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Stanniocalcin-1 is a modifier of oxygen induced retinopathy severity

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Abstract

Purpose/Aim: Abnormal activation of signaling pathways related to angiogenesis, inflammation, and oxidative stress has been implicated in the pathophysiology of retinopathy of prematurity (ROP), a leading cause of blindness in pre-term infants. Therapies for ROP include laser and anti-vascular endothelial growth factor agents. However, these therapies have side effects, and even with adequate treatment, visual acuity can be impaired. Novel therapeutic options are needed. Stanniocalcin-1 (STC-1) is neuroprotective protein with anti-inflammatory and anti-oxidative stress properties. Rodent models of oxygen-induced retinopathy (OIR) were selected to determine whether STC-1 plays a role in the development of OIR.

Materials and methods: STC-1 gene and protein expression was first evaluated in the Sprague Dawley rat OIR model that is most similar to human ROP. OIR was then induced in wild-type and *Stc-1*$^{-/-}$ mice. Retinas were isolated and evaluated for avascular and neovascular area on retinal flat mounts. Quantification of gene expression by quantitative real-time PCR was performed. VEGF was assayed by ELISA in media obtained from induced pluripotent stem cell derived retinal pigment epithelial (iPS-RPE) cells following treatment with recombinant STC-1.

Results: STC-1 was significantly upregulated in a rat model of OIR compared to room air controls at the gene ($P<0.05$) and protein ($P<0.001$) level. *Stc-1*$^{-/-}$ OIR mice showed significantly worse ROP compared to wild-type mice as assessed by avascular ($20.2 \pm 2.4\%$ vs $15.2 \pm 2.5\%$; $P=0.02$) and neovascular area ($14.3 \pm 2.7\%$ vs $8.8 \pm 3.7\%$; $P<0.05$). Transcript levels of vascular

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endothelial growth factor-A were significantly higher in *Stc-1*$^{-/-}$ OIR mice compared to wild-type controls (P=0.03). STC-1 reduced VEGF production in iPS-RPE cells (P=0.01).

Conclusions: STC-1 plays a role in the OIR stress response and development of pathologic vascular features in rodent OIR models by regulating VEGF levels.

Keywords

Retinopathy of prematurity; oxygen induced retinopathy; Stanniocalcin-1; STC-1

Introduction

Retinopathy of prematurity (ROP) is a leading cause of childhood blindness. [1–3] Aberrant vascular endothelial growth factor (VEGF) signaling contributes to neovascularization and retinal detachment in advanced disease.[4] While laser ablation of avascular retina and anti-VEGF agents can be effective treatments, [5–12] there are concerns regarding local and systemic side effects.[13, 14] Targeted therapies[15] with lower side effect profile are needed. Multiple pathways,[16] have been implicated in ROP pathogenesis, and therapies that regulate angiogenesis, inflammation, and neurodegeneration have been proposed.[17]

Stanniocalcin-1 (STC-1) is a multi-functional protein that is upregulated by cellular stresses. [18–20] STC-1 is cytoprotective in neurons,[20, 21] photoreceptors,[22] and retinal ganglion cells,[23] and reduces intraocular pressure[24], oxidative stress[22], and inflammation.[25] Its neuroprotective effects have been associated with uncoupling oxidative phosphorylation by induction of mitochondrial uncoupling protein-2, yielding anti-oxidant capacity.[22] We hypothesized that STC-1 might be a stress-response protein that is capable of decreasing inflammatory and oxidative stress underlying ROP.

Materials and methods

Rodent oxygen induced retinopathy models

To determine whether STC-1 signaling was altered in a representative ROP model,[4] we selected the rat OIR model.[26, 27] To determine the effects of STC-1 knockdown, we used the *Stc-1*$^{(-/-)}$ mouse.[28] Studies were approved by University of Utah and Mayo Clinic (Rochester, MN) IACUC and adhered to ARVO guidelines. Newborn Sprague-Dawley rats (Charles River, Wilmington, MA) and mothers were housed in cycled oxygen which alternated between 50% and 10% every 24 hours for 14 days and sacrificed (post-natal day; P14) or placed room air (RA) for 3 (P17), 4 (P18), or 6 (P20) additional days. Controls were maintained at RA throughout the experiment and sacrificed at identical time-points. *Stc-1*$^{(-/-)}$ and wild-type control mice were obtained from the Sheikh-Hamad laboratory (Baylor College of Medicine) and bred at Mayo Clinic. P7 wild-type (n=19) and *Stc-1*$^{(-/-)}$ mice (n=15, 3 separate litters)[29] with alternating surrogate mothers were maintained in 75% oxygen for 5 days (P7-P12), and then RA (21% oxygen) for 5 days (P12-17) and sacrificed at P17.

