Tumor Necrosis Factor Alpha (TNF-α) Disrupts Kir4.1 Channel Expression Resulting in Müller Cell Dysfunction in the Retina

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PURPOSE. Diabetic patients often are affected by vision problems. We previously identified diabetic retinopathy (DR) as a disease of clock gene dysregulation. TNF-α, a proinflammatory cytokine, is known to be elevated in DR. Müller cells maintain retinal water homeostasis and K⁺ concentration via Kir4.1 channels. Notably, Kir4.1 expression is reduced in diabetes; however, the interplay of TNF-α, Kir4.1, and clock genes in Müller cells remains unknown. We hypothesize that the Kir4.1 in Müller cells is under clock regulation, and increase in TNF-α is detrimental to Kir4.1.

METHODS. Long-Evans rats were made diabetic using streptozotocin (STZ). Retinal Kir4.1 expression was determined at different time intervals. Rat Müller (rMC-1) cells were transfected with siRNA for Per2 or Bmal1 and in parallel treated with TNF-α (5–5000 pM) to determine Kir4.1 expression.

RESULTS. Kir4.1 expression exhibited a diurnal rhythm in the retina; however, with STZ-induced diabetes, Kir4.1 was reduced overall. Kir4.1 rhythm was maintained in vitro in clock synchronized rMC-1 cells. Clock gene siRNA-treated rMC-1 exhibited a decrease in Kir4.1 expression. TNF-α treatment of rMCs lead to a profound decrease in Kir4.1 due to reduced colocalization of Kir4.1 channels with synapse-associated protein (SAP97) and disorganization of the actin cytoskeleton.

CONCLUSIONS. Our findings demonstrate that Kir4.1 channels possess a diurnal rhythm, and this rhythm is dampened with diabetes, thereby suggesting that the increase in TNF-α is detrimental to normal Kir4.1 rhythm and expression.

Keywords: Müller cell, circadian, Kir4.1, TNF-alpha

Diabetic patients display a variety of vision problems. For instance, patients with diabetes present with poorer reading vision early in the morning compared to the later part of the day, with spontaneous improvement in reading within 2 to 4 hours after waking.1 Vision problems of diabetic patients correspond to an overnight increase in retinal thickness that subsides as the day progresses, indicating apparent diurnal correspondence to an overnight increase in retinal thickness that 2,3 We previously reported a dysfunctional pattern of rhythm regulation of clinically evident vascular injury.6,7 Müller cells are at the core of the neurovascular unit, acting as a principal glia of the retina. The Müller cells express a diverse array of ion channels and transporters, release a variety of cytokines, provide survival and support factors, and have receptors for numerous neurotransmitters and growth factors.8 The K⁺ concentration in Müller cells is regulated by specialized inwardly rectifying K⁺ channels (Kir), and transcellular water transport is facilitated mainly through aquaporin-4 (AQP4) water channels. Approximately six to seven Kir channels (Kir1-Kir6) have been identified to date in Müller cells, among which Kir4.1 is expressed in abundance.9,10 Kir4.1 acts as a principal ion channel involved in potassium balance in Müller cells. The coexpression of Kir4.1 and AQP4, a water channel in retinal Müller cells, tightly regulates water homeostasis. Kir4.1 channels induce spatial buffering of the K⁺ currents.9 Thus, any change in the levels of Kir4.1 channels in Müller cells leads to water accumulation due to osmotic imbalance.

Diabetic retinas show a dramatic decrease in Kir4.1 expression, altered K⁺ conductance, and Müller cell swelling.5,8 In particular, the polarized pattern of Kir4.1 expression shows a strong reduction in perivascular regions of diabetic retinas expressing Müller cell markers.9,11 Genetic inactivation of Kir4.1 results in substantial depolarization of the retinal Müller cells. The electoretinogram (ERG) studies on Kir4.1 knockout mice show a lack of slow PIII response, a characteristic feature of diabetic retinopathy.

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Methods. Long-Evans rats were made diabetic using streptozotocin (STZ). Retinal Kir4.1 expression was determined at different time intervals. Rat Müller (rMC-1) cells were transfected with siRNA for Per2 or Bmal1 and in parallel treated with TNF-α (5–5000 pM) to determine Kir4.1 expression.

