Published in final edited form as:

Semin Ophthalmol. 2009 ; 24(2): 77–81. doi:10.1080/08820530902800314.

Retinopathy of Prematurity: Current Concepts in Molecular Pathogenesis

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Abstract

Retinopathy of prematurity is marked by the proliferative vascularization of the retina in preterm babies. An understanding of the molecular pathogenesis of ROP provides the basis for identifying novel therapeutic targets for treatment. Using the mouse model of oxygen-induced retinopathy, the roles of the hypoxia induced factors vascular endothelial growth factor and erythropoietin as well as the maternally derived factors insulin-like growth factor-1 and omega-3 polyunsaturated fatty acids have begun to be elucidated. Understanding the phase specific effects of these factors will serve to guide the development of non destructive treatments for ROP and for other ischemic retinopathies including diabetic retinopathy and neovascular age-related macular degeneration.

Keywords

retinopathy; prematurity; VEGF; erythropoietin; IGF-1; polyunsaturated Omega-3 Fatty Acids; neovascularization

INTRODUCTION

Retinopathy of prematurity (ROP) is marked by the proliferative vascularization of the retina in preterm babies. In spite of major advances in the understanding of the postnatal risk factors for the development of ROP since it was first described by Terry in 1942,¹ ROP continues to be one of the leading causes of childhood blindness in highly developed countries.^{2,3} With the advent of improved access to neonatal technologies and survival of premature infants, the incidence of severe ROP has begun to emerge as a major cause of blindness in newly developing countries as well.⁴ Although very low vision associated with ROP has significantly been reduced with the introduction of cryotherapy and photoreductive therapy in the last several decades,^{5–7} alternative nondestructive treatments are actively being sought, as these destructive modalities do not increase the number of children with good vision (>20/40). Studies on the molecular pathogenesis of ROP provide the basis for identifying novel therapeutic targets in the treatment of this disease and by extension for other is-chemic retinopathies such as diabetic retinopathy and neovascular age-related

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Declaration of Interest

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

macular degeneration. This review will focus on recent insights into the molecular pathogenesis of ROP.

NORMAL RETINAL VASCULARIZATION

In humans, the retinal vasculature develops almost entirely in utero with its completion at approximately 36 to 40 weeks gestational age (GA). Two processes underlie vascular development.^{8–10} The first process, termed *vasculogenesis*, involves the de novo establishment of the scaffold for the superficial retinal plexus of vessels. At approximately 12 weeks GA, vascular precursor cells emanate from the hyaloid artery at the level of the optic nerve head and migrate towards the retinal periphery in the pattern of the future retinal arcades.^{8,11,12} Immediately posterior to the leading edge, these mesenchymal cells aggregate to form vascular cords. By 20 weeks GA, red blood cells can be detected in these cords confirming their patency. These precursor cells do not migrate into the future fovea. At 21 weeks GA, they can no longer be detected and vasculogenesis is considered to be completed.¹¹

Anterior to the leading edge of vascular precursor cells, astrocyte precursor cells are also detected at 12 weeks GA migrating from the optic nerve. By 26 weeks GA, mature astrocytes have reached the retinal periphery. These cells ensheath new vessels and contribute to the formation of the blood-retinal barrier.¹³

The second mechanism of vascularization, termed *angiogenesis*, involves the development of new vessels from already existing vessels and in humans and is initiated by 17–18 weeks GA.¹¹ This mechanism generates the perifoveal vessels, the peripheral vessels, the deep plexus of retinal vessels, the capillary system, and the peripapillary radial vessels.⁸ At the site of new vessel formation, there is degradation of the extracellular matrix surrounding the preexisting vessel, migration of endothelial cells which form the new capillary sprout and proliferation of the endothelial cells linking the original vessel to the tip of the sprout.¹⁴ Angiogenesis is thought to be stimulated by “physiologic hypoxia” of the developing retina. That is, when the choroidal circulation no longer can meet the oxygen demands of the metabolically active differentiating retina, new vessels form in response to a locally secreted vasoactive factor.^{8,9,15} Remodeling or retraction of vessels continues to occur during the vascularization process; in those areas which become rich in oxygen supply, such as adjacent to arteries, there is a reduction in the density of vessels.^{8,11} Angiogenesis is completed when the superficial and deep retinal vessels reach the ora serrata near or at term.

