Stabilization of the retinal vascular network by reciprocal feedback between blood vessels and astrocytes

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Summary

Development of the retinal vasculature is controlled by a hierarchy of interactions among retinal neurons, astrocytes and blood vessels. Retinal neurons release platelet-derived growth factor (PDGFA) to stimulate proliferation of astrocytes, which in turn stimulate blood vessel growth by secreting vascular endothelial cell growth factor (VEGF). Presumably, there must be counteractive mechanisms for limiting astrocyte proliferation and VEGF production to prevent runaway angiogenesis. Here, we present evidence that the developing vessels provide feedback signals that trigger astrocyte differentiation – marked by cessation of cell division, upregulation of glial fibrillary acidic protein (GFAP) and downregulation of VEGF. We prevented retinal vessel development by raising newborn mice in a high-oxygen atmosphere, which leads, paradoxically, to retinal hypoxia (confirmed by using the oxygen-sensing reagent EF5). The forced absence of vessels caused prolonged astrocyte proliferation and inhibited astrocyte differentiation in vivo. We could reproduce these effects by culturing retinal astrocytes in a low oxygen atmosphere, raising the possibility that blood-borne oxygen itself might induce astrocyte differentiation and indirectly prevent further elaboration of the vascular network.

Ke -d : A - c e , Re i a, Bl d e el , PDGF-A, O ge , T-a ge ic mice

Introduction

Re i al a - c e a-e belie ed be-e ible f - c - lli g de el me f he-e i al a o la - e i mammal . A - c e eme-ge f- m he ic e- e head a- - d bi- h a d - ead a a - life-a i g cell - la i ac- he i e- - -face f he -e i a (S e a d D-ehe-, 1987; Wa a abe a d Raff, 1988; Li g e al., 1989; Sa de-c e e al., 1999). The -e l i g e -k f a - c e ac a a em la e f - he de el i g -e i al a o la -e, hich al -ead f- m he ic e- e head (Jia g e al., 1995; Zha g a d S e, 1997; F- ige-, 2002). The -eci e mecha i m b hich -e i al a - c e g ide e d helial cell ac- he-e i a a-e e i-el de- d b a-ece d ha h ha he adhe i m leo le R-cadhe-i , e -e ed b -e i al a - c e , i im - a f- -mal -e i al a o la- de el me (D -ell e al., 2002). Re i al a - c e al e -e a o la-e d helial cell g- h fac - (VEGF), hich - m e e d helial cell - life-a i a d mig-a i (Al e al., 1995; S e e al., 1995; Pie-ce e al., 1996; P- i e al., 1997). I ha bee h ha e d helial cell a he leadi g edge f he a o la- le e l g l dia ha cl el f ll he de-l i g a - c e caff ld (D -ell e al., 2002; Ge-ha-d e al., 2003). The di-ec ed e e i f he e l dia i media ed ia VEGF -ece - 2, a d i de e de he c -ec ai ial di -ib i f VEGF i hi he -e i a (Ge-ha-d e al., 2003). We -e i l f d ha di - ba ce he a - c e e -k - gl affec a o la- a e- i g (F- ige- e al., 1996). Th , he al a-a geme f -e i al bl d e el de e d c- i call he -e- a e- f a - c e ; fac - ha i - e ce -e i al a - c e de el me - l ima el al affec -e i al e el .

Re i al a - c e a-i e f- m a - la i f -eco - - cell i he ic e- e head ha e -e Pa 2 a d he la ele-de- i ed g- h fac - -ece - al ha (PDGFRα) (M dha- e al., 1993; O e e al., 1998; Ch e al., 2001; Dak b e al., 2003). A he e a - c e -eco - - i ade he -e i a he - life-a e- a idl a d a- e -e l le el f GFAP. A he ma - e, -e i al a - c e bec me - ie ce a d di- la - g GFAP imm -eac i i (Ch e al., 2001; Ga-ia , 2003). PDGFRα c i - e be e -e ed a all age f ma - a i a d i im - a f -e i al a - c e - life-a i a d mig-a i , bei g ac i a ed mai l b PDGFA h m dime- f- m -e i al e - (M dha- e al., 1993; F- ige- e al., 1996). I - a ge ic mice ha e -e -e PDGFA - de- he c - l f he e - - eci c e la e ge e - m e- (*E-D A* mice), he - mbe- f -e i al a - c e i g-ea l i c-ea ed, ca i g a - - i al e-g- h f he -e i al a o la -e (F- ige- e al., 1996). Thi i dica e ha PDGFA i -mall a limi i g fac - f- a - c e - life-a i a d b e - e a gi ge e i . We - eco la ed ha if hi limi a i e-e e-c me b c-ea i g a a c- i e mi ge ic l i a - c e , he he a - c e migh - life-a e i de i el a d -igge- c - lled a gi ge e i .