Results. Kir4.1 expression exhibited a diurnal rhythm in the retina; however, with STZ-induced diabetes, Kir4.1 was reduced overall. Kir4.1 rhythm was maintained in vitro in clock synchronized rMC-1 cells. Clock gene siRNA-treated rMC-1 exhibited a decrease in Kir4.1 expression. TNF-α treatment of rMCs lead to a profound decrease in Kir4.1 due to reduced colocalization of Kir4.1 channels with synapse-associated protein (SAP97) and disorganization of the actin cytoskeleton.

Conclusions. Our findings demonstrate that Kir4.1 channels possess a diurnal rhythm, and this rhythm is dampened with diabetes, thereby suggesting that the increase in TNF-α is detrimental to normal Kir4.1 rhythm and expression.

Keywords: Müller cell, circadian, Kir4.1, TNF-alpha
Diurnal Rhythm of Kir4.1 in the Retina

of functional Kir4.1 channels.10 Taken together, these studies suggest that Kir4.1 is critical for normal retinal function, and diabetes-induced changes are detrimental to K+ conductance and Kir4.1 expression. However, it is unknown whether the Kir4.1 expression in retinal Müller cells possesses a diurnal rhythm.

Proinflammatory cytokine TNF-α is known to be upregulated in DR.12 TNF-α mediates its effect on the retina through an upregulation of adhesion molecules, increase in apoptosis, and monocyte infiltration. Animal studies have shown that TNF-α is involved in a pathologic lesion of DR, such as capillary degeneration, pericyte loss, and an increase in retinal permeability.13 The polymorphism of the TNF-α gene increases the susceptibility of DR.14 TNF-α also is involved in Müller cell dysfunction observed in diabetes.15,16 However, it is unclear how the proinflammatory phenotype of TNF-α in diabetic milieu affects Kir4.1 expression. In this study, we demonstrated that Kir4.1 exhibits a diurnal rhythm in retina in vivo and in vitro. We further reported that increase in TNF-α leads to a decrease in Kir4.1 expression due to disruption of the actin cytoskeleton and Kir4.1/SAP dissociation.

METHODS

Cell Culture of Rat Müller Cells

rMC-1 cells were generous gifts from Vijay Sarthy, Northwestern University, and Timothy S. Kern, Case Western Reserve University (Cleveland, OH, USA). The rMC-1 cells were cultured in Dulbecco’s modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) before being used for the following studies.

Induction of Experimental Diabetes

Animal studies were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the Institutional Animal Care and Use Committee of Michigan State University and Indiana University. The Long-Evans rats were purchased from Harlan Laboratories (Haslett, MI, USA) and diabetes was induced using streptozotocin (STZ) (65 mg/kg) administered through the intraperitoneal (IP) route. The control animals were treated with 50% horse serum for 2 hours followed by no serum in culture for the next 48 hours, as described previously.17 The rMC-1 cells were harvested at 4-hour intervals, and the gene expression was determined as described above.

Western Blotting for Kir4.1 Protein Expression

The rMC-1 cells were harvested and lysed in RIPA buffer (Life Technologies). The concentration of protein for loading was determined using a BCA protein assay kit. Proteins were separated using Bolt Bis-Tris Plus polyacrylamide gels followed by transfer to a polyvinylidene fluoride (PVDF) membrane. The protein expression for Kir4.1 was determined by probing antibodies for Kir4.1 (Alomone Labs, Jerusalem, Israel) and β-tubulin was used (Sigma-Aldrich Corp., St. Louis, MO, USA) as a loading control.

Immunohistochemistry for Retinal Sections

The retinas were fixed in 4% paraformaldehyde. After several washing steps, the retinas were embedded in 3% agarose and sectioned using a vibratome. The retinal sections were incubated with Kir4.1 (Alomone Labs) and glutathione synthase (GS-1; Millipore, Billerica, MA, USA) followed by a secondary staining with Alexa-488 and Alexa-555 antibodies (Life Technologies). The retinal sections were imaged using a confocal scanning laser microscope (Zeiss LSM 700 confocal microscope system with Axio Observer; Carl Zeiss Meditec, Jena, Germany) and analyzed using Zen lite software for colocalization analysis.

