Caveolin-1 regulates corneal wound healing by modulating Kir4.1 activity

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Caveolin-1 (Cav1) is a 21-kDa integral membrane protein that is widely expressed in many cell types and critical in mediating numerous signaling molecules involved in cell growth, EGFR signaling, integrin-associated focal adhesion, and mobility (6, 20, 38). The relationship between Cav1 expression and ion channel activity in cell membrane has been extensively investigated (27, 31). It was shown that Cav1 was expressed in membrane microdomains and profoundly influenced ion channel activities by distinct mechanisms, such as altering channel open probability and modulating endocytosis or membrane surface expression (2, 5, 6). Although it is not completely clear whether Cav1 exerts its function exclusively through caveolae, a membrane invagination structure (3, 21), it is evidenced that Cav1 depletion reduces the surface expression of Kir4.1 in the cochlea leads to an abnormal endo-cochlear potential and, consequently, hearing loss (24, 30). We found that Kir4.1 was the major Kir channel expressed in corneal epithelial cells (29). Moreover, decreased Kir4.1 caused membrane depolarization, which promoted wound healing (29). However, little is known about the underlying mechanism by which Kir4.1 is regulated during the regeneration after wounding.

Caveolin-1 (Cav1), a 21-kDa integral membrane protein, is widely expressed in many cell types and critical in mediating numerous signaling molecules involved in cell growth, EGFR signaling, integrin-associated focal adhesion, and mobility (6, 20, 38). The relationship between Cav1 expression and ion channel activity in cell membrane has been extensively investigated (27, 31). It was shown that Cav1 was expressed in membrane microdomains and profoundly influenced ion channel activities by distinct mechanisms, such as altering channel open probability and modulating endocytosis or membrane surface expression (2, 5, 6). Although it is not completely clear whether Cav1 exerts its function exclusively through caveolae, a membrane invagination structure (3, 21), it is evidenced that Cav1 depletion reduces the surface expression of Kir4.1 in the cochlea leads to an abnormal endo-cochlear potential and, consequently, hearing loss (24, 30). We found that Kir4.1 was the major Kir channel expressed in corneal epithelial cells (29). Moreover, decreased Kir4.1 caused membrane depolarization, which promoted wound healing (29). However, little is known about the underlying mechanism by which Kir4.1 is regulated during the regeneration after wounding.

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Zhang C, Su X, Bellner L, Lin D. Caveolin-1 regulates corneal wound healing by modulating Kir4.1 activity. Am J Physiol Cell Physiol 310: C993–C1000, 2016. First published April 27, 2016; doi:10.1152/ajpcell.00023.2016.—The expression of caveolin-1 (Cav1) in corneal epithelium is associated with regeneration potency. We used Cav1−/− mice to study the role of Cav1 in modulating corneal wound healing. Western blot and whole cell patch clamp were performed to detect the phosphorylation of Cav1 in corneal wound healing. With the presence of mitomycin C (10 μg/ml) to inhibit the proliferation of corneal epithelial cells, we found that Cav1−/− mice showed a significantly reduced migration compared with control Cav1+/− mice in vitro (70 ± 10 vs. 36 ± 3%, P < 0.01). Our findings imply that the mechanism by which Cav-1 knockout promotes corneal regrowth is, at least partially, due to the inhibition of Kir4.1 which stimulates EGFR signaling.

caveolin-1; Kcnj10; corneal wound healing

Corneal epithelial cells have a vigorous ability to regenerate after injury. Migration and proliferation are distinct overlapping stages that are essential in regaining integrity of the corneal structure (7, 12). Perturbations in the process of regeneration can lead to delayed wound healing, keratitis and permanent epithelial defects. Epidermal growth factor and epidermal growth factor receptor (EGF/EGFR) signaling play an essential role in mediating corneal regeneration after wounding (8, 9, 12, 22, 31, 32, 37, 39). Insufficient EGF/EGFR stimulation after wounding in diabetic corneas results in delayed regeneration (34).

