

The combined effect with Erythropoietin and VEGF in retinal angiogenesis

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Purpose

Erythropoietin (EPO) is being considered to improve cognitive development and replenish lost red blood cells in preterm infants. However, EPO is also associated with increased risk of severe Retinopathy of prematurity (ROP). This study is to investigate the role of EPO and EPO Receptor and crosstalk with VEGF in angiogenesis in a rat oxygen-induced retinopathy (OIR) model.

Introduction

ROP is a major cause of vision loss and blindness in infants world-wide. The incidence of ROP is estimated at approximately 68% among infants born less than 1251 g in weight and 98% among infants born with a birth weight less than 750 g [1]. ROP causes damage to the retinal microvasculature with pathologic angiogenesis, vascular leakage and retinal detachment.

Vascular endothelial growth factor (VEGF) has been found to play an important role in the pathologic angiogenesis seen in ROP. However, it is also important in retinal vascular development. Inhibition of the bioactivity of VEGF in preterm infants with ROP has led to reduced pathologic angiogenesis, but also to persistent avascular retina and recurrent pathologic angiogenesis. Therefore, more study is needed to understand the role of VEGF and its interaction with other angiogenic factors in both developmental and pathologic angiogenesis.

Erythropoietin (EPO) regulates red blood cell production by binding to its cell surface receptor, EPOR, expressed on erythroid progenitor cells. Although EPO was originally believed to be an erythroid-specific hematopoietic cytokine, there is greater evidence that the biologic effects of EPO are not limited to erythropoiesis, but includes tissue protection and angiogenesis [2,3].

Methods

50/10 OIR MODEL [4]: Within 4 hours of birth, pups and their mothers were placed into the Oxycycler (Biospherix, NY), which cycled oxygen between 50% and 10% every 24 hours for 14 days. At P18, lectin stained retinal flat mounts were made and analyzed for intravitreal neovascularization/total retinal areas (IVNV) were measured with ImageJ. In fellow eyes, retinal mRNAs were extracted by Trizol, EPO and EPOR expressions were quantified by real-time PCR. Phosphorylation and expression of proteins were determined by Western Blot. Immunofluorescence was performed to detect the localization of pEPOR and pVEGFR2. Human retinal microvascular endothelial cell (HRMVEC) proliferation was measured by MTT assay.

References

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Results

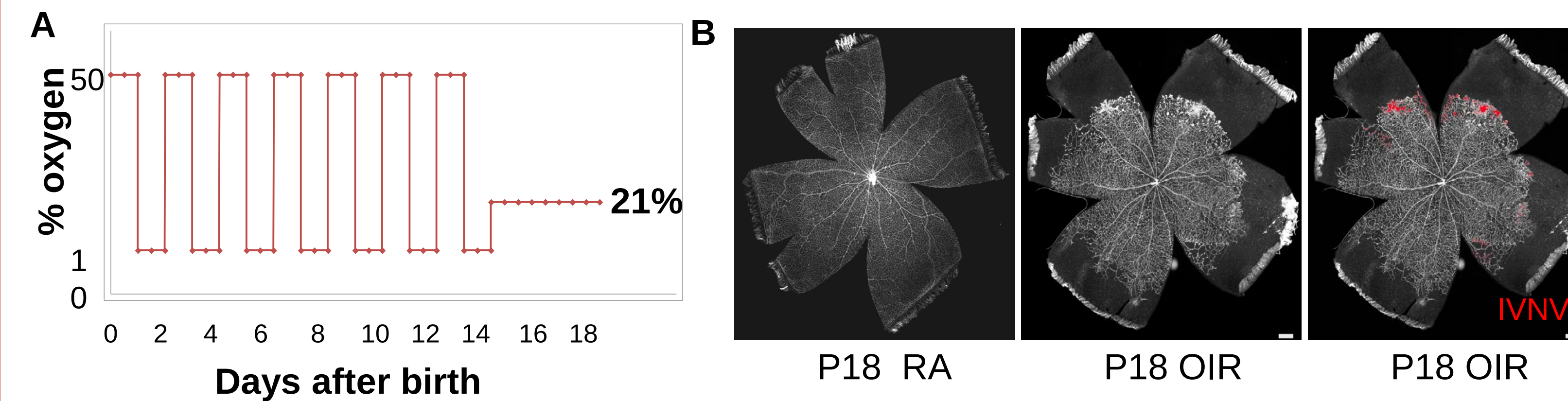


Fig 1. rat 50/10 OIR model. A. rat pups were put into the Oxycycler incubator for 14 days. After return to room air at P18 IVNV was measured. Compared to the left flat mount (P18 room air, RA), pups in OIR model show IVNV as indicated in red at the junction of avascularized and vascularized retina.

