation, whereby affected areas of the retina are cauterized. A number of future treatments, including anti-VEGF therapy and the use of antioxidants, are currently being evaluated.

### Prevention

The development of preventive and less destructive therapies for ROP such as (1) restricting tissue oxygenation to reduce oxygen toxicity [73]; (2) nutritional supplements including the use of antioxidants such as vitamin E [74] and vitamin C [75] that decrease lipid peroxidation and help maintain membrane integrity; (3) the use

of  $\omega$ -3 fatty acids [70], and (4) the potential administration of cytoprotective growth factors such as erythropoietin and/or IGF-1 could be more desirable than treatment of an established disorder. However, although promising, other than limiting post-natal hemoglobin-oxygen saturation, the other modalities remain speculative at this point (fig. 4).



## Conclusion

Preretinal neovascularization in ROP is the consequence of a progressive destruction of the retinal microvasculature. A better understanding of the underlying mechanisms implicated in extraretinal neovascularization, revascularization and vascular guidance should identify new targets and foster the development of new therapeutic approaches. Along these lines, a more profound elucidation of the complex interplay of inflammatory mediators is required. Other than pharmacologic and nutritional strategies, cell-based strategies for vascular repair are likely to emerge from advances in regenerative medicine using stem cells [76]. Finally, although recent advances in clinical trials and basic science research have led to improved knowledge of the pathophysiology of ROP, additional preventive strategies are clearly needed.

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## Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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