Expression of STC-1 in rat OIR

Rat OIR pups were euthanized at P18 (n=3 RA, n=10 OIR). Retinas were harvested, and lysates were probed by western blot for β-actin and STC-1 (R&D Systems, Minneapolis, MN),[30] and relative quantification (RQ) values for STC-1 expression normalized to β-actin were calculated with UN-SCAN-IT Gel 6.1 (Orem, UT).[27] For STC-1 RNA expression analysis, rat OIR pups were euthanized at P14 (n=3 wild-type, n=4 OIR), P17 (n=3 wild-type, n=4 OIR), or P20 (n=3 wild-type, n=3 OIR). Retinas were harvested for molecular analysis as previously described.[22] Samples were homogenized in RNA Bee (Fisher Scientific, Waltham, MA) on ice by passage through a 19.5g needle. After aqueous phase extraction, RNA was purified (RNeasy Mini; Qiagen, Valencia, CA) and cDNA generated by reverse transcription (SuperScript III; Invitrogen). Quantitative real-time PCR (qPCR) amplification was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA), and Taqman Gene Expression Assay probes (Fisher Scientific) for Stanniocalcin-1 (Stc-1-Rn00579636) and normalized to 18s ribosomal RNA (18s-Rn03928990).

Retinal flat mounts

To examine the effect of OIR on *Stc-1*$^{(-/-)}$ mice, OIR pups were euthanized at P17. One eye from each mouse was enucleated and the retina was harvested for flat mounts.[31] Eyes were fixed in 4% paraformaldehyde (0.1M phosphate buffer) for 90 minutes then transferred to phosphate buffered saline (PBS). Retinas were dissected, washed in PBS, blocked with 1% bovine serum albumin, incubated overnight with TRITC-labeled IsolectinB4 (Invitrogen, Carlsbad, CA), probed with primary antibodies against GFAP (DakoCytomation, Denmark) to label glia, and NG2 (Chemicon, Temecula, CA) to label pericytes, and incubated with secondary antibody Alexa-Fluor 488 goat anti-rabbit (Invitrogen) for 1 hour. Specimens were incubated with DAPI (Sigma, St. Lois, MO) for 10 minutes for nuclear staining, flat-mounted using FluorSave Reagent (Calbiochem, San Diego, CA), and analyzed by microscopy. Adobe Photoshop v20.0.1 (San Jose, CA) was used to delineate neovascular and avascular retina as percentage of total flat mount area (Supplementary Figure 1).[32] Three masked investigators (2 board-certified ophthalmologists, 1 American Society of Clinical Pathology certified pathology assistant) independently performed segmentation analysis, and percentages were averaged. Graders were trained to manually exclude false positive areas due to hyaloid or background autofluorescence.

Gene expression in mouse OIR

Retina from P17 mice was harvested for molecular analysis as above. qPCR amplification was performed Taqman Gene Expression Assay probes (Fisher Scientific) for tumor necrosis factor, alpha-induced protein 2 (TNFAIP2-Mm00447578_m1), placental growth factor (PGF-Mm00435613_m1), chemokine ligand 11 (CCL11-Mm00441238_m1), complement component 3 (CC3-Mm01232779_m1), Stanniocalcin-1 (STC-1-Mm01322191_m1), vascular endothelial growth factor A (VEGF-A-Mm00437306_m1), toll-like receptor 4 (TLR4-Mm00445273_m1), fibroblast growth factor 2 (FGF2-Mm01285715_m1), hypoxia inducible factor 1, alpha subunit (HIF-1α-Mm00468869_m1), kinase insert domain protein receptor (KDR/VEGFR2-Mm01222421_m1), tumor necrosis factor (TNFα-

Mm00443258_m1), complement factor B (CFB- Mm00433909_m1), complement component 1, q subcomponent (c1qb- Mm01179619_m1), and 18S ribosomal RNA (18s-Mm03928990_g1) per manufacturer's directions. RQ for qPCR values were normalized to 18s RNA and then to wild-type OIR controls.

Cell culture experiments

Induced pluripotent stem cell derived retinal pigment epithelial cells (iPSC-RPE) which produce VEGF in culture,[33] were purchased from LAgen laboratories and cultured per manufacturer's instructions. After normalization of VEGF levels in conditioned media, 24 hrs of conditioned media was collected to serve as an untreated control. STC-1 (Biovender Research and Diagnostic Products; 500ng/mL) was added, and conditioned media was collected 24 hrs following initiation of treatment. Conditioned media was centrifuged to remove cellular debris and assayed for VEGF by ELISA (R&D Systems).

Statistical analysis

Statistical analysis was performed with ANOVA and Tukey post-hoc protected tests (densitometry for western blot), Mann-Whitney U test (quantification of avascular and neovascular retina), or Student's t-test (qPCR and ELISA analysis).

Results

Compared to RA controls, OIR rat pups showed significantly increased expression of STC-1 mRNA (Fig 1A) at P17 ($P<0.01$) and P20 ($P=0.02$). This corresponded with the significant increase in STC-1 protein levels at P18 by western blot (Fig 1B) which was confirmed by densitometry (Fig 1C, $P<0.001$) .

To determine whether STC-1 upregulation was pathologic or protective, we subjected *Stc-1*$^{(-/-)}$ and wild-type mice to OIR. *Stc-1*$^{(-/-)}$ mice subjected to OIR had increased avascular (Fig 2 15.2 ± 2.5% vs 20.2 ± 2.4%, $P=0.02$) and neovascular (Fig 2, 8.8 ± 3.7% vs 14.3 ± 2.7%, $P<0.05$) areas at P17 compared to wild-type.