Kir4.1 is encoded by the gene Kcnj10, a member of the inward rectifier potassium channel family (Kir). Kir determines cell membrane potential by favoring the permissive K+ exit from the cells along with other channels, such as chloride channels (18, 23). Genetic mutations in Kcnj10 are associated with the autosomal recessive SeSAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance), also known as EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) (2). Since Kir4.1 is a major Kir channel expressed in cochlea determining membrane potential, loss of Kir4.1 in the cochlea leads to an abnormal endo-cochlear potential and, consequently, hearing loss (24, 30). We found that Kir4.1 was the major Kir channel expressed in corneal epithelial cells (29). Moreover, decreased Kir4.1 caused membrane depolarization, which promoted wound healing (29). However, little is known about the underlying mechanism by which Kir4.1 is regulated during the regeneration after wounding.

We have previously found that knockout of Cav1 inhibited Kir4.1 by abolishing the tyrosine phosphorylation of Kir4.1 in renal epithelial cells (28, 36); this suggests that Cav1 depletion reduces the surface expression of Kir4.1. Our recent study showed that Kir4.1 was the major type of Kir channels that determined cell membrane potential in corneal epithelium (29). In addition, cell membrane depolarization caused by Kcnj10 inhibition triggered EGF-like effects, such as the stimulation of EGF/TGFα secretion, tyrosine phosphorylation of EGFR, and activation of Rac1. As a consequence of the effects, membrane depolarization accelerated cell migration and wound healing in cornea (29). In this study, we find that knockout of Cav1 elevates membrane potential by inhibiting Kir4.1 activity,
which in turn enhances the phosphorylation of EGFR. Thus we hypothesize that loss of Cav1 promotes corneal wound healing through inhibiting Kir4.1.

MATERIALS AND METHODS

Animals and corneal debridement. All animal experiments were performed with a protocol approved by the Institutional Animal Care and Use Committee and in accordance with the U.S. National Institutes of Health Guide for Care and Use of Laboratory Animals. C57BL/6J Cav1−/− mice (stock no. 007083; The Jackson Laboratory, Bar Harbor, ME) were anesthetized with ketamine (50 mg/kg) and xylazine (20 mg/kg) intramuscularly, and 0.5% tetracaine-HCl eye drops were topically applied to deliver local anesthesia before animals were subjected to injury. The procedure of corneal epithelial debridement and assessment of regeneration after injury have been previously described (1). The corneal epithelium was removed up to the corneal limbal border with a 0.5-mm corneal rust ring remover (Algerbrush II; Alger Equipment, Lago Vista, TX). Reepithelialization was monitored by topical application of 1% fluorescein. Images of the anterior surface were taken with a dissecting microscope (Discovery V12; Carl Zeiss, Jena, Germany) coupled to a digital camera (AxioCam HRC; Carl Zeiss) and analyzed using software Axiovision 4.6 (Carl Zeiss).

Cell line and cell culture. The establishment of primary cultures of murine corneal epithelial cells (pMCE) and maintaining an immortalized human corneal epithelial cell line (HCE) were previously described (29). Briefly, the explants of corneal buttons from C57BL/6J wild type (WT) and Cav1−/− were initially maintained in CrT50 medium (CellTec, Bern, Switzerland) till full confluence (~2 wk) and subcultured in Epilife complete medium (Life Technologies, Grand Island, NY). All cells used for whole cell patch clamp were from passage 2 (P2) to avoid variation among the experiments. HCE was maintained in defined KFSM (Life Technologies).

Reagents and antibodies. The antibodies against pEGFR[tyrosine-845], EGFR, and Cav1 and were purchased from Cell Signaling Technologies (Beverly, MA). The antibody against α-tubulin was purchased from Rockland (Limerick, PA). All chemicals and protease/phosphatase inhibitors were obtained from Sigma Aldrich (St. Louis, MO). siRNA was designed as 19nt-length double-strand RNA ending with dTdT overhanging and screened online to avoid off-target and SNP sites (Dharmacon, Lafayette, CO). The efficiency of siRNA-Kir4.1 has been previously examined (11). The 19nt mRNA sequences were Cav1: 5'-GCTCTCCGTGATGGATTCACA; Kir4.1: 5'-GGCTATGTCCTGCTACA; and control siRNA: 5'-ACTACGTCGTTAGGGT.