PATHOGENESIS OF ROP

ROP develops when there is significant disruption of the physiologic conditions that support angiogenesis. Not only is there hyperoxia of the external environment relative to the in utero environment, but also there is the loss of maternally derived factors that contribute to normal retinal vessel formation.¹⁶ The derangement of vessel growth occurs in two sequential phases: a vaso-obliterative phase and a vaso-proliferative phase.⁹

The vaso-obliterative phase is initiated upon birth of the premature infant. In the extrauterine environment, the PaO₂ suddenly rises from the in utero levels of 30–35 mm Hg to 55–80 mm

Hg. This relative hyperoxia is exacerbated by supplemental oxygen.¹⁷ In response to the elevated levels of oxygen, expression of hypoxia-driven angiogenic factors is downregulated, vessel growth ceases, and already formed vessels constrict and retract.^{9,16}

During the vaso-proliferative phase, the incompletely vascularized areas of the retina become metabolically active and stimulate new vessel growth in response to hypoxia in these areas. This stage usually develops after 32 weeks GA. Because the vessels damaged during the vaso-obliterative phase are unable to meet the oxygen demands of the retina, previously suppressed hypoxia-induced factors are upregulated. This leads to exuberant vessel formation at the junction between avascularized and vascularized retina.¹⁶ The proliferation of vessels can either regress if adequate oxygenation is provided to the avascular retina or it will progress if the metabolic demands outpace this effort.⁹ Aberrant neovascularization or the fibrous/cicatricial changes in the retina that may occur after regression may result in macular dragging or retinal detachment and potential blindness.

To understand the mechanisms underlying the pathogenesis of ROP, animal models of oxygen-induced retinopathy (OIR) have been studied extensively in the kitten, beagle puppy, rat, and mouse.¹⁷ The mouse model of oxygen-induced retinopathy (OIR) has become the most commonly used model for investigations into the molecular pathogenesis of ROP as the model offers reproducibility of the phenotype, a reliable method of quantifying retinal neovascularization, and the ease of genetic manipulation.¹⁸ The degree of vascular development in the retina of the neonatal mouse most closely approximates the retina of a premature infant at 5 months GA.

Briefly, neonatal mice are exposed to 75% oxygen from postnatal day 7 (P7) until P12. In response to hyperoxia, the central retinal vessels vasoconstrict and regress, which recapitulates the first phase of ROP. Once the mice are returned to room air conditions, the avascular retina becomes hypoxic and drives retinal neovascularization thereby mimicking the features of the second phase of ROP. Neovascularization is maximal at P17 and thereafter regresses. The extent of vaso-obliteration and neovascularization can be quantified by evaluation of retinal flat mount preparations.^{16,18}

Role of Hypoxia Induced Factors and the Development of ROP

Vascular Endothelial Growth Factor (VEGF)—Studies in the mouse model of OIR have been extremely informative in establishing the role of the endothelial cell mitogen and vascular permeability factor VEGF in both the vaso-obliterative and vaso-proliferative phases of ROP. During normal angiogenesis, hypoxia induces upregulation of VEGF, which results in the growth of new vessels.^{19,20} VEGF expression is primarily regulated by the transcription factor hypoxia inducible factor-1 (HIF-1). Under “physiologic hypoxia,” HIF-1 α , which is degraded under normal oxygen conditions, is stabilized and results in increased expression of VEGF anterior to the growing vessels.²¹ VEGF is secreted by the supporting astrocytes that are closely associated with endothelial cells.^{15,22}

During normal vascular development in the mouse, VEGF mRNA can be detected immediately anterior to the leading vessel edge at P7. In the mouse model of OIR, levels of both VEGF mRNA and protein are decreased after 6 hours in the hyperoxic environment.