Immunofluorescence for Phalloidin, Kir4.1, and Synapse-Associated Protein (SAP97)

The rMC-1 cells were treated with rTNF-α (R & D Systems, Minneapolis, MN, USA) at the concentration of 5 to 5000 pg/ml followed by staining with anti-Phalloidin (Life Technologies), anti-SAP7 (Antibodies Incorporated, Davis, CA, USA), and Kir4.1 (Alomone Labs). The images were taken using a confocal scanning laser microscope (Zeiss LSM 700 with Axio Observer; Carl Zeiss Meditec).

Statistics and Analysis of Circadian Rhythms

The data were expressed as mean ± SEM. Statistical analysis was performed using the statistical analysis software, GraphPad Prism 6 (GraphPad Software La Jolla, CA, USA), and data were analyzed using a 1-way ANOVA followed by the Tukey-Kramer test for post hoc analysis unless otherwise specified. To analyze the biorhythm, a smooth curve for the expression of each gene was fitted by a single cosine function of f(t) = A*cos(ωt + φ) + M, where f(t) is the gene expression level at time t, A is amplitude, ω is angular frequency, φ is acrophase, and M is mesor.18 The presence of circadian rhythmicity was analyzed using COSOFF-based algorithms, as described previously.19 The data then were evaluated using a single cosine R analysis to identify the rhythmicity of gene expression. The data were considered to be statistically significant when the P value was less than 0.05 using a zero-amplitude test. The individual gene expression was analyzed using a 1-way ANOVA followed by the Tukey-Kramer test for post hoc analysis.

RESULTS

Diurnal Variation of Kcnj10 Rhythm and Dampened Response in Diabetes

Kir4.1 is a principal channel for maintaining K+ homeostasis and water balance. While previous studies have shown a
decrease in the expression of Kir4.1 in diabetes, there are no reports as to whether these channels possess diurnal variation, and whether diabetes disturbs normal biorhythm of the Kir4.1 channel. To test diurnal variation in the Kir4.1 channel, we harvested retinas of diabetic rats at a 2-hour interval. The gene expression for Kcnj10 was evaluated using quantitative (q) RT-PCR. Each time point is expressed as Zeitgeber (ZT) time, which corresponds to 12-hour light-dark cycles with lights on at 7 AM and off at 7 PM. We observed that Kcnj10 expression was at the zenith during the night (ZT-22) and at nadir earlier in the day (ZT-10; Fig. 1A, black solid line). In diabetic rats, the Kcnj10 expression was profoundly reduced at ZT-22 (peak increase for WTs; Fig. 1A, red solid line; P < 0.05). The periodicity analysis by COSOPT demonstrated a diurnal rhythm of Kcnj10 for control rats (P = 0.029); however, this diurnal rhythm of Kcnj10 was lost with diabetes (P = 0.345). The retinal clock gene expression followed an oscillatory pattern for control rats, which is suggestive of a functional clock. The oscillatory pattern for clock gene Bmal1 was phase-advanced by 2 hours in the diabetic retina. There was a strong inhibitory effect of diabetes on the negative arm of clock genes Per1 and Cry2, these results have been reported previously. Next, we evaluated the protein expression of Kir4.1 using Western blot and found that the Kir4.1 expression was at zenith at ZT-18 in control animals while at the nadir at ZT-6. Diabetes resulted in an overall decrease in Kir4.1 expression at all time points (Fig. 1B). To further explore the spectrum of Kir4.1 staining in the retina, we stained retinal transverse sections with Kir4.1 and GS antibodies (red) showing immunoreactivity of Kir4.1 in neurofilament layer (white arrowhead) and near the blood vessels (white arrow). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; n = 3 control, n = 3 diabetes.

Clock Gene-Silencing Downregulates Kcnj10 Expression

To determine whether prominent clock regulatory genes Bmal1 and Per2 have a potential role in regulation of Kcnj10 expression, we treated rMC-1 cells with siRNA for Per2 and Bmal1. We first validated our siRNA transfection using gene-specific primers for Per2 and Bmal1 (Figs. 2A, 2B). Next, we evaluated Kcnj10 expression in Per2 and Bmal siRNA-treated rMC1 cells. We observed a significant decrease in Kcnj10 expression in rMC1 cells treated with Per2 or Bmal siRNA (Fig. 2C). The decrease in mRNA levels of Kcnj10 after Per2 and Bmal siRNA treatment reflected in a similar decrease in Kir4.1 protein levels (Figs. 2D, 2E), suggesting an important role for clock genes in regulating Kir4.1 expression.
Kcnj10 and Kir4.1 Maintain Their Rhythm in Serum-Synchronized rMC-1 Cells

