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Overexpression of SIK2 Inhibits FGF2-dependent Müller Glial Reprogramming

Asli Ugurlu Bayarslan [1,2] & Kuyas Bugra [1,3]

[1] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkiye
[2] Department of Biology, Kastamonu University, Kastamonu, Turkiye
[3] Life Sciences Center, Bogazici University, Istanbul, Turkiye

Abstract

Objective: Upon injury, Müller cells re-enter the cell cycle, acquire progenitor properties, and produce new retinal neurons in zebrafish. Proliferation is an essential step in retinal regeneration. The strict regulation of Müller cell proliferation limits mammalian retinal regeneration. Growth factors such as fibroblast growth factor 2 (FGF2) can promote the proliferation of Müller glia in mammals; however, the regeneration capacity is restricted. In this study, we investigated the possible contribution of salt-inducible kinase 2 (SIK2) to Müller reprogramming through FGF2 signaling.

Materials and Methods: MIO-M1 cells were used as the model system. Modulations in cell proliferation, extracellular signal-regulated kinase (ERK)1/2 activity, and SIK2 expression during 7 days of FGF2 treatment were documented. Overexpression studies were conducted to provide clues for the potential contribution of SIK2 to MIO-M1 reprogramming.

Results: Our findings demonstrate that the expansion of Müller cells that de-differentiate into progenitors requires ERK activation. A significant reduction in the SIK2 protein level is necessary for Müller cells to proliferate. SIK2 overexpression inhibited ERK activity, cell proliferation, and reprogramming.

Conclusion: We propose that SIK2 is involved in Müller reprogramming by suppressing ERK activation.

Keywords SIK2 · FGF2 · ERK · Reprogramming · Müller glia

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Corresponding author: Asli Ugurlu Bayarslan a.z.ugurlu@gmail.com

INTRODUCTION

The description of Müller cells dates back to 1851 by Heinrich Müller, and they were later identified as the principal glial cells in the retina (1). They span the width of the retina and contact all retinal neurons (2). The major role of Müller glia is to maintain retinal homeostasis and support the survival and function of neurons. In response to damage, Müller cells undergo a series of changes including upregulated production of neuroprotective factors, such as fibroblast growth factor 2 (FGF2) (3, 4). Consistent with the upregulation of neuroprotective factors, extracellular signal-regulated kinase (ERK) activation has been detected in experimental models of various retinopathies (5, 6). ERK activation is proposed to mediate cell cycle re-entry in activated Müller cells (7).

In zebrafish, Müller glia undergo a proliferative response upon injury, gain progenitor properties, and produce all retinal cell types (8). Most of the cells produced by proliferating Müller glia remain as progenitors, whereas a few differentiate into specific neurons in chick retia (9). Müller glia can become activated in mammals, but few proliferate in response to injury and do not replenish lost neurons (10). The limited Müller cell proliferation in mammals upon injury may be due to inhibitory mechanisms or limited mitogens. Characterization of the mechanisms that limit the proliferation of mammalian Müller cells may provide clues to unlocking the dormant regenerative potential of mammals (11). Müller glia may proliferate after retinal injury in humans, but there is no evidence of neuron regeneration in the human retina. Human Müller cells (the MIO lines), isolated from different post-mortem retinas (12), express Müller and progenitor markers. Growth factors stimulate these cells to express post-mitotic neuronal markers (13, 14). FGF2 is one of the factors involved in Müller proliferation and reprogramming (13, 15). Without any damage, FGF2 and insulin stimulate Müller glia, as observed after neurotoxic damage in chicks (15). FGF2 selectively activates the Ras/MAPK/ERK signaling pathway, which regulates Müller proliferation (16).

Salt inducible kinase 2 (SIK2) was first identified in the adipose tissue of mice and has a well-defined role in regulating metabolism (17, 18). SIK2 was shown to have a role in insulin release from β-islet cells in the pancreas (19). Furthermore, SIK2 activity in cancer progression was also implicated (20, 21). In cortical neurons, oxygen-glucose deprivation causes SIK2 degradation, which activates cyclic AMP-responsive element-binding protein (CREB) and its downstream genes and promotes neuronal survival (22). By controlling Akt phosphorylation, SIK2 blocks insulin-mediated Müller cell survival in normal and chronic hyperglycaemic conditions (23).

SIK2 is expressed in Müller cells and neurons in the inner nuclear layer of the retina (23). Recently, we identified a novel regulatory role of SIK2 downstream of FGF2 signaling, induced by ERK activation (24). In this context, we provide evidence showing that SIK2 is a factor that restrain Müller proliferation and impede reprogramming.

