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Pathophysiology and Mechanisms of Severe Retinopathy of Prematurity

M. Elizabeth Hartnett, MD

Retinopathy of prematurity (ROP) affects only premature infants, but as premature births increase in many areas of the world, ROP has become a leading cause of childhood blindness. Blindness can occur from aberrant developmental angiogenesis that leads to fibrovascular retinal detachment. To treat severe ROP, it is important to study normal developmental angiogenesis and the stresses that activate pathologic signaling events and aberrant angiogenesis in ROP. Vascular endothelial growth factor (VEGF) signaling is important in both physiologic and pathologic developmental angiogenesis. Based on studies in animal models of oxygen-induced retinopathy (OIR), exogenous factors such as oxygen levels, oxidative stress, inflammation, and nutritional capacity have been linked to severe ROP through dysregulated signaling pathways involving hypoxia-inducible factors and angiogenic factors like VEGF, oxidative species, and neuroprotective growth factors to cause phases of ROP. This translational science review focuses on studies performed in animal models of OIR representative of human ROP and highlights several areas: mechanisms for aberrant growth of blood vessels into the vitreous rather than into the retina through over-activation of VEGF receptor 2 signaling, the importance of targeting different cells in the retina to inhibit aberrant angiogenesis and promote physiologic retinal vascular development, toxicity from broad and targeted inhibition of VEGF bioactivity, and the role of VEGF in neuroprotection in retinal development. Several future translational treatments are discussed, including considerations for targeted inhibition of VEGF signaling instead of broad intravitreal anti-VEGF treatment. *Ophthalmology 2015;122:200-210 © 2015 by the American Academy of Ophthalmology.*

Retinopathy of prematurity (ROP) was described in 1942 by Terry[1] as "retrolental fibroplasia," which likely represents the most severe form of ROP, stage 5. Earlier stages of ROP were not well described because the Schepens/Pomerantzeff binocular indirect ophthalmoscope[2] had not been adopted universally to examine the peripheral retina. To understand potential causes of ROP, investigators exposed newborn animals, which vascularize their retinas postnatally, to conditions similar to what human premature infants then experienced. From early studies in animals and later a clinical trial in human infants by Arnall Patz[3], it became recognized that high oxygen at birth damaged fragile, newly formed retinal capillaries, causing "vaso-obliteration." After animals were removed from supplemental oxygen to ambient air, "vasoproliferation" occurred at junctions of vascular and avascular retina. Thus, the 2-phase hypothesis was developed, almost 30 years before the classification of human ROP into zones and stages. With advances in neonatal care, including the ability to monitor and regulate oxygen, and in funduscopic imaging of the peripheral retina in preterm infants before the development of stage 5 ROP, several changes in our understanding of ROP occurred. First, the hypothesis of ROP has been revised in that there is a delay in physiologic retinal vascular development and some hyperoxia-induced, vasoattenuation in phase 1, followed by vasoproliferation into the vitreous as intravitreal neovascularization (IVNV) in phase 2 (Fig 1).[3] Second, it is recognized that the phenotype of ROP differs throughout the world in association with resources for prenatal care and oxygen regulation. Preterm infants of older gestational ages and larger birth weights than those screened in the United States now are demonstrating severe ROP in some regions with insufficient nutrition and neonatal or prenatal resources and care, and where high, unregulated oxygen is used.[4,5] Finally, heritable causes are recognized as important,[6] but candidate gene studies often have been small and have not replicated findings potentially because of phenotypic variability.

The International Classification of ROP describes stages and zones of ROP severity.[7] Because human retinal

