Molecular Vision 2025; 31:359-366 <http://www.molvis.org/molvis/v31/359>
Received 10 July 2025 | Accepted 1 October 2025 | Published 3 October 2025

Inhibition of sortilin reduces neuronal and vascular damage after ischemia/reperfusion through reduced inflammatory and autophagy actions in retinal Müller cells

Li Liu, Youde Jiang, Jena J. Steinle

Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University School of Medicine, Detroit, MI

Purpose: Our goal was to explore whether inhibition of sortilin could protect the retina against ischemia/reperfusion (I/R) damage, as well as explore whether this inhibition could reduce inflammatory mediators in retinal Müller cells.
Methods: We used both primary human Müller cells and a rat Müller cell line (rMC-1) grown in normal (5 mM) or high (25 mM) glucose. Some cells were treated with AF38469, a small-molecule inhibitor of sortilin. We performed western blotting for the inflammatory mediators, tumor necrosis factor α, and NOD-like receptor protein 3. We also measured protein levels of lysosome-associated membrane glycoprotein 2 (LAMP2), a marker of autophagy, and cleaved caspase 3, a marker of apoptosis, in the cells. We then tested the actions of eye drops containing AF38469 on mice exposed to I/R. We assessed neuronal damage at 2 days post-I/R and vascular damage at 10 days post-I/R.
Results: High-glucose culturing conditions significantly increased inflammatory, autophagic, and apoptotic markers in both primary human Müller and rat Müller cells. All markers were reduced by treating the cells with AF38469. AF38469 eye drops also significantly reduced I/R-induced neuronal and vascular damage.
Conclusion: These studies demonstrate that sortilin regulates the inflammatory, autophagic, and apoptotic pathways in Müller cells grown in high glucose. Inhibition of sortilin using AF38469 eye drops also reduced I/R-induced retinal damage.

Despite decades of research, diabetic retinopathy remains the leading cause of blindness in working-age adults. Several potential causative mechanisms have been investigated, with many focusing on inflammation [1,2]. Previous studies have shown that proneurotrophins (pro–nerve growth factor [pro-NGF] and pro–brain-derived growth factor) are linked to inflammation [3,4]. In 2006, a new receptor for the proneurotrophins was discovered and named sortilin [5].

Sortilin has been linked to over 50 different signaling pathways [6]. One function of sortilin is as a coreceptor for pro-NGF with P75NTR to mediate neuronal apoptosis [7]. Sortilin also binds pro–brain-derived growth factor, causing neuropathic pain [8]. In Parkinson's disease, sortilin activates monocytes, increasing inflammation [9], and contributes to multiple sclerosis through its role in adaptive immunity [10]. Others have suggested that sortilin is an intracellular sorting protein involved in cellular trafficking [5,11]. In addition to inflammation, sortilin has been linked to other cellular actions. During retinal development, sortilin, along with P75NTR, regulates apoptosis in retinal ganglion cells [12]. Sortilin has also been implicated in the death of inner ear neurons [13]. In addition to apoptosis, inhibition of sortilin-mediated lysosomal degradation and its actions has reduced progranulin levels in microglial cells [14].

In a model of retinal ischemia, sortilin was localized to Müller cells with reduced expression in retinal ganglion cells [15]. In studies of patients with proliferative diabetic retinopathy, significantly increased levels of sortilin were observed in the retinal lysates of the patients [16,17]. A recent study showed increased sortilin levels in the human diabetic retina and in the retina of streptozotocin (STZ)-treated diabetic mice, an increase associated with the actions of pro-NGF/P75NTR in diabetic mice for 8 weeks [18].

Based on the literature, we hypothesized that inhibition of sortilin would protect the retina in an ischemia/reperfusion (I/R) model. Sortilin would regulate inflammatory and apoptotic pathways in retinal Müller cells grown in high glucose.

METHODS

Diabetic mice: C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Diabetes was induced as previously done [19]. Glucose levels >250 mg/dl were considered diabetic. Five control and five diabetic mice were sacrificed for immunostaining. Mice were allowed free access to water and food and kept at a constant temperature for all experiments. Euthanasia was completed by CO_2

Correspondence to: Jena Steinle, Department of Ophthalmology, Visual and Anatomical Sciences, Wayne State University School of Medicine, 9320 Scott Hall, Detroit MI 48202; email: jsteinle@med.wayne.edu

overdose and cervical dislocation. All mouse experiments were approved by the Wayne State University Institutional Animal Care and Use Committee and adhered to the rules provided by the Association for Research in Vision and Ophthalmology.