MATERIALS AND METHODS

Cell Culture

MIO-M1 cells were provided by Prof. Astrid Limb (University College London, Institute of Ophthalmology) and grown in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Scientific, USA) and 0.1% penicillin/streptomycin (Thermo Scientific, USA) at 37°C and under 5% CO_2. For FGF2 treatment, 500 MIO-M1 cells per cm^2 were cultured on matrigel-coated plates and serum-starved overnight. Subsequently, the cells were stimulated with 40 ng/mL FGF2 (R&D Systems, USA) for 7 days (13). The FGF2-containing medium was replenished every 2 days.

RNA Isolation and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA isolation was performed using the Ouick-RNA MiniPrep Kit (Zymo Research, USA) according to the manufacturer's instructions. RNA samples were treated with DNase I on a column for 30 minutes at 37°C. Total RNA was reverse-transcribed using the RevertAid First Strand cDNA synthesis kit (Thermo Scientific, USA). RT-qPCR amplifications were performed on a Piko Real 96 Real-time Thermal Cycler (Thermo Scientific, USA) in a reaction mixture containing SYBR Green (Takara, Japan) and the primer pairs listed in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a normalisation standard. The amplification reactions were started with initial denaturation at 95°C for 5 min. This was followed by 40 cycles of denaturation at 95°C for 10 s, annealing of primers for 10 s, and extension at 72°C for 10 s. Relative transcript levels were calculated using the ΔΔCT method.

Lysate Preparation and Western Blotting

Cell pellets were resuspended in ice-cold lysis buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 supplemented with protease and phosphatase inhibitor cocktails and lysed using ceramic beads (Roche, Germany) with MagnaLyser homogenizer (Roche, Germany) at 6500 rpm for 30 s. Centrifugation for 20 min at 13,200 rpm at 4°C removed cell debris.

Table 1. List of primers used in qPCR

Gene ID	Forward primer	Reverse primer
GAPDH	5'-GGAAGGTGAAGGTCGGAGTC-3'	5'-AACATGTAAACCATGTAGTTGAGGT-3'
Pax6	5'-CCGAGATTTCAGAGCCCCAT-3'	5'-AGACACCACCGAGCTGATTC-3'
Vimentin	5'-CGGGAGAAATTGCAGGAGGA-3'	5'-AAGGTCAAGACGTGCCAGAG-3'
Calretinin	5'-ATCCTGCCAACCGAAGAGAAC-3'	5'-GCAGGAAGTTTTCCTGGACAG-3'
Chx10	5'-AGTGTCATGGCGGAGTATGG-3'	5'-TTTTGTGCATCCCCAGTAGCC-3'
Hesr2	5'-GAGAGCGACATGGACGAGAC-3'	5'-CGACGCCTTTTCTCTATAATCCCT-3'
Prox1	5'-TGACTTTGAGGTTCCAGAGAGAT-3'	5'-AGGCAGTTCGGGGATTTGAA-3'

Total protein extracts or immunoprecipitated samples were separated on a polyacrylamide gel, electroblotted on a polyvinylidene fluoride (PVDF) membrane (Roche, Germany), blocked by either 1% skimmed milk powder or 5% bovine serum albümin (BSA) in 150 mM NaCl, 20 mM Tris-HCl (pH 8.0), 0.1% Tween 20 and probed with primary antibodies overnight at 4°C. Anti-SIK2 (Cell Signaling, 1/1000), anti-phospho-extracellular signal-regulated kinase (pERK) (Cell Signaling, 1/1000), anti-ERK (Santa Cruz, 1/1000), anti-β-actin (Santa Cruz, 1/5000) antibodies were used. The membranes were then washed and incubated with horseradish peroxidase (HRP)-tagged secondary antibodies. The antibody-antigen interactions were visualized by ImmunoCruz luminol reagent (Santa Cruz, USA) using the Stella imaging system (Raytest, Germany).

Cell Proliferation Assay

Proliferating cells were detected using an *in situ* cell proliferation kit, FLUOS (Roche, Germany). Briefly, 10 µM bromodeoxyuridine (BrdU) was added to the culture medium 5 h before fixation of the cells with 70% ethanol and 30% glycine (50 mM, pH 2). Cellular DNA was denatured with 4M HCl. The samples were incubated with the fluorescein-conjugated anti-BrdU antibody for 45 min at 37°C in the dark. 4′,6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei. Samples were analysed using Zeiss Axio Observer Z1 Inverted fluorescence microscope (Zeiss, USA). For each image, 200 cells were counted from distinct areas. For SIK2 overexpressed MIO-M1 cells, anti-BrdU antibody (Roche, Germany) and Alexa Fluor 555-conjugated secondary antibody were used.