http://dx.doi.org/10.1016/j.ophtha.2014.07.050
ISSN 0161-6420/14

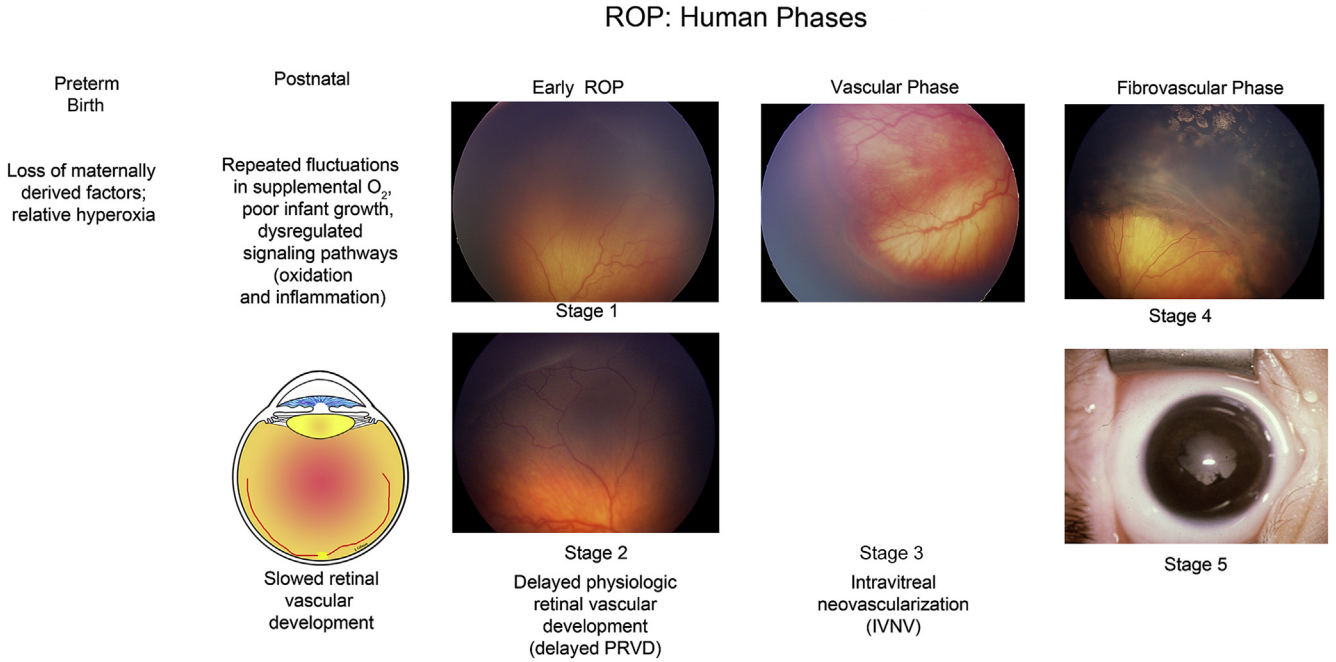

Figure 1. Human retinopathy of prematurity (ROP) classified by zone, stage, and the presence of plus disease. To facilitate comparing phases of ROP with experimental studies, ROP can be divided into Early Phase ROP, which comprises delayed physiologic retinal vascular development, and stages 1 and 2 ROP; Vascular Phase ROP, which comprises stage 3 ROP and, in severe ROP, plus disease; and Fibrovascular Phase ROP, which comprises stages 4 or 5 ROP with partial or total retinal detachment, respectively. Drawing by James Gilman, CRA, FOPS.

vasculature is not complete until term birth, an infant born prematurely initially has incomplete vascular coverage of the retina. The zones of ROP define the area of retina covered by physiologic retinal vascularization. The stages often progress sequentially and describe the severity of disease. Stages 1 and 2 represent early ROP, and stage 3 represents the vascular phase in which IVNV occurs (Fig 1). Stages 4 and 5 ROP represent the fibrovascular phase with retinal detachment.[8] Laser treatment for severe ROP, now described as type 1 ROP in the Early Treatment for Retinopathy of Prematurity Study,[9] can reduce the risk of a poor outcome in approximately 90% of eyes. In some infants, aggressive posterior ROP occurs, in which stage 3 and severe plus disease develops—without prior stages 1 or 2—in zone 1 or posterior zone 2.

It is important to consider human retinal vascular development when studying what goes awry in ROP. Because of the difficulty in obtaining intact human preterm infant eyes, studies on human retinal vascular development have been limited, but reports indicate that the initial retinal vasculature develops through vasculogenesis in the posterior pole from precursor cells that migrate out of the deep retina and into inner layers.[10,11] At approximately 15 weeks of gestation[11] until at least 22 weeks of gestation, these precursors become angioblasts and form the inner vascular plexus that extends to approximately zone 1. After 22 weeks of gestation, when it is difficult to obtain fetal human tissue, the ensuing development of the vascular plexi is based on studies carried out in other species and believed to occur through budding angiogenesis, that is, the proliferation and growth of blood vessels from existing blood vessels. In several species, astrocytes sense a physiologic hypoxia[12] and upregulate vascular endothelial growth factor (VEGF). Ensuing endothelial cells proliferate and migrate toward the gradient of VEGF and thereby extend the inner vascular plexus toward the ora serrata. Angiogenesis also is important in the development of the deep retinal plexi. Besides astrocytes, glia, like Müller cells, and neurons, such as ganglion cells, are also important.[13–15] The process is complex and requires interactions between different cell types and regulation of signaling pathways through several family members of VEGF and other pathways, including delta-like 4/notch and robo/slit, as examples, which regulate interactions between the sensing, endothelial tip cells and the proliferating stalk cells.[16] Of all the factors involved in physiologic retinal vascular development, it is clear that VEGF is essential.

Animal Models to Study Retinopathy of Prematurity

It is not safe to experiment on human preterm infant eyes because of risks of bleeding and retinal detachment. Therefore, models of oxygen-induced retinopathy (OIR) are performed in animals that vascularize their retinas postnatally to study disease mechanisms. Most OIR models recreate only some aspects of human ROP. All models have limitations because they use newborn, instead of premature, animals. Newborn animals are healthy and do not have the comorbidities of human preterm infants, such as necrotizing enterocolitis, sepsis, bronchopulmonary dysplasia, shunting of oxygenated and deoxygenated blood, and immature lung development. Animals experience much higher arterial oxygen levels when given similar inspired oxygen levels as premature infants with