To understand whether TNF-α possesses a direct effect on Kir4.1 expression, we first evaluated in vitro rhythm of Kcnj10 in rMC-1 cells. We used an established protocol for synchronizing clock genes by treating rMC-1 cells with 50% horse serum for 2 hours followed by the absence of serum for the remainder of the study. The cells were harvested at 4-hour time intervals, and the mRNA expression of Kcnj10, Kir4.1, Bmal, and Per2 was determined. We observed that Kcnj10 maintains the biorhythm in vitro similar to our in vivo studies in which rats have a regular pattern of peaks and troughs every 24 hours (Fig. 3A). Serum-synchronized rMC-1 cells exhibited a functional clock as evidenced by a rhythmic change in Bmal and Per2 expression (Fig. 3B). Next, we examined protein expression of Kir4.1 by Western blot; the Kir4.1 expression followed a similar pattern as that of retinal Kcnj10 expression. The highest Kir4.1 expression was observed at ZT-16, ZT-20, ZT-40, and ZT-44 (Fig. 3C).

**FIGURE 2.** Clock gene silencing decreases Kcnj10 expression. rMC-1 cells were treated with Per2, Bmal1, or scrambled siRNA and the mRNA expression for (A) Per2, (B) Bmal1, and (C) Kncj10 was determined using qRT-PCR. Bar chart showing gene silencing for (A) Per2 and (B) Bmal1. (C) The siRNA treatment of rMCs with these clock genes showing a decrease in Kncj10 expression. Western blot for Kir4.1 showing a decrease in Kir4.1 expression after treatment with (D) Per2 siRNA and (E) Bmal siRNA.
Clock Gene-Silencing Upregulates TNF-α Expression

To study whether TNF-α exhibits a clock rhythm similar to Kir4.1, we performed a serum synchronization of rMC-1 cells and the mRNA expression of TNF-α was evaluated using qRT-PCR. As shown in Figure 4A, TNF-α exhibited an oscillatory pattern; however, we did not observe a peak or trough every 24 hours. Overall, the oscillatory pattern was bimodal for TNF-α.

To test if clock gene silencing has any effect on TNF-α levels, we treated the rMC-1 cells with siRNA for Bmal and Per2, and the mRNA expression of TNF-α was evaluated using qRT-PCR. Treatment with Bmal siRNA increased TNF-α by 1.5-fold (P < 0.01), while treatment with Per2 siRNA increased TNF-α by 1.3-fold (P < 0.05; Fig. 4B).

TNF-α Downregulates Kcnj10 Expression in Dose-Dependent Manner

To study the effect of TNF-α on Kcnj10 and Kir4.1 expression, we treated rMC-1 cells with rTNF-α in vitro. We performed a dose response using 5 to 5000 pg/mL of TNF-α. We observed a significant decrease in Kcnj10 expression at all concentrations tested. The greatest decrease of Kcnj10 was observed at 5000 pg/mL of TNF-α (Fig. 5A, P < 0.001). The Kir4.1 protein expression also was decreased in a dose-dependent manner (Fig. 5B). Based on a previous study, which reports TNF-α level in diabetic patients (without or with complications) in a range of 30 to 80 pg/mL, TNF-α (50 pg/mL) was used in the following experiments. To test whether the decrease in Kir4.1 is causal, we treated rMC-1 cells with a TNF-α receptor 1 inhibitor, R7050 before treatment with TNF-α. R7050 treatment blocked the effect of TNF-α, suggesting that the downregulation of Kir4.1 is mediated through TNF-α receptors (Fig. 5C).

TNF-α Downregulates Kcnj10 Expression by Disorganizing Actin Cytoskeleton

Previous studies suggest that TNF-α treatment leads to an actin depolymerization in arterial endothelial cells, but it is unknown whether TNF-α treatment has a similar response on actin depolymerization in Müller cells. To test this, we treated the rMC-1 cells with TNF-α and stained with phalloidin to visualize the actin cytoskeleton. Untreated rMC-1 cells showed a characteristic staining for phalloidin with continuous trans cytoplasmic actin filaments (Fig. 6A). The TNF-α treatment resulted in disruption of the F-actin lattice with disorganization of a filamentous pattern of actin staining. At higher concentrations, the cells were almost devoid of filamentous actin, and the actin was clustered mostly in bundles.