Scrape wound healing assay in vitro. Scrape wound healing assay was performed as previously described (11). Briefly, HCE cells were seeded in 24-well plates and maintained at 70% confluence before the siRNA transfection. For preparing transfection mixture, 100 nM siRNA (applied to all gene silencing conditions) or control were mixed with 2 l lipofectamine 2000/well in 50 l medium and incubated at room temperature (RT) for 15 min. The transfection-mixture was then added to HCE. After a 48-h incubation, scrape injury was made by using a 10-μl tip with the presence of 10 μg/ml mitomycin C. The images were acquired with an inverted microscope Axiovert25 (Carl Zeiss) equipped with a camera (CMOS; Q Imaging, Surrey, BC, Canada). The regrowth efficiency was measured at the specified time points using the formula: (% restoration = (S0 - S1)/S0 × 100, where S0 represents the bare surface area at starting time point and S1 represents the bare surface area at specified time points after injury.

Whole cell patch-clamp electrophysiology. The pMCE at P2 were used in patch-clamp experiments. The perforated whole cell patch protocol has been described in detail previously (11). Perforated patch-clamp experiments were carried out at RT. For measurement K+ currents, we added 0.6 mM BaCl2 at the end of experiments. The cells were incubated with a bath solution containing 140 mM KCl, 0.5 mM MgCl2, 1.5 mM CaCl2, and 10 mM HEPES (pH 7.4). For the measurement of K+ reversal potential, the cells were bathed in a solution containing 5 mM K+/140 mM Na. Borosilicate glass (1.7-mm OD; Harvard Apparatus, Holliston, MA) was used to make the patch-clamp pipettes that were pulled with a Narishige electrode puller (Narishige, Long Island, NY). The pipette had a resistance of 2 to 4 MΩ when filled with 140 mM KCl. The tip of the pipette was filled with pipette solution containing 140 mM KCl, 2 mM MgCl2, 1 mM EGTA, and 5 mM HEPES (pH 7.4). The pipette was then back-filled with pipette solution containing amphotericin B (20 μg/0.1 ml). After a high-resistance seal was formed (>2 GΩ), the membrane capacitance was monitored until the whole cell patch configuration was formed. The cell membrane capacitance was measured and compensated. This compensated value indicated the membrane capacitance of each cell and it was used for normalizing K+ currents for each measurement. K+ currents were measured by an Axon 200A patch-clamp amplifier (Molecular Devices, Sunnyvale, CA). The currents were low-pass filtered at 1 KHz and digitized by an Axon interface (Digidata 1200; Molecular Devices). Data were stored in a Dell PC and were analyzed using the pClamp software version 9 at sampling rate of 4 KHz (Molecular Devices). K+ currents were presented as picoamperes/25 picofarads.

Transwell migration assay in Boyden chamber. pMCE (1×104) at P2 cultured from C57BL/6J WT and Cav1−/− mice were seeded in an 8-μm pore-size insert-chamber (BD Falcon, Franklin Lakes, NJ). After 6 h, the insert was cleaned with Q-tip on the top side, fixed with 4% paraformaldehyde for 5 min, stained with 0.1% crystal violet for 30 min, and washed with tap water. After the insert dried, images were taken with an inverted microscope Axiovert25 (Carl Zeiss) and the cells were manually counted.