To examine differential gene expression between wild-type and *Stc-1*$^{(-/-)}$ OIR mice, we selected known upregulated genes in the mouse OIR model at P17 as markers of disease induction.[34] VEGF-A was upregulated in *Stc-1*$^{(-/-)}$ vs. wild-type controls (Fig 3, $P=0.03$). There were no significant differences between wild-type and *Stc-1*$^{(-/-)}$ mice for HIF-1α, VEGFR2, TNFα, Cfb, C1qb, FGF2, TLR4, TNFAIP2, PGF, or CCL11 (Supplementary Fig 2). However, CC3 was higher in *Stc-1*$^{(-/-)}$ mice (Supplementary Fig 2, $P<0.05$), and as expected, STC-1 gene expression was undetectable in *Stc-1*$^{(-/-)}$ mice (Supplementary Fig 2, $P<0.001$).

To determine whether STC-1 has an effect on VEGF production, we selected iPS-RPE cells which produce VEGF in standard culture conditions. Treatment with STC-1 significantly reduced VEGF concentration in iPS-RPE conditioned media at 24 hours (Fig 4, $P=0.01$).

Discussion

STC-1 is induced by pathologic stresses, including inflammation,[35] oxidation,[36] and hypoxia.[18–20] We have now shown that STC-1 is induced by OIR at the gene and protein level, adding OIR to the list of cellular stresses that induce STC-1 expression. Under physiologic conditions, *Stc-1*$^{(-/-)}$ mice appear phenotypically indistinguishable from wild-type including ocular anatomy. [24] However, when subjected to pathologic stress, *Stc-1*$^{(-/-)}$ mice develop worse disease compared to controls, as previously demonstrated in models of acute kidney injury[37] and stroke.[20] This study adds ROP to the list of disease models with worse outcomes in the absence of STC-1.

Assessment of genes previously reported to be altered in OIR compared to wild-type mouse models showed only VEGF-A and CC3 with increased expression. While increased VEGF-A levels are known to be upregulated in rodent OIR models, we also found that they were significantly higher in *Stc-1*$^{(-/-)}$ mice compared to wild-type mice subjected to OIR, consistent with worse ROP in these animals. Our finding that STC-1 treated iPS-RPE cells resulted in decreased levels of VEGF in conditioned media suggest that STC-1 may be a regulator of VEGF expression. Because we did not find any expression change in HIF1α in *Stc-1*$^{(-/-)}$ mice compared to wild-type control, this suggests STC-1 may regulate VEGF levels downstream of HIF1α. Consistent with this hypothesis, STC-1 expression can be induced by binding of HIF1α to the STC-1 promoter.[38] Paradoxically, STC-1 has been reported to induce VEGF signaling leading to increased angiogenesis in other model systems.[39–41] Additional studies in multiple cell types and disease models are needed to clarify the role of STC-1 in VEGF expression and signaling.

Regarding higher levels of CC3 in *Stc-1*$^{(-/-)}$ mice, it remains unclear whether this is related to VEGF regulation (VEGF can affect complement regulation[42]), or secondary to direct anti-inflammatory effects of STC-1. A recent clinical study found increased CFH, CC3, and C4 in addition to VEGF at the protein level in the vitreous of ROP patients.[16]