Human Müller cells: Human Müller cells (AcceGen, Fairfield, NJ) were plated in the ABM-TM133L medium kit containing their supplemental growth support reagent. While cells were not subjected to STR analyses, there were no other cells grown in the same environment during these studies, so no risk of contamination. Cells were grown in normal (5 mM) and high glucose (25 mM) conditions for at least 10 days.

Rat Müller cells: Rat Müller cells (rMC-1) were plated and grown in normal (5 mM) and high glucose (25 mM) Dulbecco's modified Eagle's medium supplemented 5% fetal bovine serum, 10 µg/ml gentamycin, and 0.25 µg/ml amphotericin B (Invitrogen, Carlsbad, CA). Cells were grown in normal and high glucose medium for at least 10 days. A subset of both primary human Müller cells and rMC-1 cells was treated with AF38469 at a dose of 20 µM for 24 h before cell lysate collection.

Immunostaining: Two-month-old male and female diabetic (STZ) C57BL/6 mice and corresponding control mice were euthanized by CO_2, followed by cervical dislocation. After confirmation of death, the eyes were removed and placed into 4% paraformaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4) for 6 h. Whole globes were transferred to 0.1 M PBS with 30% sucrose overnight for cryoprotection. The following day, 10-µm cryosections were collected and stored in −20 ºC for further analysis. Slides were rinsed in PBS and placed into 5% normal goat serum for 2 h at room temperature to block nonspecific staining, followed by incubation with rabbit antisortilin (1:400; Abcam, Cambridge, UK) and mouse antiglutamine synthetase (1:500; Abcam) overnight at 4 °C. After rising in 0.3% Triton/PBS, slides were incubated with secondary antibody goat antirabbit conjugated to Alexa Fluor 555 (1:500; Life Technologies, Carlsbad, CA) and goat antimouse conjugated to Alexa Fluor 488 (1:500; Life Technologies) overnight at 4 °C. Slides were then rinsed in PBS, counterstained with DAPI, mounted with FluorSave Reagent (Calbiochem, San Diego, CA), and examined on a Leica (Wetzlar, Germany) confocal microscope.

Western blotting: Cell lysates were collected in lysis buffer containing both protease and phosphatase inhibitors. Equal amounts of protein were separated using precast Tris-glycine gels (Invitrogen) and blotted onto nitrocellulose membranes. After blocking in TBST (10 mM Tris-HCl buffer [pH 8.0], 150 mM NaCl, 0.1% Tween-20) and 5% (w/v) bovine serum albumin, membranes were treated with antibodies to sortilin (ab16640, rabbit 1:500; Abcam), NOD-like receptor protein 3 (NLRP3) (ab263899, rabbit, 1:500; Abcam), tumor necrosis factor α (TNFα) (ab183218, rabbit, 1:300; Abcam), lysosome-associated membrane glycoprotein 2 (LAMP2; ab203224, rabbit 1:500; Abcam), cleaved caspase 3 (MAB835, rabbit 1:1,000; R&D Systems), P75NTR (ab52987, rabbit, 1:1,000; Abcam), and beta-actin (Santa Cruz Biotechnology, Santa Cruz, CA) primary antibodies, followed by incubation with secondary antibodies tagged with horseradish peroxidase. Blots were visualized with an Azure C500 machine (Azure Biosystems, Dublin, CA) via chemiluminescence (Thermo Scientific, Pittsburgh, PA). Western blot band densities were measured using Image Studio Lite software.

Ischemia/reperfusion: Animals were anesthetized with an injection of ketamine and xylazine. Once without toe reflex, a 32-gauge needle attached to an infusion line of sterile saline was used to pierce the anterior chamber of the eye. Hydrostatic pressure of 80 to 90 mm Hg (TonoPen; Medtronic, Jacksonville, FL, USA) was maintained for 90 min to induce retinal ischemia, observed as blanching of the iris and loss of red reflex [20,21]. After 90 min of infusion, the needle was withdrawn and intraocular pressure normalized. The contralateral eye was an intra-animal control. After the 90-min infusion, a subset of mice received eye drops of AF38469 (10 µg/ml in ~2 µl) into both eyes as a treatment. This treatment was repeated at the same time each day for up to 10 days.