TNF-α Treatment Downregulates Kir4.1 Expression Via Dislocalization of SAP97 and Kir4

SAP97 is a mammalian membrane-associated guanylate kinase family member protein. Previous studies suggest that Kir4.1 is
expression of TNF-α for solid line function is shown as a dotted line determined using qRT-PCR (dotted line). rMC-1 cells were serum-synchronized and TNF-α for TNF-α treatment downregulated Kir4.1 expression, and we observed Kir4.1 in the pathogenesis of DR. We previously identified DR as a disease associated with clock gene dysregulation and disturbed diurnal rhythm. In this study, we emphasize on clock gene regulation in Müller cells. Our studies highlight that the Kir4.1 follows a diurnal rhythm in the retina, which is dampened in diabetes. The proinflammatory cytokine TNF-α inhibits the expression of Kcnj10 via depolymerization of the actin cytoskeleton and dissociation of Kir4.1 and SAP-97 complex.

Kir4.1 acts as a principal ion channel involved in potassium balance and fluid clearance from the retinal tissues. The complex in situ hybridization, immunofluorescence, and immunogold studies demonstrate that the large enrichment of Kir4.1 to Müller cell endfeet processes envelop the retinal blood vessels. As transport of water in Müller cells is coupled to K+ currents, downregulation of K+ channels by retinal Müller cells impairs the transectal water transport, resulting in swelling of the Müller cells. The rhythmic change in expression of Kir4.1 can influence the K+ conductance and the amount of water entering the Müller cells. Interestingly, previous studies performed on human retinas suggest that retinal thickness varies by time of day. In diabetic individuals with macular edema, the retina is thickest at night (resting phase) and thins out as the day progresses (activity phase). Changes in retinal thickness correspond to poorer reading vision early in the morning compared to the later part of the day, with reading improving spontaneously within 2 to 4 hours after waking.

An increase in overnight thickness of the retina can be partly explained by fluctuations in arterial blood pressure; however, other mechanisms, like the osmotic effect of extravasated plasma macromolecules and increased permeability of retinal vessels, also are suggested to have a role in regulating retinal thickness. Whether osmotic changes in Müller cells have any role in macular edema or overnight change in retinal thickness remains largely unknown. Our study, indeed, suggests that the expression Kir4.1 peaks at the beginning of the resting phase in nocturnal animals, like rats, which is in line with studies performed on diabetic individuals, where assessment of retinal thickness was performed between 8 and 10 PM. We speculated that changes in the K+ channel dynamic may also have an important role in retinal thickness and visual acuity in diabetic individuals.

The circadian rhythms have an important role in our day-to-day activities. The core of the circadian clock contains a set of clock genes, which form autoregulatory transcriptional-translational loops that regulate a range of physiologic functions and metabolic perturbations of the body. Approximately 10% of the transcriptome is known to be under the regulation of clock genes. Genetic studies suggest that clock gene dysfunction results in diverse pathophysiologic disorders, such as metabolic syndrome, obesity, premature aging, and abnormal sleep-wake cycles. The clock gene synthesis is regulated by a tightly controlled self-regulatory transcriptional and translational loop. The positive arm of clock genes constitutes by Clock and Bmal1 is at its highest during the day, while the negative arm, comprising Per and Cry genes, is highest during the night time. Notably, we observe that siRNA silencing of Bmal and Per2 downregulates Kcnj10 expression, suggesting the direct impact of the clock on Kir4.1 channels.
Previous studies report that the plasma concentration of TNF-α is approximately 80 pg/mL in patients with diabetic complications, while it is 30 pg/mL in diabetic patients without complications, which is slightly higher than in nondiabetic patients (22 pg/mL). In the aforementioned study, it is clear that complications, like DR, show particularly high levels of TNF-α. It is noteworthy that even a modest treatment of TNF-α (5 pg/mL) is detrimental in causing a 2-fold reduction in Kcnj10 expression. Although not tested, we speculate that TNF-α treatment also will directly affect clock regulation based on a previous report, which suggests that TNF-α suppresses the expression of clock gene, Per2, by directly inhibiting CLOCK-BMAL1-induced activation of E-box regulatory elements-dependent clock gene promoters.