Our data suggests STC-1 is a regulator of OIR severity in part due to its effect on VEGF production. Reduction in both avascular and neovascular retina in the presence of STC-1 could be related to a combined effect of neuroprotective and anti-VEGF properties. Further studies are needed to evaluate the therapeutic potential of STC-1 in ROP and other retinal vascular disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Faia LJTM. Retinopathy of prematurity In: Ryan SJSS, Hinton DR, Schachat AP editor. Retinopathy of prematurity. Retina: Saunders, an imprint of Elsevier Inc; 2013.
2. Lepore DQG, Molle F, Baldascino A, Orazi L, Sammartino M, Purcaro V, Giannantonio C, Papacci P, Romagnoli C. Intravitreal bevacizumab versus laser treatment in type 1 retinopathy of prematurity: Report on fluorescein angiographic findings. Ophthalmology 2014:121(11): 2212–2219. [PubMed: 25001158]
3. Fu ZNS, Li SY, Wong D, Chung SK, Lo AC. Deficiency of aldose reductase attenuates inner retinal neuronal changes in a mouse model of retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2015: Epub ahead of print.
4. Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. Ophthalmology 2015:122(1): 200–210. [PubMed: 25444347]
5. Group CfRoPC. Multicenter trial of cryotherapy for retinopathy of prematurity: Preliminary results. Pediatrics 1988:81(5): 697–706. [PubMed: 2895910]
6. Good WV ETfRoPCG. Final results of the early treatment for retinopathy of prematurity (etrop) randomized trial. Trans Am Ophthalmol Soc 2004:102:233–248. [PubMed: 15747762]
7. Chung EJKJ, Ahn HS, et al. Combination of laser photocoagulation and intravitreal bevacizumab (avastin) for aggressive zone i retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2007:245:1727–1730. [PubMed: 17690897]
8. Travassos ATS, Ferreira P, et al. Intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Ophthalmic Surg Lasers Imaging 2007:38:233–237. [PubMed: 17552391]
9. Quiroz-Mercado HM-CM, Hernandez-Rojas ML, et al. Antiangiogenic therapy with intravitreal bevacizumab for retinopathy of prematurity. Retina 2008:28:S19–S25. [PubMed: 18317339]
10. Menke MNFC, Nelle M, Berger MR, Sturm V, Wolf S. Intravitreal ranibizumab monotherapy to treat retinopathy of prematurity zone ii, stage 3 with plus disease. BMC Ophthalmol 2015:15:20. [PubMed: 25886603]
11. Bührer CBD. Oral propranolol: A new treatment for infants with retinopathy of prematurity? Neonatology 2015:108(1): 49–52. [PubMed: 25968340]
12. Fierson WM, Ophthalmology AAOPSo, American Academy Of O, American Association For Pediatric O, Strabismus, American Association Of Certified O. Screening examination of premature infants for retinopathy of prematurity. Pediatrics 2018:142(6).
13. Kong LBA, Demny AB, Coats DK, Li A, Rahman EZ, Smith OE, Steinkuller PG. Pharmacokinetics of bevacizumab and its effects on serum vegf and igf-1 in infants with retinopathy of prematurity. Invest Ophthalmol Vis Sci 2015:56(2): 956–961. [PubMed: 25613938]
14. Zhou YJY, Bai Y, Wen J, Chen L. Vascular endothelial growth factor plasma levels before and after treatment of retinopathy of prematurity with ranibizumab. Graefes Arch Clin Exp Ophthalmol 2015: Epub ahead of print.
15. Wang HZS, Hartnett ME. Signaling pathways triggered by oxidative stress mediate features of severe retinopathy of prematurity. JAMA Ophthalmol 2013:131(1): 80–85. [PubMed: 23307212]
16. Rathi S, Jalali S, Patnaik S, Shahulhameed S, Musada GR, Balakrishnan D, Rani PK, Kekunnaya R, Chhablani PP, Swain S et al. Abnormal complement activation and inflammation in the pathogenesis of retinopathy of prematurity. Front Immunol 2017:8:1868. [PubMed: 29312345]
17. Rivera JC, Holm M, Austeng D, Morken TS, Zhou TE, Beaudry-Richard A, Sierra EM, Dammann O, Chemtob S. Retinopathy of prematurity: Inflammation, choroidal degeneration, and novel promising therapeutic strategies. J Neuroinflammation 2017:14(1): 165. [PubMed: 28830469]
18. Ito Y, Zemans R, Correll K, Yang IV, Ahmad A, Gao B, Mason RJ. Stanniocalcin-1 is induced by hypoxia inducible factor in rat alveolar epithelial cells. Biochemical and biophysical research communications 2014:452(4): 1091–1097. [PubMed: 25251473]
19. Shi X, Wang J, Qin Y. Recombinant adeno-associated virus-delivered hypoxia-inducible stanniocalcin-1 expression effectively inhibits hypoxia-induced cell apoptosis in cardiomyocytes. J Cardiovasc Pharmacol 2014:64(6): 522–529. [PubMed: 25490418]