SIK2 Overexpression in MIO-M1 Cells

MIO-M1 cells were transfected with the enhanced green fluorescent protein-salt inducible kinase 2 plasmid (pEGFP-SIK2) using X-treme Gene HP DNA transfection reagent (Roche, Germany) at a 3:1 ratio as instructed by the manufacturer. An empty pEGFP vector was used as the control. After 24 h, the culture medium was replenished. To select stable clones, cells were grown in 500 µg/mL neomycin (Sigma, USA) containing medium for 2 weeks, and the medium was replenished every 3 days. Neomycin-resistant colonies were collected using cloning cylinders. Western blotting analysis verified SIK2 overexpression in neomycin-resistant colonies.

Statistical Analyses

Differences between the untreated cells and FGF2-treated cells, and mock-transfected cells and pEGFP-SIK2 transfected cells were analysed with Student's t-test using Graphpad Prism 7. $p<0.05$ was considered as significant.

RESULTS

FGF2 Stimulates Müller Proliferation Through Early ERK Activation

Müller reprogramming consists of multiple stages, including the cell cycle re-entry of Müller cells, their conversion into progenitors, progenitor amplification, and differentiation (25). The ERK pathway plays a major role in FGF2-dependent MIO-M1 proliferation (26). Hence, we analysed the effect of FGF2 on the cell proliferation and ERK activity profiles of MIO-M1 cells. The proliferation assays indicated that 25% of the cells were BrdU positive in the untreated culture. This percentage increased up to 42% after 4 h of FGF2 treatment and stayed there for 24 h (Figure 1A). A second proliferation peak with 37% of the cells expressing BrdU was observed after 6 days of FGF2 stimulation (Figure 1A).

Parallel with cell proliferation, a 1.4-fold increase in ERK activity was detected after 4 h and 12 h of FGF2 stimulation (Figure 1B). In the remaining time points, the active ERK levels were near basal.

SIK2 Protein Expression is Diminished Upon FGF2 Induction

SIK2 plays a role in the negative regulation of FGF2-dependent ERK activation and proliferation in Müller glia (24). Based on the central role of SIK2 in Müller proliferation, we investigated the possible role of SIK2 in Müller reprogramming. Western blot analysis revealed that SIK2 levels decreased by

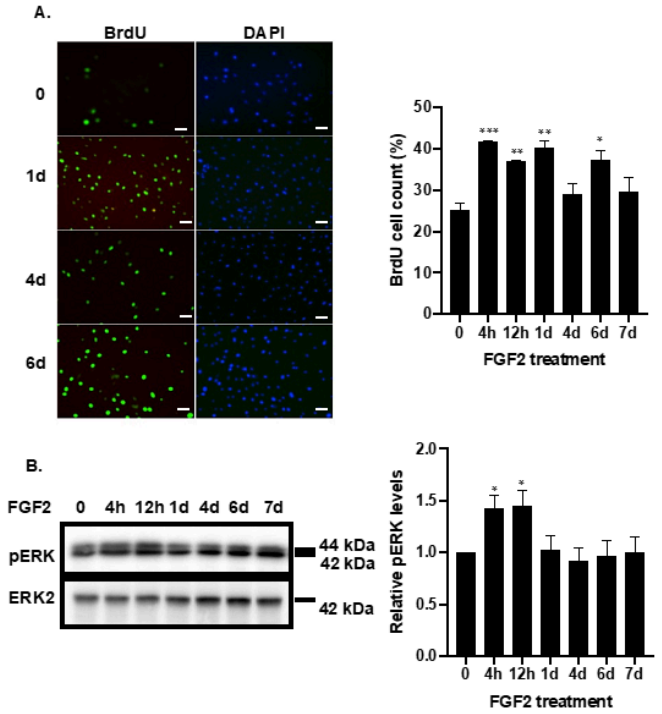

Figure 1. FGF2-dependent cell proliferation and ERK activity profile. MIO-M1 cells were treated with FGF2 for 7 days and the untreated cells were used as control group (0). A. BrdU incorporation was performed to detect proliferating cells, and DAPI staining was used to label the nuclei. The number of BrdU-positive cells was normalised to the number of DAPI-stained nuclei in the same samples. B. ERK activity was analysed by western blotting. The band intensities of pERK were normalised to that of ERK. Data are the means ± standard error; n=3. FGF2 treated cells were compared to the control group; *p<0.05, **p<0.01, ***p<0.001. Scale bar: 50 µm. BrdU: Bromodeoxyuridine; DAPI: 4′,6-diamidino-2-phenylindole; ERK2: Extracellular signal-regulated kinase 2; pERK: Phospho-extracellular signal-regulated kinase; FGF2: Fibroblast growth factor 2; h: hours, d: days.