Neuronal and vascular analyses: Two days after exposure to I/R, a subset of each group of mice was sacrificed for measurements of neuronal thickness, as we have done previously [22]. Ten-micrometer sections were taken from throughout the retina. Multiple sections from each animal were assessed for retinal thickness and cell numbers in the ganglion cell layer [22,23]. Ten days after I/R exposure, all remaining mice were sacrificed to measure degenerate capillaries, as we have done previously [21,24].

Statistics: Statistics were calculated using Prism 7.0 (GraphPad Software, San Diego, CA). A one-way analysis of variance with Tukey's post hoc test was used for data analyses. $P < 0.05$ was considered statistically significant.

RESULTS

Sortilin is localized to retinal Müller cells: The literature suggested that sortilin is localized in retinal Müller cells in diabetic mice [18]. To confirm these findings in our laboratory, we also used retinal sections from control and STZ-treated diabetic mice to localize sortilin in the retina. Figure 1 shows that sortilin (red) colocalized with glutamine synthase (green), which is a Müller cell marker.

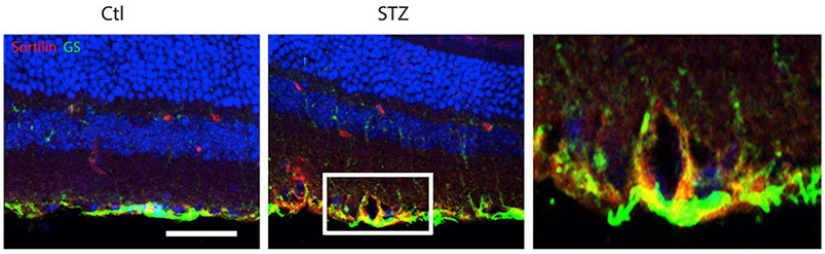

Figure 1. Sortilin immunostaining (red) and glutamine synthase (GS, green) in sectioned retinal samples from control and STZ-treated mice. Sortilin is localized in Müller cells. *N* = 5. Scale bar is 50 µm. The right panel is an enlargement of the area of the box in STZ.

Sortilin is increased in retinal Müller cells grown in high glucose, which is blocked by AF38469: Since sortilin was localized in the Müller cells of diabetic mice, we chose to grow human Müller cells (A) and rat Müller cells (rMC-1) in normal and high glucose, treating some cells in each glucose condition with the sortilin small-molecule inhibitor, AF38469. Figure 2 shows that sortilin levels were significantly increased in Müller cells from both humans and rats, and this increase was significantly reduced by AF38469 treatment. AF38469 also reduced sortilin levels in rMC-1 cells grown in normal glucose but not in the primary human cells.

AF38469 reduces high glucose–induced increases in NLRP3 and TNFα: We have previously reported that diabetes increases both NLRP3 and TNFα [23,25]. To test whether sortilin regulated these pathways in retinal Müller cells, we grew human and rMC-1 cells in normal and high glucose and treated some cells with AF38469. Figure 3 shows that high glucose increased NLRP3 and TNFα in both cell types, which were reduced by AF38469 treatment, suggesting that sortilin regulates these inflammatory pathways in Müller cells.

P75NTR is regulated by AF38469 in Müller cells: Sortilin is known to be a coreceptor with P75NTR for the proneurotrophins [26]. To test this in Müller cells, we treated human and rMC-1 cells with AF38469 to measure P75NTR. Figure 4 shows that high glucose increased P75NTR in both cell types. Treatment with AF38469 reduced P75NTR in these cells.

Sortilin regulates LAMP2 and cleaved caspase 3 in retinal Müller cells: Sortilin has been linked to autophagic proteins and apoptosis in other systems [6,7]. To investigate this in our system, human and rMC-1 cells grown in normal and high glucose alone and treated with AF38469 were collected, and levels of LAMP2 and cleaved caspase 3 were measured. Figure 5 shows that both LAMP2 (A, B) and cleaved caspase 3 (C, D) were increased in the Müller cells grown in high glucose. Both proteins were reduced by AF38469 treatment in both cell types.