Corneal protein sample preparation and Western blot. One-hundred microliters of lysis buffer (1% Triton-X100, 25 mM Tris, and 1× protease and phosphatase inhibitor cocktail) were applied to each cornea in a microtissue grinder (item no. 358204; Wheaton, Millville, NJ). After 20 times of up-and-down manual strokes on ice, the sample was centrifuged with 8,000 g for 20 min. The supernatant was aliquoted and stored at −80°C until use. Total protein (20 μg) was loaded onto SDS-PAGE gel to resolve the sample and then transferred to an NC membrane with 100 V for 1 h. The membrane was blocked and probed with the first antibody, washed three times with 0.1% PBST, and followed with the IRDye secondary antibody (LI-COR, Lincoln, NE). The blot image was finally scanned with an Odyssey infrared imager and the densitometry was analyzed by Image Studio software (LI-COR). To compare the expression levels of a given protein between different groups, we used the band density of a housekeeping protein determined by densitometry as standard and this value was used to normalize loading variations among the groups. We then obtained the density of the relevant protein band, which was divided by corresponding standard of the housekeeping gene to obtain the ratio. We rescaled the ratio of the control group as 1 (reference group) and this ratio was used to normalize the expression of a protein among different groups.

Statistical analyses. Student’s t-test (unpaired groups) was used to compare the differences between two groups. P < 0.05 was considered as statistical significance.

RESULTS

Genetic deletion of Cav1 suppressed Kir4.1 in corneal epithelial cells. Since it was demonstrated that disruption of Cav1 decreased Kir4.1 activity in renal tubular cells (28), we predicted that the lack of Cav1 would lead to inhibition of endogenous K+ channels in cornea. Therefore, we examined the effect of Cav1 knockout on Kir4.1 activity in primary cultured murine corneal epithelial cells (pMCE) from WT and...
Cav1<sup>−/−</sup> mice. Figure 1A represents a typical whole cell Ba<sup>2+</sup>-sensitive K<sup>+</sup> current measured with the perforated whole cell recording at clamping potential from −100 to 100 mV in 10 s. The results from four experiments summarized in Fig. 1B show that the Ba<sup>2+</sup>-sensitive whole cell K<sup>+</sup> current in pMCE from Cav1<sup>−/−</sup> mice (602 ± 96 pA, mean ± SE, P < 0.01) was significantly lower than in cells from WT mice (1,300 ± 80 pA). The similar results were obtained with step protocol (data not shown). In addition, K<sup>+</sup> reversal potential was measured with perforated whole cell recording. It is summarized in Fig. 1C and shows that K<sup>+</sup> reversal potential from Cav1<sup>−/−</sup> was increased than that from WT mice (−43 ± 7.5 vs. −58 ± 4.0 mV, respectively, P < 0.05, n = 4). Since K<sup>+</sup> reversal potential is very close to the cell membrane potential, a less positive K<sup>+</sup> reversal potential indicates the depolarization of pMCE. Our results suggest that the disruption of Cav1 reduces the endogenous inward K<sup>+</sup> currents and depolarizes cell membrane in pMCE. Since the major inwardly rectifying K<sup>+</sup>
channel is Kir4.1 in cornea (29), we conclude that genetic knock out Cav1 suppresses Kir4.1.

We then used Western blot to further test the effect of Cav1 knockout on expression of Kir4.1 in corneas from Cav1\(^{−/−}\) mice and WT counterpart. Figure 2A shows that Kir4.1 expression in corneal tissues was suppressed in Cav1\(^{−/−}\) mice. The results analyzed with Image Studio are summarized in Fig. 2B showing that the normalized expression of Kir4.1 from Cav1\(^{−/−}\) was decreased compared with that in WT (0.09 ± 0.03- vs. 1.25 ± 0.68-fold, respectively, mean ± SD, \(P < 0.01, n = 4\)).