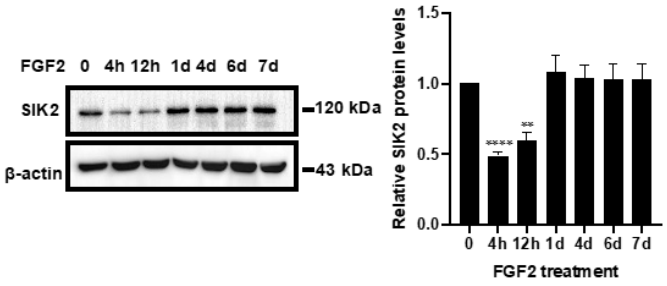

Figure 2. FGF2-dependent modulations in SIK2 expression. MIO-M1 cells were treated with FGF2 for 7 days and the untreated cells were used as control group (0). The SIK2 protein level was analysed with western blotting. Data are the means ± standard error; n=3. FGF2 treated cells were compared to the control group; ** p<0.01, ****p<0.0001. FGF2: Fibroblast growth factor 2; SIK2: Salt-inducible kinase 2; h: hours; d: days.

approximately 50% at 4 h and 12 h of FGF2 stimulation, recovered to basal levels after 24 h of FGF2 stimulation, and did not change at later time points (Figure 2).

SIK2 Overexpression Blocks FGF2-Dependent Cell Proliferation by Inhibiting ERK Activity

Because we observed a significant decrease in its protein level, we decided to focus on the expression level of SIK2 to modulate ERK activity and FGF2-dependent proliferative response in Müller cells. The pEGFP-SIK2 vector was used to transfect MIO-M1 cells for SIK2 overexpression. The presence of both the 120-kDa endogenous SIK2 band and the 150-kDa GFP-SIK2 band confirmed the SIK2 overexpression (Figure 3A). Only the endogenous SIK2 band was detected in the control cells.

We examined the effect of SIK2 overexpression on proliferation and ERK activation to test whether SIK2 inhibits proliferation through ERK in MIO-M1 cells. As in the wild-type cells, an increase in cell proliferation and ERK activity was observed at 4 h and 12 h in the mock-transfected cells. When SIK2 was overexpressed, the percentage of BrdU-positive cells was lower than the baseline at all times and strikingly near