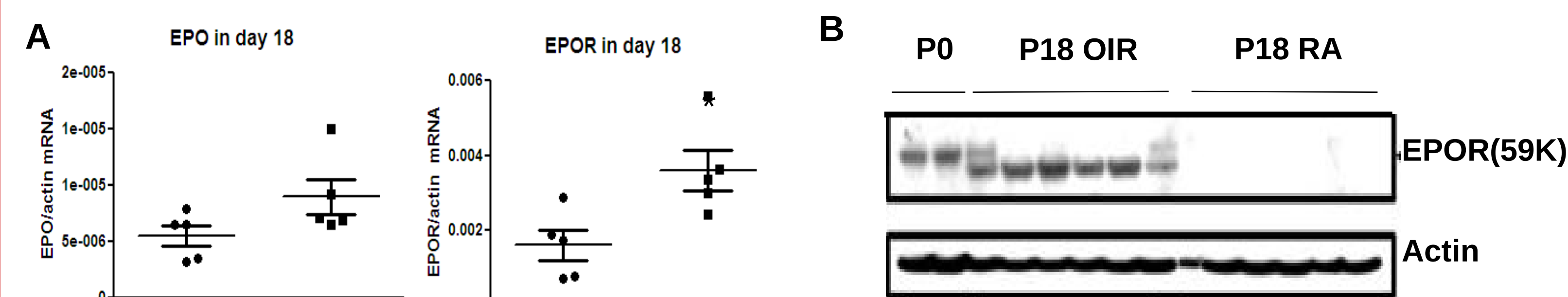


Fig 2. EPO receptor (EPOR) increased in rat 50/10 OIR model compared with room air (RA) at p18. A. mRNA were extracted from rat retina, EPO and EPOR mRNA level were measured by real-time PCR. (*:P<0.05 EPOR in OIR vs. RA). B. EPOR protein was detected by western blot and increased in P18 OIR.