We e ed hi b e gi ee- i g -a ge ic mice ha e -e

a *D A* -a ge e de- c - l f he *A* ge e - m e- (*A* - *D A* mice) (F- ige- e al., 2000), ha a - c e - ide hei- PDGFA. De i e d-ama ic ea-l h e- - life-a i f a - c e a d bl d e el , cell - life-a i ill l ed d a d ed i hi a eek af e- bi- h j a i ild e mice al h gh al cell mbe- e- e m ch highe- i he -a ge ic . We f d e ide ce ha e- e i f he *D A* -a ge e - i -ece - PDGFRα i e i g i hed i he -a ge ic , he- e m be he- fac - ha limi -e i al a - c e - life-a i e e he PDGFA i i e ce .

I he e e d , e ied ide if fac - he- ha PDGF ha limi -e i al a - c e - life-a i . We iced ha, i e a al mice, m a - c e - life-a i a d VEGF e e i co - i he e-i he-al e i a, ahead f he ad a ci g a o la -e. Thi gge ed ha bl d e el - hei- c e migh ega i el -eg la e b h a - c e - life-a i a d VEGF e e i . We e ed hi b ai i g e b - mice i a high- ge a m he-e, hich bl ck de el me f-e i al bl d e el a d c e e l lead i e h ia. Thi a acc m a ied b e ha ced a - c e - life-a i a d VEGF - d c i , gge i g ha bl d e el -mall limi hei- f -ma i h- gh a feedback ig al( ) ha e- e o ff he VEGF l . The ega i e feedback c ld be media ed b a m leo le( ) -elea ed f- m e d helial cell - he bl d. We f d ha a - c e - life-a i a d diffe-e ia i c ld be c - lled i i - b ma i la i g ge e i , -ai i g he ibili ha ge i elf migh be a ac i e m ie .

e e ie ci g m ia, de i e he ab e ce f e i al e el . A likel e la a i i ha he ic e e head i ge a ed di ec l b he maj h al id a e ha a e al g he ic e e, h gh he ce al e i a he le (Cla a d F ige , 2003). N e ha hi h al id a e i i ible i h le-m e a a i .

Retinal oxygen tension measured with the hypoxia marker EF5

I de i ali e ge a i i h le-m e i ae e ed he EF5 h ia ma-ke em (K ch, 2002). M e

-m ic o l-e , b -emai ed ele a ed h- gh he o l-e e-i d (Fig. 5I).

Discussion

A l f-e ea-ch ha f o ed l cal fac - ha c - l bl d e el g- h. H e e-, li le i k ab he-e e- e: h bl d e el affec de el me f - di g i e. I hi d , e- ide e ide ce ha bl d e el e e- a ega i e

e-idi g fac - f - i hibi i g -e i al a - c e - life-a i . O - di g -ai e he - e i f he he- -e i al a - c e ill - life-a e - checked i *A - D* *A* mice if e ca im l a e - l -e e de el me f he-e i al a o la -e. T e hi , e ied bl ck e el g- h i e a al *A - D* *A* mice b -ai i g hem i a 80% O_2 a m he-e b i h - cce , e-ha beca e he g-ea l i c-ea ed - mbe- f-e i al a - c e i he-a ge ic - ided - g a a gi ge ic im l be e-c me b h e- ia.

I he- e e d e al -e e e ide ce - gge i g ha i - e ge a i migh be he c-i ical fac - ha d-i e diffe-e ia i f-e i al a - c e . I i- , l ge ke -e i al a - c e i a - life-a i g, imma - e a e, he-ea i c-ea ed ge i hibi ed hei- - life-a i a d i d ced a m -e ma - e, GFAP- i i e he e. F- m - e e-ime i i ible de e-mi e he he- ge le el affec a - c e di-ec l - i di-ec l ; f - e am le, b im la i g he-elea e f diffe-e ia i fac - f- m he-e i al cell . I

(Mal e e a d Sim , 1998). The-ef -e, ma cell e de el a d diffe-e ia e i a e i- me f cha gi g ge c ce -a i a d i eem likel ha m -e e am le ill be f d f cell ma i g i -e e -i i g ge le el .

H d die f-e i al a c e a l he--egi f he CNS? A c e i he g-a ma e- f he b-ai i al c d a-e all GFAP- ega i e, a a- f- m h e ha eci call c ac bl d e el , hich e -e GFAP gl (Big ami a d Dahl, 1974). I i ible ha a c e i c ac i h bl d e el a-e i d ced e -e GFAP b diffe-e ia i fac ec-e ed b a o la- cell -, al e- a i el , beca e he e e-ie ce highe- ge c ce -a i ha a c e fi -he- a a f- m e el . Se e ce a al i f he *A* m e- -egi -e eal e e-al h ia -e e eleme (h), b da e i ha bee e abli hed h he he- he e affec *A* -a c-i i i i .

I fi -e i ill be i e-e i g e l -e he de el me al -ela i hi be ee a c e , bl d e el a d ge i he b-ai a d i al c d. I i al a i -ig i g ibili ha eci cal feedback be ee e el-i d ci g cell a d hei- a cia ed e el migh g e- a o la- de el me i he- a f he emb ide he CNS.

We ha k S a e Cla f a di g ech ical a i a ce a d c lleag e f di o i a d c c i e c-i ici m. We al ha k