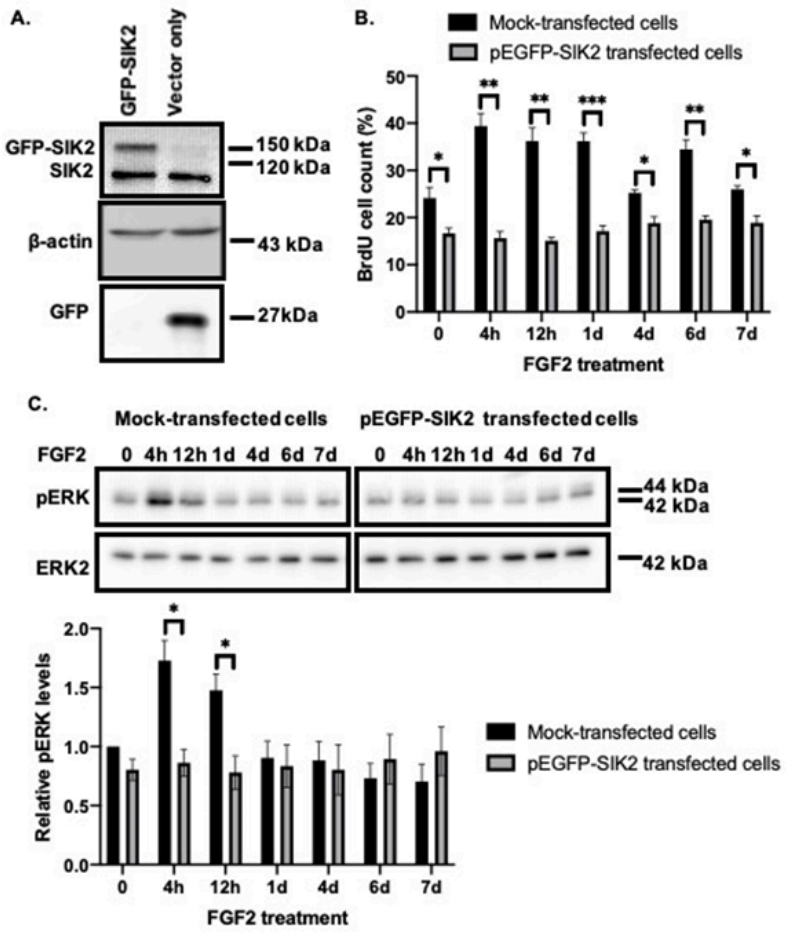

Figure 3. SIK2 overexpression in MIO-M1 cells. A. MIO-M1 cells were transfected with pEGFP-SIK2 for SIK2 overexpression and with pEGFP for mock transfection. The SIK2 protein level was analysed by western blotting. B. pEGFP-SIK2 transfected and mock-transfected MIO-M1 cells were treated with FGF2 for 7 days and the untreated cells were used as control group (0). The effect of SIK2 overexpression on cell proliferation was analysed by BrdU incorporation C. The effect of SIK2 overexpression on ERK activation was analysed by western blotting. Data are the means ± standard error; n=3. pEGFP-transfected cells were compared to mock-transfected cells; *p<0.05, **p<0.01, ***p<0.001. GFP: Green fluorescent protein; SIK2: Salt inducible kinase 2; FGF2: Fibroblast growth factor 2; pEGFP: Enhanced green fluorescent protein plasmid; pERK: Phospho-extracellular signal-regulated kinase; BrdU: Bromodeoxyuridine; ERK2: Extracellular signal-regulated kinase 2; h: hours; d: days.

half in the early days (Figure 3B). There was no significant increase in active ERK levels in the SIK2-overexpressed MIO-M1 cells, and SIK2 overexpression alone blocked the early activation of ERK2 in 4 h and 12 h (Figure 3C). These results show that during the early phases of FGF2 exposure, SIK2 overexpression inhibits cell proliferation through ERK activity, and blocking cell proliferation might hamper the reprogramming.

SIK2 Overexpression Alters FGF2-Dependent Transcriptional Activity

RT-qPCR experiments were performed to determine the FGF2-dependent transcriptional changes.

We observed an increase in the levels of the progenitor markers *paired box 6 (Pax6)* and *visual system homeobox 2 (Chx10)* that peaked at 12 h and 1 day of FGF2 treatment in mock-transfected MIO-M1 cells (Figure 4A, 4B). The simultaneous rise in the *Pax6* and *Chx10* levels shows that FGF2 stimulation induces progenitor features in MIO-M1 cells. The transcript levels of the Müller marker *vimentin* decreased after 4 days of FGF2 treatment in mock-transfected cells (Figure 4C). In the retina, Hes related family bHLH transcription factor with YRPW motif 2) Hesr2 promotes Müller development. The *Hesr2* transcript level was detectable in MIO-M1 cells as expected; however, we could not observe any significant change in the *Hesr2* level upon FGF2 treatment in mock-transfected cells (Figure 4D). After 6 days of FGF2 treatment, the expression of the neuronal marker *calretinin* increased in the mock-transfected cells (Figure 4E). We detected *Prospero homeobox 1 (Prox1)* expression, the regulator of horizontal cell development, in the mock-transfected cells. *Prox1* transcript levels were increased after 4 days of FGF2 treatment and remained high at subsequent