Concomitant with downregulation of VEGF, there is cessation of vessel growth and loss of already formed vessels.²³ Administration of exogenous VEGF or the VEGFR-1 specific ligand placental growth factor-1 (PlGF-1) can inhibit vaso-obliteration in these mice confirming the prominent role played by VEGF in phase one of ROP.^{23,24}

The role of VEGF in phase two of ROP is suggested by the induction of VEGF mRNA and protein expression prior to the development of neovascularization. VEGF levels rise approximately 6–12 hours after returning the neonatal mice to hypoxic conditions and these levels are sustained until neovascularization commences.²⁵ Targeted inhibition of VEGF using antisense oligodeoxynucleotides or small interfering RNA (siRNA) prevents retinal neovascularization.^{26,27} Clinical interventions utilizing an anti-VEGF strategy are underway in the treatment of ROP.

Erythropoietin (Epo)—Although manipulation of VEGF can reduce the extent of retinal neovascularization in the mouse model, inhibiting VEGF expression does not completely eliminate aberrant vessel growth. Investigations into another oxygen sensitive growth factor, erythropoietin (Epo), have suggested an independent role in retinal angiogenesis distinct from VEGF-mediated vascular growth.¹⁶ Epo is a hormone secreted by the fetal liver and subsequently by the adult kidney with pleiotropic effects including stimulation of erythropoiesis in the bone marrow, inhibition of apoptosis in vascular cells and neurons, and regulation of angiogenesis.²⁸ In an *in vitro* angiogenesis model, Epo and VEGF were shown to have equal potential as angiogenic factors.²⁹ Epo expression is hypoxia induced and its expression is regulated by the transcription factors HIF-1 α and HIF-1 α -like factor (HLF).³⁰

In the mouse, Epo protein and the Epo receptor are expressed both in retinal vessels as well as in the inner retina at P8. In the mouse model of retinopathy, under hyperoxic conditions, Epo mRNA expression is markedly reduced, suggesting that loss of Epo may contribute to vessel loss. Administration of exogenous Epo is vasoprotective during phase one of OIR. Not only does restoration of Epo prevent vaso-obliteration but also it exerts a protective effect on neuronal apoptosis in the retina.³¹ This cytoprotective effect has been shown to be mediated locally via direct inhibition by Epo on caspase mediated apoptosis as well as systemically through recruitment of endothelial progenitor cells to the retina from the bone marrow.³¹

The role of Epo in phase two of ROP is suggested by the increase in Epo mRNA expression during neovascularization in the mouse model of OIR, which can be prevented by exogenous administration of Epo during the hyperoxia phase of ROP.³¹ In HLF knockdown mice subjected to hyperoxia then relative hypoxia, neovascularization of the retina does not occur; the failure to progress to vaso-proliferation is related specifically to reduced levels of Epo mRNA.³⁰ Finally, targeted inhibition of Epo with siRNA has been demonstrated to prevent retinal neovascularization.³²

Currently, recombinant Epo (rhEPO) has been advocated for the treatment of anemia in premature infants to reduce the need for repeated blood transfusions.^{16,33} In a recent retrospective analysis of premature infants who received rhEPO as part of the standard treatment for anemia, however, administration of rhEPO was determined to be an

independent risk factor for developing ROP and further for ROP which necessitated intervention with photoablative therapy.³³ Animal studies suggest that the timing of intervening with Epo is critical as it appears to have a protective role during phase one of ROP but may exacerbate neovascularization if administered during the proliferative phase of ROP.^{16,34}

Role of Maternally Derived Factors and the Development of ROP

Insulin-like Growth Factor-1 (IGF-1)—IGF-1 is part of a family of polypeptides that are implicated in human fetal growth; levels of IGF-1 found in the maternal serum and fetus continue to increase throughout gestation.³⁵ In preterm infants, however, there is a deficit in maternally derived IGF-1 supplied in the placenta and amniotic fluid; it has been hypothesized that the most important risk factors for ROP, gestational age (prematurity) and low birth weight may correlate with the loss of a biochemical factor such as IGF-1, which is important in fetal growth and development.^{13,16} Using mouse models, the impact of IGF-1 on the pathogenesis of ROP has begun to be elucidated.¹⁶