Inhibition of sortilin reduces neuronal and vascular damage associated with ischemia/reperfusion: Cell data suggested that inhibition of sortilin would protect the retina. To test this in vivo, we employed the I/R model, where you get neuronal

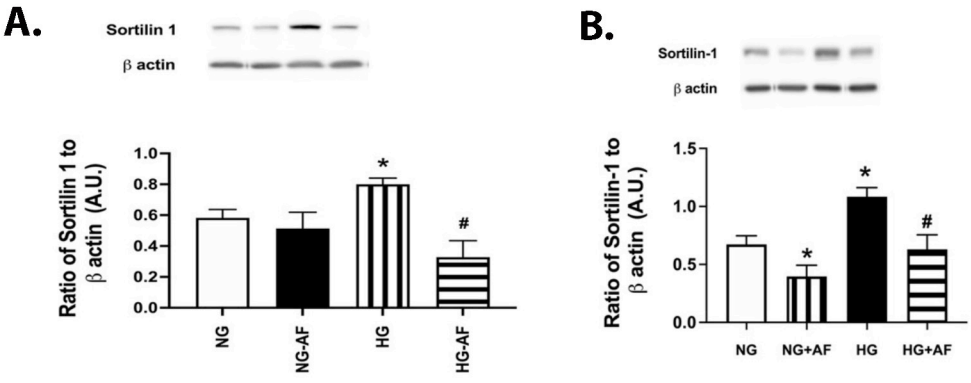

Figure 2. Sortilin protein levels in human Müller cells (**A**) and rat Müller cells (rMC-1, **B**) grown in normal glucose (NG) or high glucose (HG) and treated with AF38469. *$p < 0.05$ versus NG, #$p < 0.05$ versus HG, assessed by one-way analysis of variance. $n = 4$.

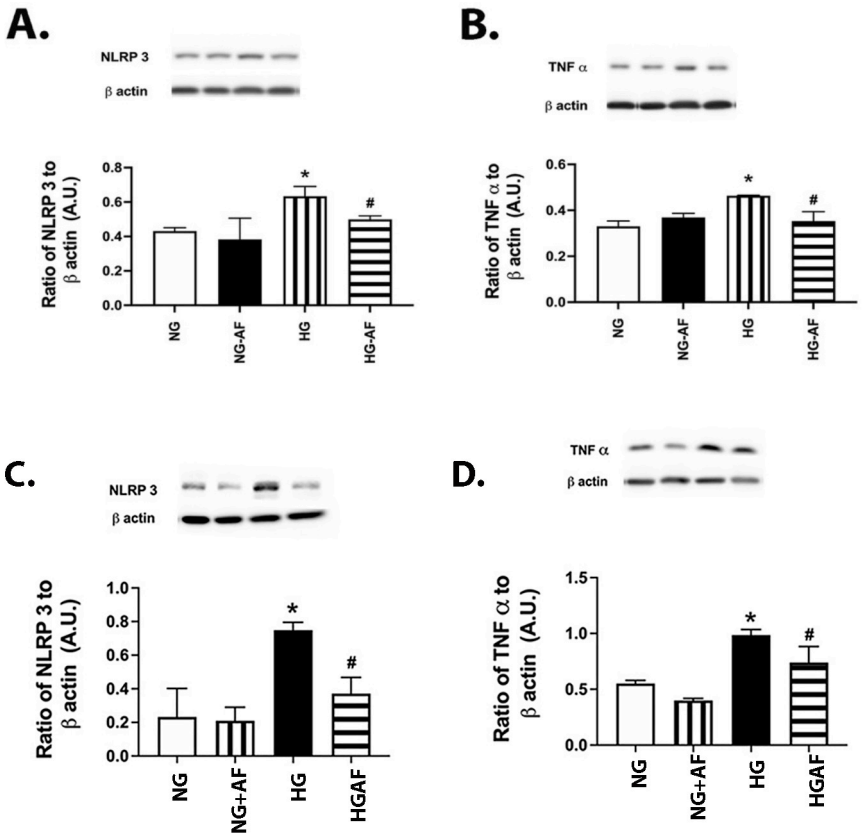

Figure 3. Inflammatory mediators in Muller cells. NLRP3 (**A**) and TNFα (**B**) levels in human Müller cells and rat Müller cells (**C**, **D**) grown in normal glucose (NG) and high glucose (HG) alone and treated with AF38469. *$p < 0.05$ versus NG, #$p < 0.05$ versus HG, assessed using a one-way analysis of variance. $n = 4$.