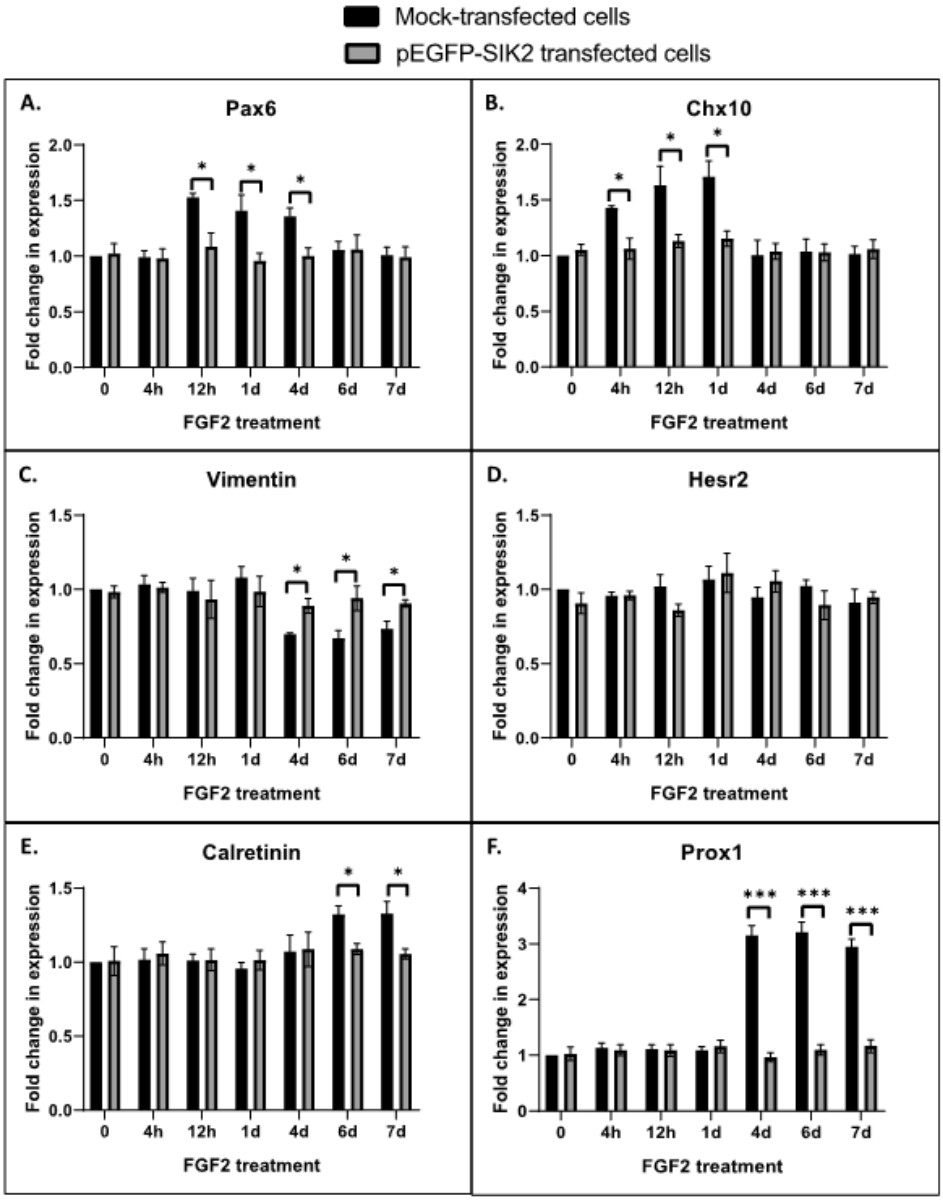

Figure 4. Effect of SIK2 overexpression on marker gene expression. pEGFP-SIK2 transfected and mock-transfected MIO-M1 cells were treated with FGF2 for 7 days and the untreated cells were used as control group (0). Relative mRNA expression levels were assessed by qPCR. The Ct of target gene/the Ct of reference gene (*GAPDH*) ratio for the untreated mock-transfected sample was set as a baseline value to which all time points were normalised. Data are the means ± SE; n=3. pEGFP transfected cells were compared to mock-transfected cells; *p<0.05, ***p<0.001. A. Pax6, B. Chx10, C. Vimentin, D. Hesr2, E. Calretinin, F. Prox1. SIK2: Salt inducible kinase 2; FGF2: Fibroblast growth factor 2; pEGFP: Enhanced green fluorescent protein plasmid; Pax6: Paired box 6; Chx10: Visual system homeobox 2; Hesr2: Hes related family bHLH transcription factor with YRPW motif 2; Prox1: Prospero homeobox 1; h: hours; d: days.

times (Figure 4F). Although MIO-M1 cells acquire certain neuronal features, as indicated by an increase in *calretinin*, our data highlight a considerable tendency towards horizontal cell fate.

In SIK2-overexpressing MIO-M1 cells, the expression of *Pax6*, *Chx10*, *vimentin*, *calretinin*, and *Prox1* remained at a basal level compared with the mock-transfected cells (Figure 4). These results support the hypothesis that SIK2 overexpression inhibits the cell cycle re-entry and reprogramming of MIO-M1 cells.

DISCUSSION

In zebrafish, Müller cells respond to injury by reprogramming, which results in the repair of the damaged retina. Mammalian Müller cells become activated, but their regeneration ability is highly restricted. Cell cycle re-entry is necessary for the functional regenerative response. The limited Müller proliferation is considered a barrier to retinal regeneration in mammals. FGF2 is one of the factors that stimulate Müller reprogramming through ERK activation (13, 15, 16). SIK2 negatively regulates FGF2-dependent Müller proliferation by

inhibiting ERK activity in MIO-M1 cells (24). Therefore, we hypothesised that SIK2 might be a crucial factor that regulates Müller proliferation and block reprogramming.

Initially, we investigated the temporal profile of proliferation and ERK activation during reprogramming. The number of BrdU-positive MIO-M1 cells was significantly increased between 4 h and 24 h of FGF2 stimulation; a second proliferation peak was detected after 6 days of FGF2 treatment. The parallel increase in ERK activity at 4-12 hours of FGF2 treatment demonstrated that enhanced proliferation at 4-12 hours involves the ERK pathway. In addition, SIK2 protein levels were nearly halved after 4 h and 12 h of FGF2 treatment and returned to basal levels after 24 h. SIK2 might regulate ERK activation during the early stages of reprogramming, as evidenced by the correlation between the rise in active ERK and the reduction in SIK2 protein levels. Consistent with this scenario, pERK levels in SIK2-overexpressing MIO-M1 cells remained at a basal level, and we could not detect any increase in the number of proliferating cells upon FGF2 treatment. In addition, the expression levels of the progenitor markers *Pax6* and *Chx10*, as well as the neuronal markers *calretinin* and *Prox1* did not increase in response to FGF2 in SIK2-overexpressing MIO-M1 cells.