Hypoxia is a critical regulator of clock genes and a variety of studies suggest that hypoxic environments leads to PER2 degradation. Hypoxia suppresses the levels of Per2 in hepatocellular carcinoma, renal carcinoma, and breast cancer. The hypoxia-mediated suppression of PER2 leads to an increase in epithelial-mesenchymal cell transition, which may be associated with an increase in tumor invasion, malignancy, and poor clinical outcome for breast cancer patients. Hypoxia also is a potent stimulator of TNF-α. Retinal ischemia leads to elevated levels of TNF-α in the vitreous and increases the mRNA levels of TNF-α in the neuroretina. Inhibition of TNF-α in the retina protects from ischemic retinopathy and reduces retinal neovascularization. Taken together, hypoxia has a profound effect on TNF-α and Per2; however, to our knowledge there are no studies highlighting how hypoxia signaling might influence Per2 gene by TNF-α. Considering the interplay of circadian rhythms, TNF-α, and Kir4.1, our study further paves a way for evaluating the effect of hypoxia on TNF-α modulation of Per2 and Kir4.1.

The mechanism by which TNF-α downregulates Kcnj10 expression is not known. Previous studies suggest that insulin and laminin treatment enhance the expression of Kir4.1. Laminin provides an anchoring domain for cells to adhere via integrins and focal adhesion kinase (FAK), and insulin,
together with laminin, helps in regulating the expression of Kir4.1 channels. F-actin, which is upstream of FAK, helps to strengthen the cellular skeleton and normal function of Kir4.1 channels. SAP97 is a critical member in clustering the Kir4.1 channel, and the coexpression of Kir4.1 with SAP97 enhances the expression of Kir4.1 and functional K⁺ currents.23 Taken together, we speculated that TNF-α treatment first causes depolymerization of the actin cytoskeleton, followed by dislocalization of SAP97 and Kir4.1, resulting in an overall decrease in colocalization for Kir4.1 and SAP97.

**TABLE.** Body Weight, Blood Glucose, and Glycated Hemoglobin for Diabetic Rats

<table>
<thead>
<tr>
<th>Metabolic Parameters</th>
<th>Control</th>
<th>Diabetes</th>
<th>Statistical Significance</th>
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<tr>
<td>Body weight</td>
<td>479.3 ± 6.6</td>
<td>422.4 ± 16.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>139.8 ± 3.4</td>
<td>321.4 ± 41.1</td>
<td>&lt;0.0001</td>
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<tr>
<td>Glycated hemoglobin</td>
<td>4.9 ± 0.1</td>
<td>7.7 ± 0.7</td>
<td>&lt;0.0001</td>
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**FIGURE 6.** TNF-α downregulates Kir4.1 expression by disorganization of F-actin and dissociation of SAP97. rMC-1 cells were treated with rTNF-α and stained for the following antibodies: phalloidin, Kir4.1, and SAP97. (A) The control cells exhibited continuous trans cytoplasmic actin filaments. The rTNF-α treatment resulted in disorganization of actin skeleton and the actin was observed mostly in clumps in the cytoplasm of rMC-1 cells. (B) rTNF-α (50 pg/mL)-treated rMC-1 cells were stained for Kir4.1 (red) and SAP97 (green) antibodies. The control cells showing the cytoplasmic and nuclear distribution for Kir4.1 along with colocalization for SAP97. The rTNF-α treatment resulted in a decrease in Kir4.1 staining with reduced colocalization of Kir4.1 and SAP97. (C) The colocalization analysis was performed using Zen Lite software showing a profound decrease in colocalization for Kir4.1 and SAP97.
Decrease in the expression of SAP97 and Kir4.1. Although not studied, FAK and laminin-mediated signaling also may have an important role in downregulation of Kir4.1 and is worth investigating in the future.

Overall, our studies suggested that the principal inwardly rectifying K+ ion channel, Kir4.1 in Müller cells, follows a diurnal rhythm in the retina, and diabetes-induced inflammatory milieu, such as an increase in TNF-α, can disrupt this physiologic rhythm and expression of the Kir4.1 channel, resulting in Müller cell dysfunction in the retina. Further, our studies showed the importance of time-based treatment in counteracting fluctuations in retinal thickness and macular edema in DR.

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References