**Inhibition of Cav1 or Ba\(^{2+}\) incubation stimulated the phosphorylation of EGFR at Tyr845 in corneas.** Our previous study showed that the downregulation of Kir4.1 stimulated EGF/EGFR-dependent signaling in cornea (29). Since the disruption of Cav1 also inhibited Kir4.1, we speculated that the lack of Cav1 should mimic the effect of Kir4.1 inhibition by stimulating EGF/EGFR signaling. Thus we examined EGFR phosphorylation levels in corneas from Cav1\(^{−/−}\) and Kcnj10\(^{−/−}\) mice (used as positive control) compared with WT mice control, respectively. Figure 2C shows a representative blot from four independent experiments. It shows that EGFR\(^{Y845}\) phosphorylation was significantly increased in both Cav1\(^{−/−}\) and Kcnj10\(^{−/−}\) mice contrasted with a very weak band in WT (Fig. 2D). Total EGFR and \(α\)-tubulin blotting indicated identical total protein loading. The effect of Cav1 inhibition on EGFR activities was further tested by HCE cells transfected with siRNA-Cav1 and negative control. Figure 3A shows that EGFR\(^{Y845}\) phosphorylation in HCE transfected with siRNA-Cav1 was upregulated compared with control (4.57 ± 0.34- vs. 1.0 ± 1.78-fold, mean ± SD, \(P < 0.01, n = 4\); Fig. 3B). The silencing efficiency of siRNA-Cav1 is shown in Fig. 3C. To examine whether membrane depolarization elevated EGFR phosphorylation, HCE cells were preincubated with 0.6 mM BaCl\(_2\) for 2 h before being lysed for western blot. Figure 3D shows that Ba\(^{2+}\) incubation enhanced the phosphorylation of EGFR\(^{Y845}\). The results from Fig. 3D are analyzed in Fig. 3E. It shows that the phosphorylation of EGFR\(^{Y845}\) was significantly stimulated by Ba\(^{2+}\) incubation compared with control (1.47 ± 0.11- vs. 1.07 ± 0.12-fold, mean ± SD, \(P < 0.05, n = 3\)).

**Cav1 inhibition promoted cell migration in corneal epithelial cells.** To determine whether the disruption of Cav1 influenced cell migration, we used a transwell migration assay as described by other investigators (16, 29). Figure 4A is a representative microscopic field from both Cav1\(^{−/−}\) (right) and WT (left), showing that cells from Cav1\(^{−/−}\) mice had a larger number of cells migrating to the chamber side compared with

![Fig. 3.](image-url)

**Fig. 3.** Inhibition of Cav1 by siRNA or inhibition of Kir4.1 by Ba\(^{2+}\) incubation enhances the phosphorylation of EGFR\(^{Y845}\) in human corneal epithelial cells (HCE). A, top: expression of phosphorylation of EGFR\(^{Y845}\) from both control and siRNA-Cav1 groups. A, bottom: total EGFR expression in the groups. B: normalized expression of EGFR\(^{Y845}\) was quantitated by densitometry (\(P < 0.01, n = 4\)). C: silencing efficiency of siRNA-Cav1 was examined by statistical analysis. D: Ba\(^{2+}\) incubation enhanced the expression of EGFR\(^{Y845}\) in HCE cells. E: normalized expression of EGFR\(^{Y845}\) from Ba\(^{2+}\) incubation and control was quantitated by densitometry (\(P < 0.05, n = 3\)).
cells from WT mice. The results are summarized in Fig. 4B indicating that the number of cells migrating to chamber side at 6 h was significantly higher in Cav1−/− mice than in WT (WT vs. Cav1−/−, 173 ± 12.1 vs. 271 ± 15.5, mean ± SD, P < 0.01, n = 4).

Deletion of Cav1 promoted wound healing of corneal epithelial cells. After demonstrating that the disruption of Cav1 promoted EGF/EGFR signaling, we used Cav1−/− mice to determine whether the lack of Cav1 accelerated wound healing process in vivo. Mouse corneal debridement (Fig. 5A) was performed to evaluate the effect of Cav1 genetic deletion on wound healing. As seen in Fig. 5B, healing of corneal wound was markedly accelerated in Cav1−/− compared with WT mice (70 and 90 vs. 30 and 50% at day 2 and day 6 after injury, respectively, mean ± SD, P < 0.01, n = 6).