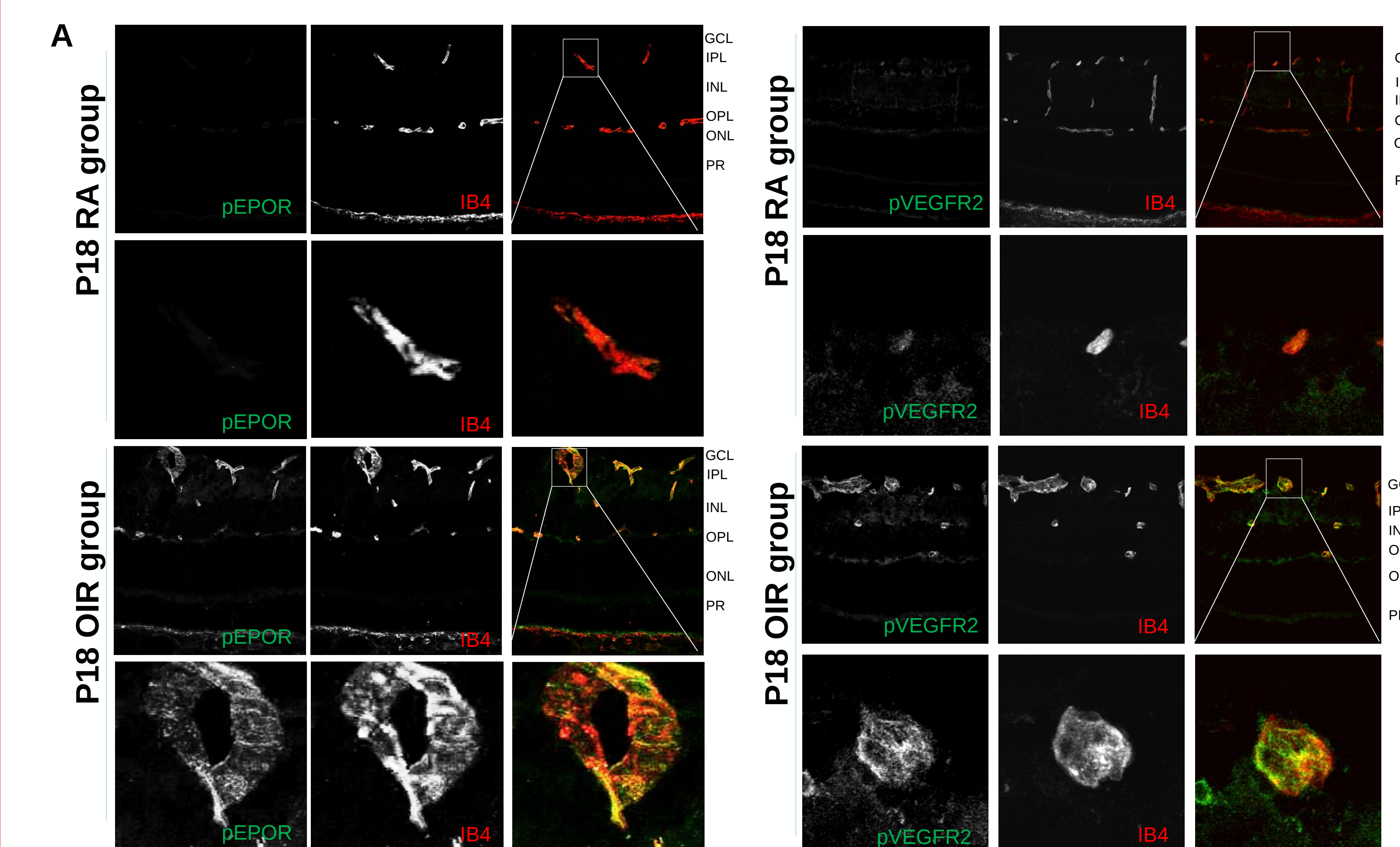


Fig 3. pEPOR increased in rat 50/10 OIR at P18 A. IHC was performed to detect the localization of pEPOR and pVEGFR2. Isolectin B4 (IB4) was used to label blood vessels. Both pEPOR and pVEGFR2 co-labeled with IB4 and appeared to be increased at P18 in the 50/10 OIR group compared to RA.