Based on these results, we found that in the absence of FGF2, high levels of SIK2 prevent proliferation and reprogramming. After FGF2 stimulation, the reduction in SIK2 protein levels allows ERK activation at an early phase, and Müller cells proliferate and de-differentiate into progenitor cells, possibly through Notch, pCREB, and early growth response 1 (EGR1) (16).

De-differentiated MIO-M1 cells proliferate and enrich the progenitor pool, and a different signaling pathway may regulate this enrichment. At later stages, Notch, which functions downstream of forkhead box N4 (Foxn4), might promote horizontal cell formation (27).

Müller de-differentiation has recently been associated with changes in miRNA expression profiles (28). It has also been demonstrated that miRNAs targeting SIK2 expression, including miR143 and miR125, are involved in both de-differentiation and differentiation events (29, 30). miR-125 regulates retinal progenitor cells during development (30), and miR143 is differentially regulated in Müller glia during de-differentiation (29). It is likely that higher levels of these miRNAs result in decreased SIK2 expression in the early phase of the MIO-M1 reprogramming.

CONCLUSION

Our study provided evidence for the first time that SIK2 is involved in FGF2-induced MIO-M1 reprogramming. Increased SIK2 levels prevent cell cycle re-entry and reprogramming in the absence of FGF2. Following FGF2 treatment, the significant reduction in SIK2 levels allowed ERK activity, Müller cells re-enter the cell cycle, de-differentiate into progenitors, and differentiate into horizontal cells.

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Author Details

Asli Ugurlu Bayarslan

[1] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkiye

[2] Department of Biology, Kastamonu University, Kastamonu, Turkiye

0000-0003-2131-2823 a.z.ugurlu@gmail.com

Kuyas Bugra

[1] Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Turkiye

[3] Life Sciences Center, Bogazici University, Istanbul, Turkiye

0000-0003-1327-5938

REFERENCES

1. Vecino E, Rodriguez FD, Ruzafa N, Pereiro X, Sharma SC. Glia–neuron interactions in the mammalian retina. Prog Retin Eye Res 2016; 51: 1–40.
2. Bringmann A, Pannicke T, Grosche J, Francke M, Wiedemann P, Skatchkov S, et al. Müller cells in the healthy and diseased retina. Prog Retin Eye Res 2006; 25(4): 397–424.
3. Frasson M, Picaud S, Léveillard T, Simonutti M, Mohand-Said S, Dreyfus H, et al. Glial cell line-derived neurotrophic factor induces histologic and functional protection of rod photoreceptors in the rd/rd mouse. Invest Ophthalmol Vis Sci 1999; 40(11): 2724–34.
4. Honjo M, Tanihara H, Kido N, Inatani M, Okazaki K, Honda Y. Expression of ciliary neurotrophic factor activated by retinal Müller cells in eyes with NMDA- and kainic acid-induced neuronal death. Invest Ophthalmol Vis Sci 2000; 41(2): 552–60.
5. Takeda M, Takamiya A, Yoshida A, Kiyama H. Extracellular signal-regulated kinase activation predominantly in Müller cells of retina with endotoxin-induced uveitis. Invest Ophthalmol Vis Sci 2002; 43(4): 907–11.