20. Durukan Tolvanen A, Westberg JA, Serlachius M, Chang AC, Reddel RR, Andersson LC, Tatlisumak T. Stanniocalcin 1 is important for poststroke functionality, but dispensable for ischemic tolerance. Neuroscience 2013:229:49–54. [PubMed: 23159313]
21. Zhang K, Lindsberg PJ, Tatlisumak T, Kaste M, Olsen HS, Andersson LC. Stanniocalcin: A molecular guard of neurons during cerebral ischemia. Proc Natl Acad Sci U S A 2000:97(7): 3637–3642. [PubMed: 10725397]
22. Roddy GW, Rosa RH Jr., Oh JY, Ylostalo JH, Bartosh TJ Jr., Choi H, Lee RH, Yasumura D, Ahern K, Nielsen G et al. Stanniocalcin-1 rescued photoreceptor degeneration in two rat models of inherited retinal degeneration. Mol Ther 2012:20(4): 788–797. [PubMed: 22294148]
23. Kim SJ, Ko JH, Yun JH, Kim JA, Kim TE, Lee HJ, Kim SH, Park KH, Oh JY. Stanniocalcin-1 protects retinal ganglion cells by inhibiting apoptosis and oxidative damage. PLoS One 2013:8(5): e63749. [PubMed: 23667669]
24. Roddy GW, Viker KB, Winkler NS, Bahler CK, Holman BH, Sheikh-Hamad D, Roy Chowdhury U, Stamer WD, Fautsch MP. Stanniocalcin-1 is an ocular hypotensive agent and a downstream effector molecule that is necessary for the intraocular pressure-lowering effects of latanoprost. Invest Ophthalmol Vis Sci 2017:58(5): 2715–2724. [PubMed: 28538979]
25. Huang L, Garcia G, Lou Y, Zhou Q, Truong LD, DiMattia G, Lan XR, Lan HY, Wang Y, Sheikh-Hamad D. Anti-inflammatory and renal protective actions of stanniocalcin-1 in a model of anti-glomerular basement membrane glomerulonephritis. Am J Pathol 2009:174(4): 1368–1378. [PubMed: 19246645]
26. Penn JS, Henry MM, Tolman BL. Exposure to alternating hypoxia and hyperoxia causes severe proliferative retinopathy in the newborn rat. Pediatr Res 1994:36(6): 724–731. [PubMed: 7898981]
27. Wang H, Yang Z, Jiang Y, Hartnett ME. Endothelial nadph oxidase 4 mediates vascular endothelial growth factor receptor 2-induced intravitreal neovascularization in a rat model of retinopathy of prematurity. Mol Vis 2014:20:231–241. [PubMed: 24623966]
28. Chang AC, Cha J, Koentgen F, Reddel RR. The murine stanniocalcin 1 gene is not essential for growth and development. Mol Cell Biol 2005:25(23): 10604–10610. [PubMed: 16287871]
29. Stahl A, Chen J, Sapieha P, Seaward MR, Krah NM, Dennison RJ, Favazza T, Bucher F, Lofqvist C, Ong H et al. Postnatal weight gain modifies severity and functional outcome of oxygen-induced proliferative retinopathy. Am J Pathol 2010:177(6): 2715–2723. [PubMed: 21056995]
30. Ohkouchi S, Block GJ, Katsha AM, Kanehira M, Ebina M, Kikuchi T, Saijo Y, Nukiwa T, Prockop DJ. Mesenchymal stromal cells protect cancer cells from ros-induced apoptosis and enhance the warburg effect by secreting stc1. Mol Ther 2012:20(2): 417–423. [PubMed: 22146344]
31. Moreno-Miralles I, Ren R, Moser M, Hartnett ME, Patterson C. Bone morphogenetic protein endothelial cell precursor-derived regulator regulates retinal angiogenesis in vivo in a mouse model of oxygen-induced retinopathy. Arterioscler Thromb Vasc Biol 2011:31(10): 2216–2222. [PubMed: 21737784]
32. Connor KM, Krah NM, Dennison RJ, Aderman CM, Chen J, Guerin KI, Sapieha P, Stahl A, Willett KL, Smith LE. Quantification of oxygen-induced retinopathy in the mouse: A model of vessel loss, vessel regrowth and pathological angiogenesis. Nat Protoc 2009:4(11): 1565–1573. [PubMed: 19816419]
33. Marmorstein AD, Johnson AA, Bachman LA, Andrews-Pfannkoch C, Knudsen T, Gilles BJ, Hill M, Gandhi JK, Marmorstein LY, Pulido JS. Mutant best1 expression and impaired phagocytosis in an ipsc model of autosomal recessive bestrophinopathy. Sci Rep 2018:8(1): 4487. [PubMed: 29540715]
34. Sato T, Kusaka S, Hashida N, Saishin Y, Fujikado T, Tano Y. Comprehensive gene-expression profile in murine oxygen-induced retinopathy. Br J Ophthalmol 2009:93(1): 96–103. [PubMed: 18838407]
35. Tang SE, Wu CP, Wu SY, Peng CK, Perng WC, Kang BH, Chu SJ, Huang KL. Stanniocalcin-1 ameliorates lipopolysaccharide-induced pulmonary oxidative stress, inflammation, and apoptosis in mice. Free Radic Biol Med 2014:71:321–331. [PubMed: 24685991]
36. Nguyen A, Chang AC, Reddel RR. Stanniocalcin-1 acts in a negative feedback loop in the prosurvival erk1/2 signaling pathway during oxidative stress. Oncogene 2009:28(18): 1982–1992. [PubMed: 19347030]

37. Pan JS, Huang L, Belousova T, Lu L, Yang Y, Reddel R, Chang A, Ju H, DiMattia G, Tong Q et al. Stanniocalcin-1 inhibits renal ischemia/reperfusion injury via an amp-activated protein kinase-dependent pathway. J Am Soc Nephrol 2015:26(2): 364–378. [PubMed: 25012175]

38. Law AY, Ching LY, Lai KP, Wong CK. Identification and characterization of the hypoxia-responsive element in human stanniocalcin-1 gene. Mol Cell Endocrinol 2010:314(1): 118–127. [PubMed: 19628018]

39. Zhao M, Xie W, Tsai SH, Hein TW, Rocke BA, Kuo L, Rosa RH Jr. Intravitreal stanniocalcin-1 enhances new blood vessel growth in a rat model of laser-induced choroidal neovascularization. Invest Ophthalmol Vis Sci 2018:59(2): 1125–1133. [PubMed: 29490350]

40. Law AY, Wong CK. Stanniocalcin-1 and −2 promote angiogenic sprouting in huvecs via vegf/vegfr2 and angiopoietin signaling pathways. Mol Cell Endocrinol 2013:374(1-2): 73–81. [PubMed: 23664860]

41. He LF, Wang TT, Gao QY, Zhao GF, Huang YH, Yu LK, Hou YY. Stanniocalcin-1 promotes tumor angiogenesis through up-regulation of vegf in gastric cancer cells. J Biomed Sci 2011:18:39. [PubMed: 21672207]

42. Keir LS, Firth R, Aponik L, Feitelberg D, Sakimoto S, Aguilar E, Welsh GI, Richards A, Usui Y, Satchell SC et al. Vegf regulates local inhibitory complement proteins in the eye and kidney. J Clin Invest 2017:127(1): 199–214. [PubMed: 27918307]