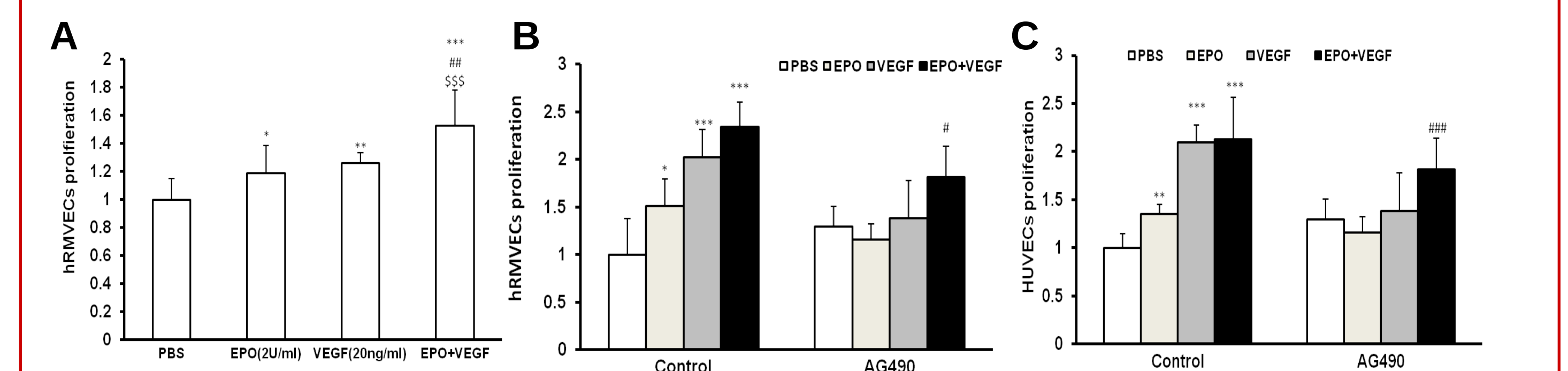
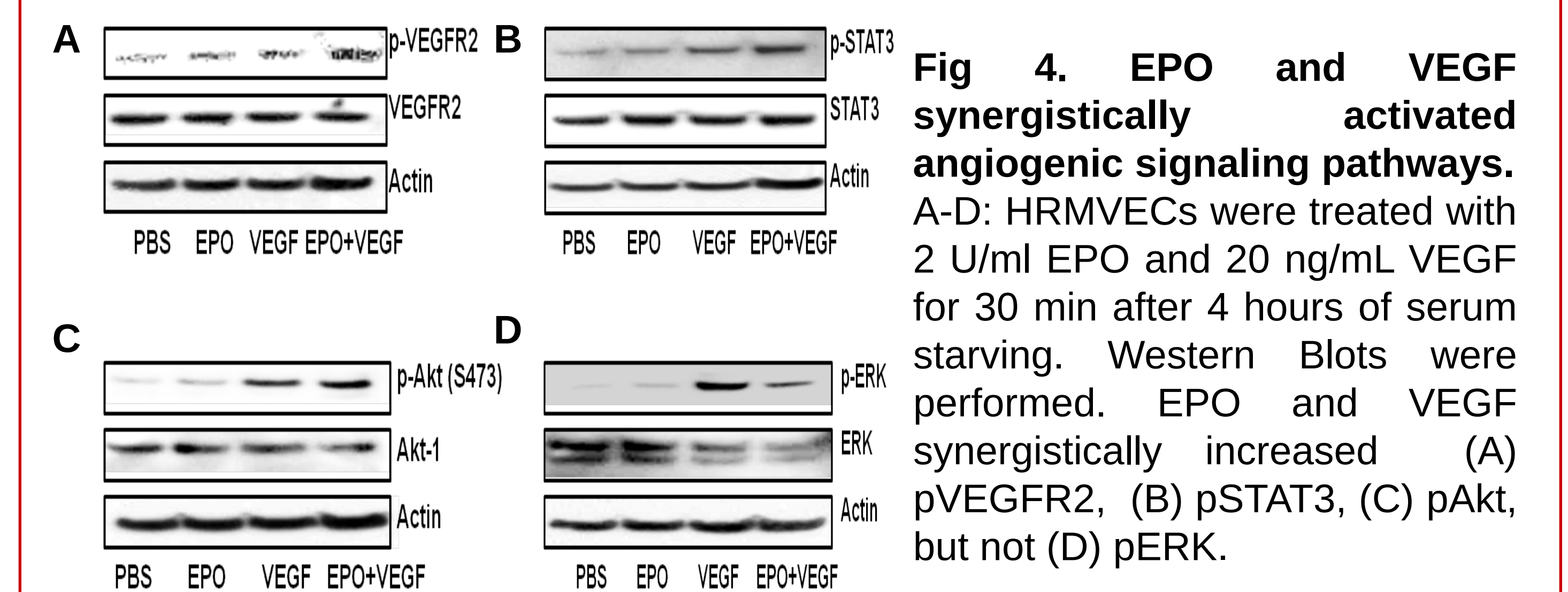
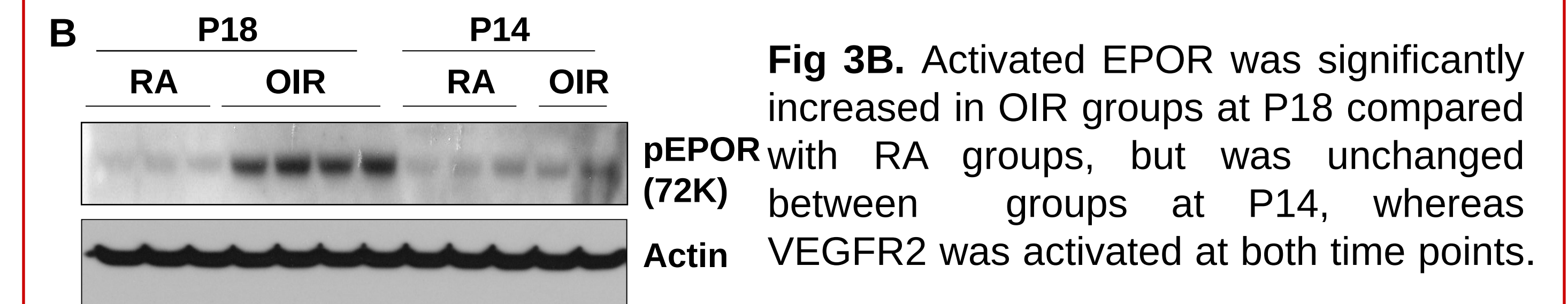


Fig 5. EPO and VEGF synergistically increased HRMVEC proliferation through pSTAT3.

A. HRMVECs treated with EPO, VEGF or both for 24 hours following serum starvation. Proliferation was measured by the MTT assay. B-C: Inhibition of pSTAT3 reduced proliferation from stimulation with EPO, VEGF or both. *P<0.05 vs. PBS; **P<0.01 vs. PBS; ***P<0.005 vs. PBS; #:P<0.05 vs. PBS; ###:P<0.01 vs. EPO; ####:P<0.005 vs. PBS; \$\$\$P<0.005 vs. VEGF

Conclusion

1. pEPOR increased in lectin-stained vessels at P18 in the 50/10 OIR model when VEGF has been reported to be upregulated.[5].
2. EPO and VEGF synergistically activated a number of angiogenic signals.
3. EPO and VEGF mediated HRMVEC proliferation via activation of pSTAT3.

Determining the mechanisms for the interaction between VEGF and EPO may lead to a safe method to use EPO for cognitive development in preterm infants without worsening VitNV.

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