data at 2 days post-I/R and vascular changes at 10 days post-I/R. Mice were exposed to I/R for 90 min. At that time, the first eye drop of AF38469 (10 µg/ml) was applied. Mice were then treated daily at the same time with the AF38469 eye drops for up to 10 days. Figure 6A-C shows that I/R significantly reduced neuronal thickness and cell numbers in the ganglion cell layer. In mice exposed to I/R and treated with AF38469 eye drops for 2 days, neuronal thickness and cell numbers were significantly increased compared to I/R alone. Figure 6D-E shows that I/R significantly increased the

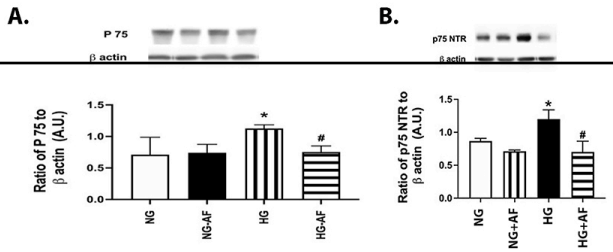

Figure 4. P75 levels in Muller cells. p75NTR protein levels in human Müller cells (**A**) and rat Müller cells (**B**) grown in normal glucose (NG) or high glucose (HG) and treated with AF38469. *$p < 0.05$ versus NG, #$p < 0.05$ versus HG assessed using a one-way analysis of variance. $n = 4$.

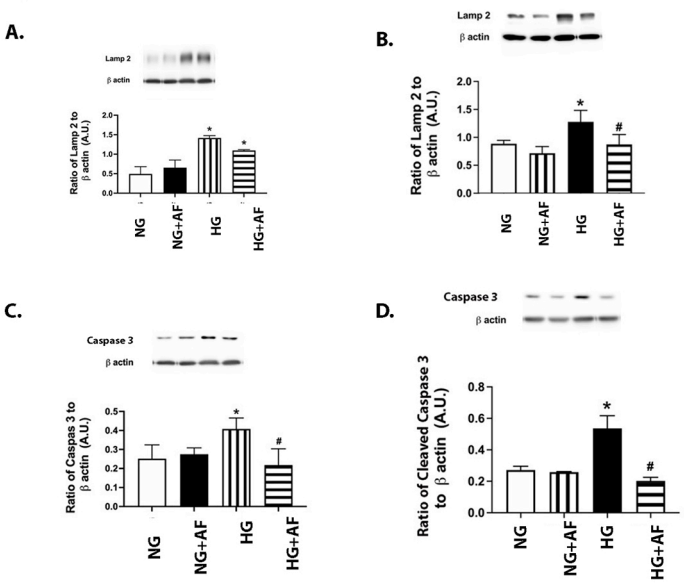

Figure 5. Autophagy and apoptotic markers in Muller cells. LAMP2 (**A**) and cleaved caspase 3 (**C**) levels in human Müller cells and rat Müller cells (**B**, **D**, respectively) grown in normal glucose (NG) and high glucose (HG) alone and treated with AF38469. *$p < 0.05$ versus NG, #$p < 0.05$ versus HG, assessed using a one-way analysis of variance. $n = 4$.

degeneration of retinal capillaries at 10 days. Treatment with AF38469 eye drops significantly reduced the loss of retinal capillaries in the retina. These data suggest that inhibition of sortilin could protect the retina against acute damage.

DISCUSSION

We and others have previously reported that I/R is an effective way to observe neuronal and vascular damage due to retinal stress [20,27,28]. In these studies, we used the I/R model to investigate the effectiveness of sortilin inhibition against retinal damage. We found that daily administration of AF38469, a small-molecule inhibitor of sortilin, significantly improved retinal thickness and cell numbers in the ganglion cell layer. It also reduced the number of degenerate capillaries, suggesting that AF38469 protected the retina against I/R-induced damage. Our findings on reduced neuronal damage agree with the literature in acute diabetic models using a different sortilin inhibitor [18].