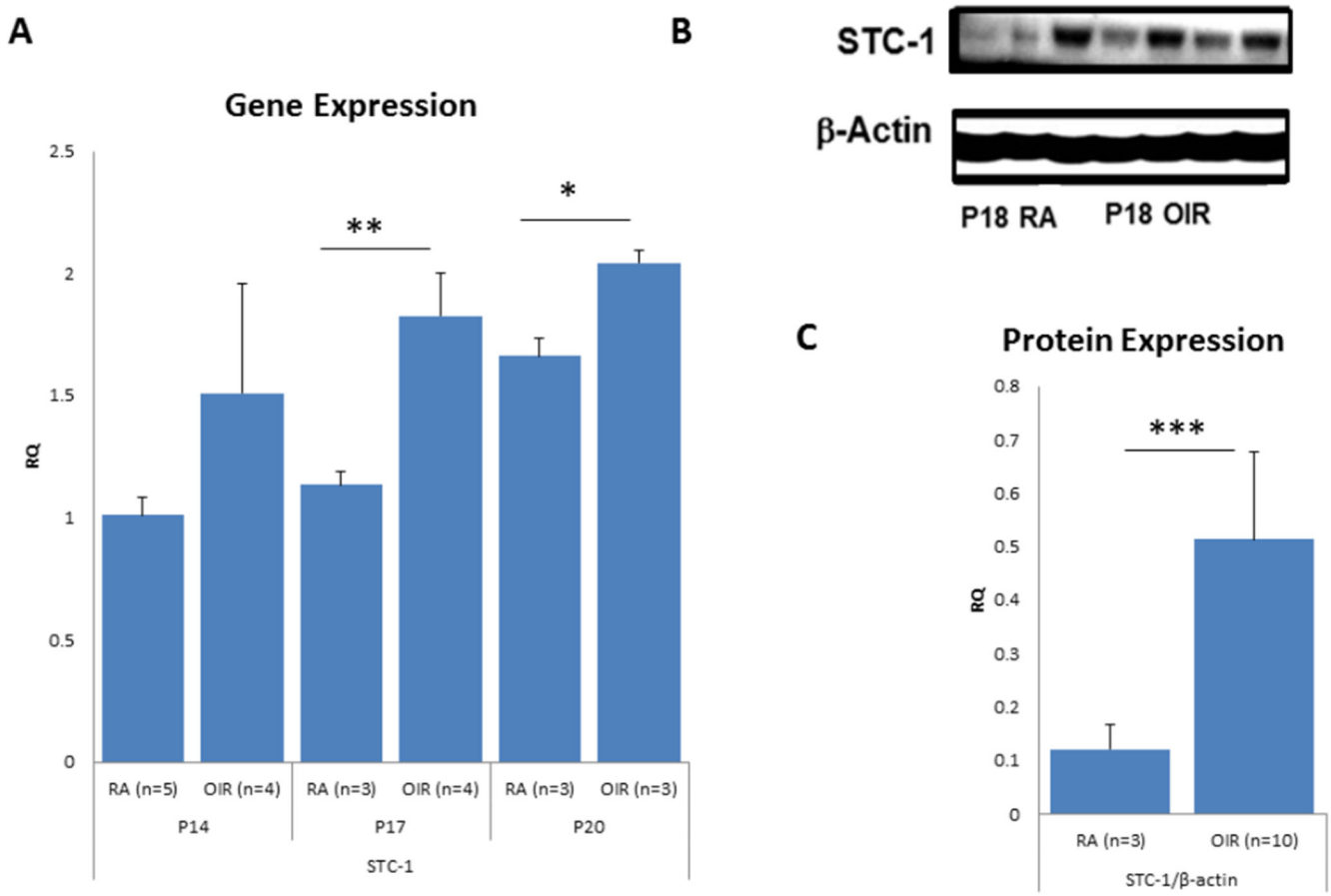

Figure 1. Stanniocalcin-1 is upregulated in a rat model of oxygen induced retinopathy.

(A) Quantitative real-time PCR shows a significant increase in STC-1 expression at P17 ($P<0.01$) and P20 ($P=0.02$) in wild-type oxygen induced retinopathy (OIR) rats compared to room air (RA) controls. Note there was no significant difference at P14. (B) Representative western blot reveals that STC-1 is upregulated in retinal lysates obtained from P18 OIR rats compared to RA. (C) Densitometry of STC-1 normalized to β-actin (Relative Quantification; RQ) revealed significantly increased STC-1 in OIR (n=10, $p<0.001$)*** compared to P18 RA controls (n=3). Error bars represent SEM.