6. Tezel G, Chauhan BC, LeBlanc RP, Wax MB. Immunohistochemical assessment of the glial mitogen-activated protein kinase activation in glaucoma. Invest Ophthalmol Vis Sci 2003; 44(7): 3025-33.
7. Dyer MA, Cepko CL. Control of Müller glial cell proliferation and activation following retinal injury. Nat Neurosci 2000; 3(9): 873–80.
8. Bernardos RL, Barthel LK, Meyers JR, Raymond PA. Late-stage neuronal progenitors in the retina are radial Müller glia that function as retinal stem cells. J Neurosci 2007; 27(26): 7028–40.
9. Fischer AJ, Reh TA. Müller glia are a potential source of neural regeneration in the postnatal chicken retina. Nat Neurosci 2001; 4(3): 247–52.
10. Karl MO, Reh TA. Regenerative medicine for retinal diseases: activating endogenous repair mechanisms. Trends Mol Med 2010; 16(4): 193–202.
11. Hamon A, Roger JE, Yang XJ, Perron M. Müller glial cell-dependent regeneration of the neural retina: An overview across vertebrate model systems. Dev Dyn 2016; 245(7): 727–38.
12. Limb GA, Salt TE, Munro PMG, Moss SE, Khaw PT. In vitro characterization of a spontaneously immortalized human Müller cell line (MIO-M1). Invest Ophthalmol Vis Sci 2002; 43(3): 864–9.
13. Lawrence JM, Singhal S, Bhatia B, Keegan DJ, Reh TA, Luthert PJ, et al. MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. Stem Cells 2007; 25(8): 2033–43.
14. Bhatia B, Jayaram H, Singhal S, Jones MF, Limb GA. Differences between the neurogenic and proliferative abilities of Müller glia with stem cell characteristics and the ciliary epithelium from the adult human eye. Exp Eye Res 2011; 93(6): 852–61.
15. Fischer AJ, McGuire CR, Dierks BD, Reh TA. Insulin and fibroblast growth factor 2 activate a neurogenic program in Müller glia of the chicken retina. J Neurosci 2002; 22(21): 9387–98.
16. Fischer AJ, Scott MA, Tuten W. Mitogen-activated protein kinase-signaling stimulates Müller glia to proliferate in acutely damaged chicken retina. Glia 2009; 57(2): 166–81.
17. Horike N, Takemori H, Katoh Y, Doi J, Min L, Asano T, et al. Adipose-specific expression, phosphorylation of Ser794 in insulin receptor substrate-1, and activation in diabetic animals of salt-inducible kinase-2. J Biol Chem 2003; 278(20): 18440–7.
18. Zhang ZN, Gong L, Lv S, Li J, Tai X, Cao W, et al. SIK2 regulates fasting-induced PPARα activity and ketogenesis through p300. Sci Rep 2016; 6(1): 23317.
19. Sakamaki JI, Fu A, Reeks C, Baird S, Depatie C, Al Azzabi M, et al. Role of the SIK2–p35–PJA2 complex in pancreatic β-cell functional compensation. Nat Cell Biol 2014; 16(3): 234–44.
20. Zohrap N, Saatci Ö, Ozes B, Coban I, Atay HM, Battaloglu E, et al. SIK2 attenuates proliferation and survival of breast cancer cells with simultaneous perturbation of MAPK and PI3K/Akt pathways. Oncotarget 2018; 9(31): 21876–92.
21. Miranda F, Mannion D, Liu S, Zheng Y, Mangala LS, Redondo C, et al. Salt-inducible kinase 2 couples ovarian cancer cell metabolism with survival at the adipocyte-rich metastatic niche. Cancer Cell 2016; 30(2): 273–89.
22. Sasaki T, Takemori H, Yagita Y, Terasaki Y, Uebi T, Horike N, et al. SIK2 is a key regulator for neuronal survival after ischemia via TORC1-CREB. Neuron 2011; 69(1): 106–19.
23. Küser-Abali G, Ozcan F, Ugurlu A, Uysal A, Fuss SH, Bugra-Bilge K. SIK2 is involved in the negative modulation of insulin-dependent Müller cell survival and implicated in hyperglycemia-induced cell death. Invest Ophthalmol Vis Sci 2013; 54(5): 3526.
24. Kuser-Abali G, Ugurlu-Bayarslan A, Yilmaz Y, Ozcan F, Karaer F, Bugra K. SIK2: A novel negative feedback regulator of FGF signaling. Adv Biology 2024; 8(11): 240003.
25. Goldman D. Müller glial cell reprogramming and retina regeneration. Nat Rev Neurosci 2014; 15(7): 431–42.
26. Hollborn M, Jahn K, Limb GA, Kohen L, Wiedemann P, Bringmann A. Characterization of the basic fibroblast growth factor-evoked proliferation of the human Müller cell line, MIO-M1. Graefes Arch Clin Exp Ophthalmol 2004; 242(5): 414–22.
27. Luo H, Jin K, Xie Z, Qiu F, Li S, Zou M, et al. Forkhead box N4 (Foxn4) activates Dll4-Notch signaling to suppress photoreceptor cell fates of early retinal progenitors. Proc Natl Acad Sci U S A 2012; 109(9): E553-62.
28. Quintero H, Gómez-Montalvo AI, Lamas M. MicroRNA changes through Müller glia dedifferentiation and early/late rod photoreceptor differentiation. Neurosci 2016; 316: 109–21.
29. Katsman D, Stackpole EJ, Domin DR, Farber DB. Embryonic stem cell-derived microvesicles induce gene expression changes in Müller cells of the retina. PLoS One 2012; 7(11): e50417.
30. Tao Z, Zhao C, Jian Q, Gillies M, Xu H, Yin ZQ. Lin28B promotes Müller glial cell de-differentiation and proliferation in the regenerative rat retinas. Oncotarget 2016; 7(31): 49368–83.