Normal angiogenesis requires growth hormone (GH) and its downstream effector IGF-1. The observation of subnormal retinal vascularization in patients with genetic deficiencies in the growth hormone/IGF-1 axis supports this.³⁶ Studies in IGF-1 knockout mice demonstrate an absence of normal vessel growth. In these mice, VEGF levels are comparable to those in wild type IGF-1 mice, suggesting that although VEGF is necessary for angiogenesis, VEGF is not sufficient to induce angiogenesis.³⁷

The role of GH in the proliferative phase of ROP is supported by a reduction of retinal neovascularization in transgenic mice engineered to express a growth hormone antagonist; the inhibition of vaso-proliferation is eliminated by exogenously treating the same mice with IGF-1 confirming that the effect of GH is mediated through IGF-1.³⁸ In the mouse model of OIR, mice treated exogenously with an IGF-1 receptor antagonist also exhibit a reduction of neovascularization of the retina. Reduction in IGF-1 levels does not directly impact VEGF expression but rather suppresses VEGF-mediated MAPK pathway activation and Akt pathway activation, required for endothelial cell survival and proliferation.³⁹ A minimal level of IGF-1 is required for VEGF to act on endothelial cells.

Based on these findings, it has become clear that IGF-1 and VEGF are intimately linked in the process of angiogenesis. IGF-1 appears to play a permissive role in VEGF-mediated new vessel growth. In the preterm infant, low IGF-1 levels limit new vessel growth. Hypoxia of the avascular retina, which is exacerbated by supplemental oxygen, results in increased levels of VEGF expression. As the infant matures, the body begins to produce endogenous IGF-1 and this sudden elevation in IGF-1 levels allows for VEGF-driven neovascularization to ensue. The timing for potential therapeutic restoration of IGF-1 is therefore critical as it could either prevent vessel loss if administered during phase one of ROP or promote aberrant vessel growth if administered during phase two of ROP.³⁴

ω -3 Polyunsaturated Fatty Acids (PUFAs)—The important role of maternally derived ω -3 PUFAs in the prevention of ROP has begun to emerge through recent studies in the mouse model of OIR.¹⁶ Like IGF-1, this factor is depleted in preterm infants, who miss the

normal massive transfer of PUFAs in the third trimester from mother to infant. The primary ω -3 PUFA in the retina is docosahexaenoic acid (DHA) and the primary ω -6 PUFA is arachidonic acid (AA) and it is the delicate ratio of ω -3 to ω -6 PUFAs, which appears to promote neuronal and vascular cell survival in the retina.^{16,40–42}

The neuroprotective role of ω -3 PUFAs in phase one of ROP is supported by a statistically significant decrease in the extent of vaso-obliteration in pups raised on an isocaloric diet with 2% of total fatty acids from ω -3 PUFAs compared with a diet enriched in ω -6 PUFAs. This change is affected through an increase in vessel regrowth during phase one of ROP by the suppression of the inflammatory cytokine, tumor necrosis factor- α (TNF- α), which in the retina is secreted by microglia and macrophages.⁴² Further exogenous administration of downstream effectors of ω -3 PUFAs, neuroprotectin D1, resolvins D1 and E1, conferred protection from both vaso-obliteration as well as retinal neovascularization. The suppressive effects of ω -3 PUFAs supplementation has been demonstrated to be comparable to the effects of anti-VEGF therapy in the mouse model of OIR offering a promising new therapy for the prevention of ROP.¹⁶

SUMMARY

ROP is a clinically multifactorial process that can lead to potentially devastating consequences for vision in the preterm infant. In spite of improved morbidity from current ablative therapies in the retina, the development of non-destructive therapies is actively being sought. Studies of the molecular pathogenesis of ROP have helped to identify therapeutic targets including factors that are hypoxia regulated (VEGF and Epo) and those maternally derived factors that are lost in the preterm infant (IGF-1 and ω -3 PUFAs). It is critical that any strategy that seeks to affect the levels of these factors must consider their phase-specific effects on the development of ROP.

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