Since we found that AF38469 was effective against I/R-induced injury, we wanted to determine potential mechanisms by which sortilin mediated retinal damage. Previous work has shown that sortilin is primarily expressed in retinal Müller cells [18], a finding that we confirmed in our studies. Using both primary human Müller and a rat Müller cell line, we showed that high glucose culturing conditions significantly increased inflammatory mediators, which were reduced by AF38469. These findings agree with the literature on Parkinson's disease, showing that sortilin activated monocytes to increase inflammation [9]. In multiple sclerosis, sortilin contributed to inflammation through its role in adaptive immunity. In addition to inflammatory markers, we also found that high glucose increased markers of apoptosis and autophagy in both human and rat Müller cells. When these cells were treated with AF38469, both apoptotic and autophagy markers were significantly reduced. These findings on autophagy agree with studies in hippocampal neurons treated with amyloid β1-42, showing that sortilin contributed to the elimination of clustrin protein by targeting it into lysosomes [29]. Sortilin also regulates macrophage accumulation of cholesterol by lysosomal degradation [30]. Due to its role in regulating the proneurotrophins, sortilin can increase apoptosis. We found that inhibition of sortilin reduced the cleavage of caspase 3. This matches findings in retinal ganglion cells [12] and inner ear neurons [13].

While our studies showed the effectiveness of AF38469 in reducing sortilin in the retina, further studies are needed to examine the penetration and concentration of AF38469 after eye drop application. We also need to examine the chronic effects of sortilin inhibition in the diabetic retina, as well as AF38469 side effects on other organs.

In conclusion, we found that a small-molecule inhibitor of sortilin, AF38469, reduced retinal neuronal and vascular damage in response to I/R injury. Sortilin is primarily expressed in retinal Müller cells. Treatment of primary

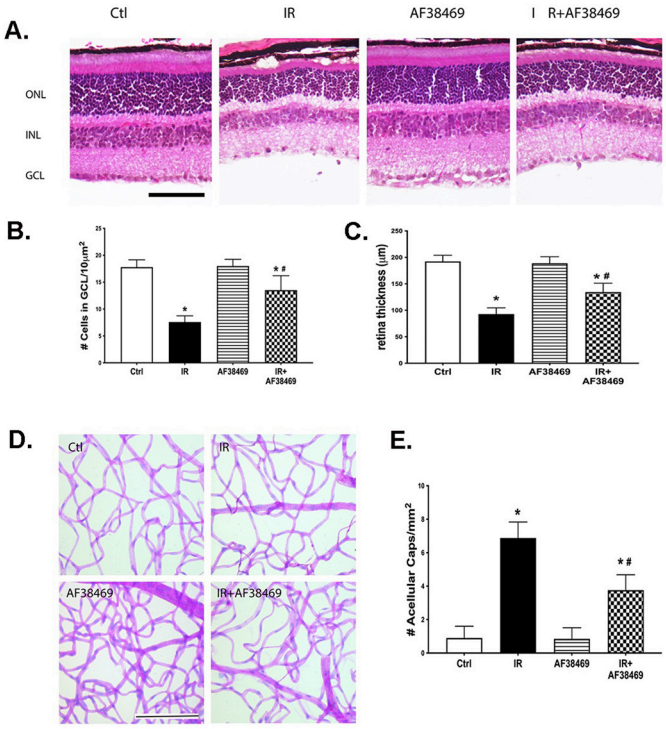

Figure 6. Neuronal and vascular analyses after exposure to ischemia/reperfusion (I/R). Mice are untreated (ctrl), exposed to I/R only (IR), treated with AF38469 only (AF38469), or exposed to I/R and treated with AF38469 eye drops. **A**. A representative image of neuronal changes. **B**. Number of cells in the ganglion cell layer. **C**. Retinal thickness. **D**. A representative image of the vascular changes showing degenerate capillaries. **E**. Counts of acellular capillaries. $n = 5$ in all groups. *$p < 0.05$ versus Ctrl, #$p < 0.05$ versus IR only, as measured by one-way analysis of variance, followed by Tukey's test. Data are mean ± SEM. Scale bar for neuronal analyses is 50 µm.

human Müller cells or a rat Müller cell line with AF38469 significantly reduced high glucose–induced increases in inflammatory, autophagy, and apoptotic markers. Further studies are needed on the use of AF38469 as a therapeutic for retinal damage.

ACKNOWLEDGMENTS

These studies were funded by R01EY030284 (JJS), P30EY04068 Core grant (LDH, PI of Core grant), an unrestricted grant from Research to Prevent Blindness. Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Author Contributions: LL did all mouse work; YJ performed the western blotting work; JJS designed the experiments and wrote the text.