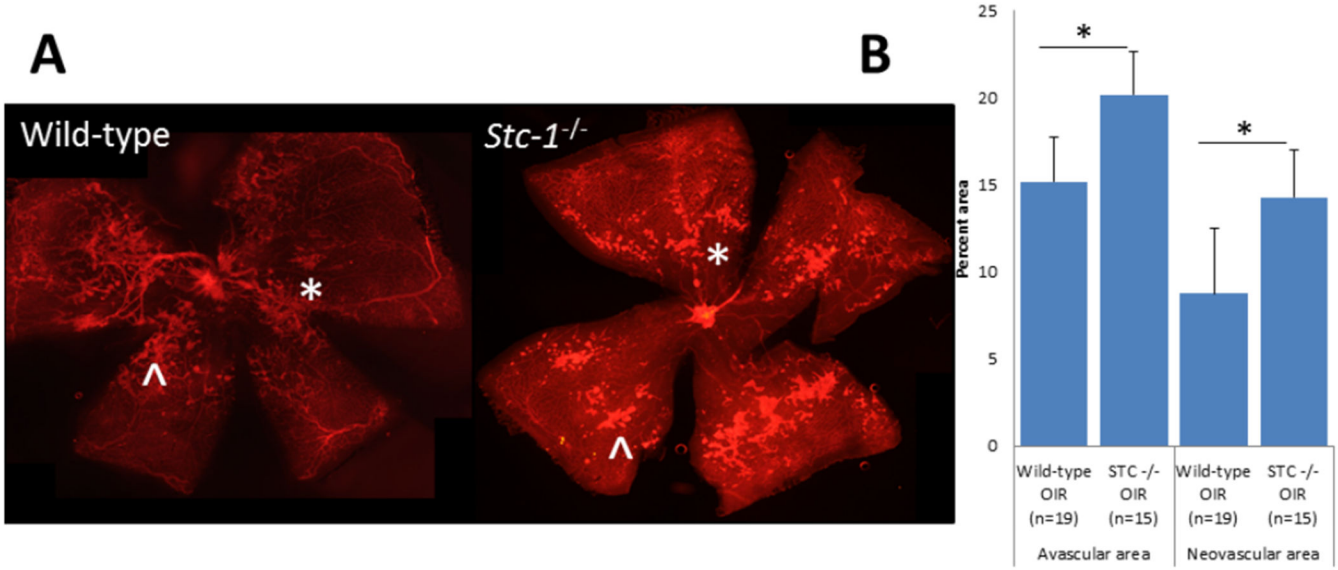

Figure 2. *Stc-1*$^{-/-}$ mice develop worse retinopathy in a mouse model of oxygen induced retinopathy.

(A) Representative retinal flat mount of mice subjected to OIR shows avascular (asterisk) and neovascularization (arrow head) in wild-type mice (left panel) that is less severe compared to *Stc-1*$^{-/-}$ mice (right panel) at P17. (B) Graph showing increased avascular (P=0.02) and neovascular (P<0.05) regions in *Stc-1*$^{-/-}$ mice compared to wild-type controls at P17. Error bars represent SEM.