Fig. 4. Knockout of Cav1 significantly stimulates migration in pMCE compared with WT. A: migrating pMCE cultured from WT (left) and Cav1−/− mice (right). B: migrating cells from Cav1−/− and WT were counted and are shown (P < 0.01, n = 4).

Fig. 5. Knockout of Cav1 accelerates corneal wound healing in vivo. Corneal debridement was performed in Cav1−/− and WT. A: images from both Cav1−/− and WT were acquired at the specified time points indicated. The regeneration after wounding was analyzed and is shown in B (n = 6, P < 0.01).
Scratch wounding assay was also employed to test whether decreased expression of Cav1 promoted corneal epithelial cell migration/regeneration via suppression of Kir4.1. Figure 6A shows that in the presence of mitomycin C, which eliminated the influence of cell proliferation, migration of HCE transfected with siRNA-Cav1 was significantly enhanced compared with control. Clustered migrating cells appeared to be polarized and protruded along wounding edges in HCE transfected with siRNA-Cav1 (Fig. 6A, right, arrow). In contrast, few scattered migrating cells were observed in HCE transfected with negative control (Fig. 6A, left, arrow). The migration is analyzed in Fig. 6B. It shows that siRNA-Cav1 promoted migration to 22.9 ± 10.2% compared with negative control 11.3 ± 5.5% (mean ± SD). We next tested whether such stimulatory effect on migration was further enhanced by cotransfection with siRNA-Kcnj10. Figure 6C shows that HCE transfected with siRNA-Cav1 stimulated regeneration compared with negative control at 8 h after wounding (72.9 ± 11.7 vs. 58.3 ± 14.2%, mean ± SD, P < 0.01, n = 4). HCE transfected with siRNA-Kcnj10 promoted regrowth compared with negative control (78.3 ± 11.6 vs. 60.6 ± 13.6%, mean ± SD, P < 0.01, n = 4). However, HCE cotransfected with siRNA-Cav1 and -Kcnj10 failed to further accelerate the regrowth after wounding compared with either siRNA-Cav1 or siRNA-Kcnj10, respectively. (n = 4, P = NS).

DISCUSSION

The corneal wound healing process is characterized as multiple overlapping stages including epithelial cell migration, proliferation, and differentiation (10, 14, 31–35). Among these stages, cell migration, in which EGFR signaling plays the essential role, from adjacent cells is the initial event occurring after wounding. A better understanding of novel modulators to promote cell migration would positively contribute to the accelerated regeneration for corneal complications, such as corneal persistent epithelial defects (PEDs).

Cav1 is ubiquitously expressed in many cell types, including corneal epithelial cells (20). It exerts profound influence in mediating membrane lipid metabolism and membrane protein trafficking. A recent study (5) demonstrated that Cav1 is indispensable for Gq protein-mediated Kir4.1 activities in renal epithelial cells. The study suggested that Cav1 may influence cell membrane potential by modulating Kir4.1 function

![Fig. 6. Cav1 inhibition by siRNA promotes cell migration in HCE. A: image from negative control (left) shows that few scattered cells (arrows) started to migrate to the bare area. In contrast, image from siRNA-Cav1 (right) shows that populated polarized cells appeared to clustered (arrows) crossing the injury edge. B: Student’s t-test was used to compare the difference between groups from both negative control and siRNA-Cav1 (P < 0.05, n = 4). C: scrape wound healing assay was performed on HCE transfected with siRNA-Cav1, -Kcnj10, -Cav1/Kcnj10, and control, respectively. The image shows that siRNA-Cav1 or -Kcnj10 significantly stimulated the regrowth (P < 0.01, n = 4). However, cotransfection with both siRNA-Cav1 and -Kcnj10 could not further accelerate this stimulation effect when comparing them with either siRNA-Cav1 or -Kcnj10 (P = N.S., n = 4).](http://ajpcell.physiology.org/)
through recruiting G-protein signaling. In addition cell depolarization potentially affected many cell physiological activities, such as cell migration and proliferation.