REFERENCES

1. Joussen AM, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Döhmen S, Adamis AP. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J 2002; 16:438-40. [PMID: 11821258].
2. Tang J, Kern TS. Inflammation in diabetic retinopathy. Prog Retin Eye Res 2011; 30:343-58. [PMID: 21635964].
3. Mazella J. Deciphering Mechanisms of Action of Sortilin/Neurotensin Receptor-3 in the Proliferation Regulation of Colorectal and Other Cancers. Int J Mol Sci 2022; 23:23-[PMID: 36233189].
4. Mohamed R, El-Remessy AB. Imbalance of the Nerve Growth Factor and Its Precursor: Implication in Diabetic Retinopathy. J Clin Exp Ophthalmol 2015; 6:6-[PMID: 26807305].
5. Mazella J, Zsürger N, Navarro V, Chabry J, Kaghad M, Caput D, Ferrara P, Vita N, Gully D, Maffrand JP, Vincent JP. The 100-kDa neurotensin receptor is gp95/sortilin, a non-G-protein-coupled receptor. J Biol Chem 1998; 273:26273-6. [PMID: 9756851].
6. Mitok KA, Keller MP, Attie AD. Sorting through the extensive and confusing roles of sortilin in metabolic disease. J Lipid Res 2022; 63:100243[PMID: 35724703].

7. Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, Jacobsen C, Kliemannel M, Schwarz E, Willnow TE, Hempstead BL, Petersen CM. Sortilin is essential for proNGF-induced neuronal cell death. Nature 2004; 427:843-8. [PMID: 14985763].
8. Richner M, Pallesen LT, Ulrichsen M, Poulsen ET, Holm TH, Login H, Castonguay A, Lorenzo LE, Gonçalves NP, Andersen OM, Lykke-Hartmann K, Enghild JJ, Rønn LCB, Malik IJ, De Koninck Y, Bjerrum OJ, Vægter CB, Nykjær A. Sortilin gates neurotensin and BDNF signaling to control peripheral neuropathic pain. Sci Adv 2019; 5:eaav9946[PMID: 31223654].
9. Georgoula M, Ntavaroukas P, Androutsopoulou A, Xiromerisiou G, Kalala F, Speletas M, Asprodini E, Vasilaki A, Papoutsopoulou S. Sortilin Expression Levels and Peripheral Immunity: A Potential Biomarker for Segregation between Parkinson's Disease Patients and Healthy Controls. Int J Mol Sci 2024; 25:25-[PMID: 38339069].
10. Reuter E, Weber J, Paterka M, Ploen R, Breiderhoff T, van Horssen J, Willnow TE, Siffrin V, Zipp F. Role of Sortilin in Models of Autoimmune Neuroinflammation. J Immunol 2015; 195:5762-9. [PMID: 26566674].
11. Barnes JW, Aarnio-Peterson M, Norris J, Haskins M, Flanagan-Steet H, Steet R. Upregulation of Sortilin, a Lysosomal Sorting Receptor, Corresponds with Reduced Bioavailability of Latent TGFβ in Mucolipidosis II Cells. Biomolecules 2020; 10:10-[PMID: 32357547].
12. Nakamura K, Namekata K, Harada C, Harada T. Intracellular sortilin expression pattern regulates proNGF-induced naturally occurring cell death during development. Cell Death Differ 2007; 14:1552-4. [PMID: 17541425].
13. Tauris J, Gustafsen C, Christensen EI, Jansen P, Nykjaer A, Nyengaard JR, Teng KK, Schwarz E, Ovesen T, Madsen P, Petersen CM. Proneurotrophin-3 may induce Sortilin-dependent death in inner ear neurons. Eur J Neurosci 2011; 33:622-31. [PMID: 21261755].
14. Long S, Liu Z, Wang Y. Sortilin inhibition in microglial cells cannot alleviate ischemia and hypoxia-induced neuronal injury in co-culture. Neuroreport 2024; 35:320-7. [PMID: 38305117].
15. Xu F, Wei Y, Lu Q, Zheng D, Zhang F, Gao E, Wang N. Immunohistochemical localization of sortilin and p75(NTR) in normal and ischemic rat retina. Neurosci Lett 2009; 454:81-5. [PMID: 19429059].
16. Abu El-Asrar AM, Mohammad G, De Hertogh G, Nawaz MI, Van Den Eynde K, Siddiquei MM, Struyf S, Opdenakker G, Geboes K. Neurotrophins and neurotrophin receptors in proliferative diabetic retinopathy. PLoS One 2013; 8:e65472[PMID: 23762379].
17. Boss JD, Singh PK, Pandya HK, Tosi J, Kim C, Tewari A, Juzych MS, Abrams GW, Kumar A. Assessment of Neurotrophins and Inflammatory Mediators in Vitreous of Patients With Diabetic Retinopathy. Invest Ophthalmol Vis Sci 2017; 58:5594-603. [PMID: 29084332].
18. Jakobsen TS, Østergaard JA, Kjolby M, Birch EL, Bek T, Nykjaer A, Corydon TJ, Askou AL. Sortilin Inhibition Protects Neurons From Degeneration in the Diabetic Retina. Invest Ophthalmol Vis Sci 2023; 64:8-[PMID: 37272764].
19. Liu L, Jiang Y, Steinle JJ. Epac1 and Glycyrrhizin Both Inhibit HMGB1 Levels to Reduce Diabetes-Induced Neuronal and Vascular Damage in the Mouse Retina. J Clin Med 2019; 8:8-[PMID: 31159195].
20. Abcouwer SF, Lin CM, Shanmugam S, Muthusamy A, Barber AJ, Antonetti DA. Minocycline prevents retinal inflammation and vascular permeability following ischemia-reperfusion injury. J Neuroinflammation 2013; 10:149-[PMID: 24325836].
21. Liu L, Jiang Y, Steinle JJ. Compound 49b Restores Retinal Thickness and Reduces Degenerate Capillaries in the Rat Retina following Ischemia/Reperfusion. PLoS One 2016; 11:e0159532[PMID: 27439004].
22. Steinle JJ, Kern TS, Thomas SA, McFadyen-Ketchum LS, Smith CP. Increased basement membrane thickness, pericyte ghosts, and loss of retinal thickness and cells in dopamine beta hydroxylase knockout mice. Exp Eye Res 2009; 88:1014-9. [PMID: 19176214].
23. Zhang Q, Guy K, Pagadala J, Jiang Y, Walker RJ, Liu L, Soderland C, Kern TS, Ferry R Jr, He H, Yates CR, Miller DD, Steinle JJ. Compound 49b prevents diabetes-induced apoptosis through increased IGFBP-3 levels. Invest Ophthalmol Vis Sci 2012; 53:3004-13. [PMID: 22467575].
24. Veenstra A, Liu H, Lee CA, Du Y, Tang J, Kern TS. Diabetic Retinopathy: Retina-Specific Methods for Maintenance of Diabetic Rodents and Evaluation of Vascular Histopathology and Molecular Abnormalities. Curr Protoc Mouse Biol 2015; 5:247-70. [PMID: 26331759].
25. Liu L, Jiang Y, Steinle JJ. PKA and Epac1 Reduce Nek7 to Block the NLRP3 Inflammasome Proteins in the Retinal Vasculature. Invest Ophthalmol Vis Sci 2022; 63:14-[PMID: 35006270].
26. Li Q, Hu YZ, Gao S, Wang PF, Hu ZL, Dai RP. ProBDNF and its receptors in immune-mediated inflammatory diseases: novel insights into the regulation of metabolism and mitochondria. Front Immunol 2023; 14:1155333[PMID: 37143663].
27. Caballero S, Sengupta N, Afzal A, Chang KH, Li Calzi S, Guberski DL, Kern TS, Grant MB. Ischemic vascular damage can be repaired by healthy, but not diabetic, endothelial progenitor cells. Diabetes 2007; 56:960-7. [PMID: 17395742].
28. Liu L, Jiang Y, Steinle JJ. Epac1 protects the retina against ischemia/reperfusion-induced neuronal and vascular damage. PLoS One 2018; 13:e0204346[PMID: 30235337].
29. Wang Y, Qin X, Paudel HK. Amyloid β peptide promotes lysosomal degradation of clusterin via sortilin in hippocampal primary neurons. Neurobiol Dis 2017; 103:78-88. [PMID: 28396259].

30. Lv Y, Yang J, Gao A, Sun S, Zheng X, Chen X, Wan W, Tang C, Xie W, Li S, Guo D, Peng T, Zhao G, Zhong L. Sortilin promotes macrophage cholesterol accumulation and aortic atherosclerosis through lysosomal degradation of ATP-binding cassette transporter A1 protein. Acta Biochim Biophys Sin (Shanghai) 2019; 51:471-83. [PMID: 30950489].