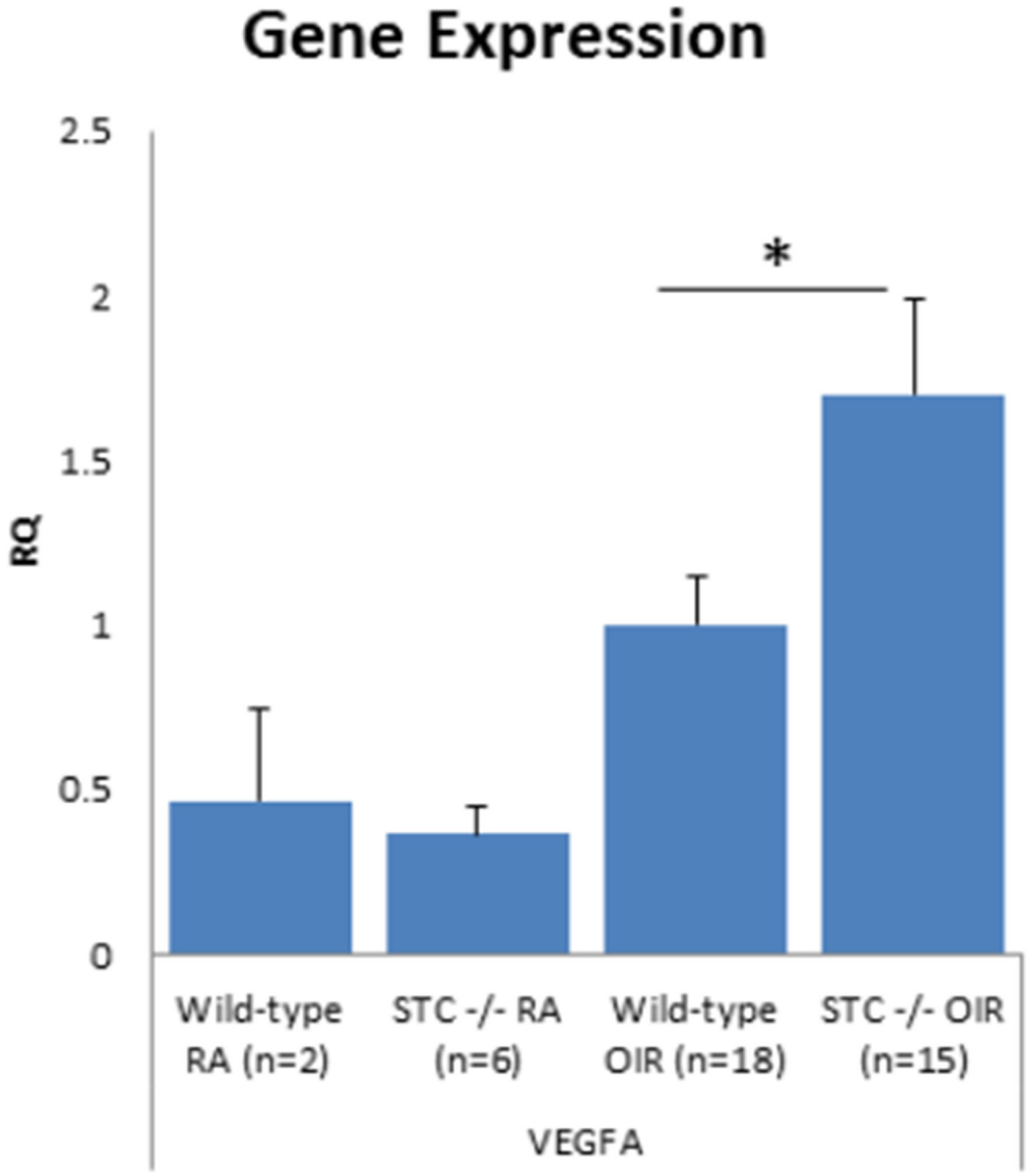

Figure 3. *Stc-1*$^{-/-}$ mice show higher levels of VEGFA compared to wild-type in a mouse model of oxygen induced retinopathy

Quantitative real-time PCR shows that *Stc-1*$^{-/-}$ mice had a significant increase in expression of VEGF-A (P=0.03)* at P17 compared to wild-type controls. Error bars represent SEM.

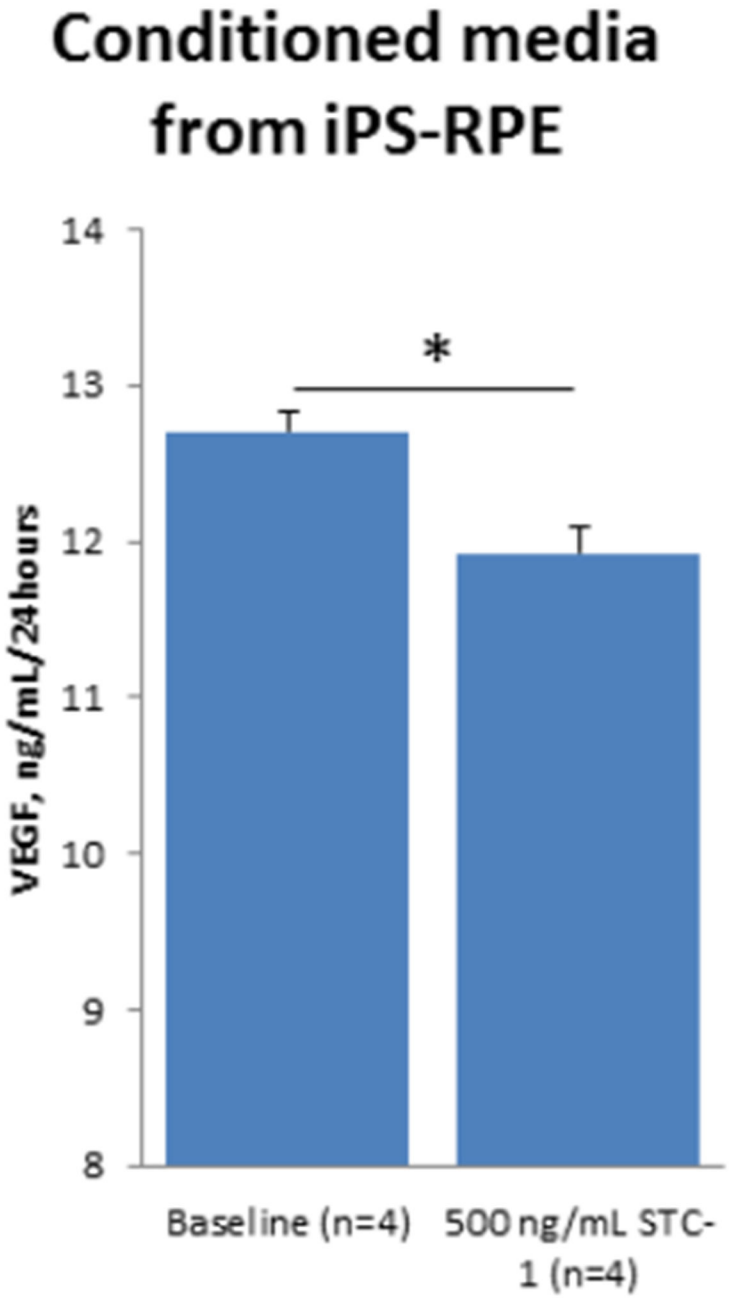

Figure 4. VEGF is decreased in cultures of induced pluripotent stem cell derived retinal pigment epithelium cell cultures following addition of STC-1.

ELISA for VEGF in conditioned media from induced pluripotent stem cell derived retinal pigment epithelium (iPS-RPE) shows a decrease in VEGF expression 24 hours after addition of recombinant human STC-1 (500ng/mL) (n=4, P=0.01). Error bars represent SEM.