We currently find that Cav1 is abundantly expressed in both mouse cornea and human corneal epithelial cells (HCE). Furthermore, the Western blot from Cav1−/− mouse corneas shows that genetic deletion of Cav1 significantly suppresses the expression of Kir4.1, a key determinant of corneal epithelial cell membrane potential, indicating a close relationship between the expression of Cav1 and Kir4.1 in corneas. Since we previously showed that suppression of Kir4.1 expression was associated with increased EGFR (29), it is reasonable to predict that knockout of Cav1 induces cell membrane depolarized due to decreasing Kir4.1 function. Here we demonstrate that knockout of Cav1 elevates the cell membrane potential in cornea. Moreover, suppression of either Cav1 or Kir4.1 significantly increases EGFR Y845 phosphorylation, which is a critical player in mediating corneal wound healing. The enhanced EGFR signaling may constitute the mechanism by which suppression of Cav1 accelerates corneal regeneration following injury. Both mouse corneal debridement from WT and Cav1−/− mice and transwell migration assay support the conclusion that inhibition of Cav1 promotes corneal cell migration and wound healing. Since we have previously demonstrated that inhibition of Kir4.1 promotes corneal cell migration after injury, we fail to observe the additional stimulatory effect of cotransfection with siRNA-Cav1 and -Kcnj10 on cell migration. We think that the beneficial effect of Cav1 suppression on cell migration probably depends on cell depolarization induced by inhibiting Kir4.1 activity.

Increasing evidence shows that membrane depolarization caused by the repression of Kirs, such as Kir2.1 and Kir4.1, widely modulates cellular physiological processes, such as stimulation of cell proliferation and inhibition of apoptosis by activating RhoA small GTPase and Ca2+ signaling (13, 15, 19, 25–27). We previously found that Kir4.1 was the major Kir channel expressed in corneal epithelial cells, and it determined membrane potential by allowing K+ exit from cells. Moreover, wounding inhibited Kir4.1 expression by increasing miR-205 expression. Additionally, inhibition of Kir4.1 facilitated corneal neogrowth after injury and promoted cell migration (11, 29). The underlying mechanism by which Kir4.1 modulates corneal wound healing is that depolarization enhances cell mobility and proliferation by stimulating GTP-Rac1 production, thus, causing EGF-like effects, which include, but are not limited to, the stimulation of EGFR and its downstream signaling. Regarding the mechanism by which Kir4.1 activities were regulated during regeneration after wounding, we found that miR-205 overexpression inhibited Kir4.1 posttranscriptionally (11). The current study reveals that the expression and presence of membrane adapter protein Cav1 are prerequisites for Kir4.1 function, suggesting that Cav1 plays a critical role in corneal epithelial wound healing, presumably by supplying the lipid-raft enriched docking site for Kir4.1 channel expression and functional opening.

Here, we have identified a novel mechanism shown in Fig. 7. We find that Cav1 depletion in corneal epithelium accelerates corneal wound healing by Kir4.1 inhibition through diminishing K+ conductance that results in membrane depolarization. These depolarization-induced EGF-like effects need to be further explored. Our current limitation is the lack of the specific stimulator for Kir4.1 to test the specificity of the mechanism by which Cav1 inhibition exclusively suppresses Kir4.1. Regarding the underlying mechanism by which Cav1 regulated Kir4.1 activity, our previous study demonstrated that the disruption of caveolin-1 inhibited the stimulatory effect of src-family protein kinase on Kir4.1 in HEK cells and in the kidney (28). We surmise that the same mechanism may exert a role in the corneas (28, 36).

In conclusion, Cav1 expression is correlated with Kir4.1 activity and inhibition of Cav1 promotes corneal wound healing and migration, largely through reducing Kir4.1 activities, which in turn stimulate depolarization-induced EGFR phosphorylation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Caveolin-1 Regulates Corneal Wound Healing

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