Corneal Wound Healing Requires IKB kinase β Signaling in Keratocytes

Liang Chen[1¤a], Maureen Mongan[1], Qinghang Meng[1¤b], Qin Wang[1], Winston Kao[2], Ying Xia[1]*

1 Department of Environmental Health and Center of Environmental Genetics, University of Cincinnati Medical Center, Cincinnati, Ohio, 45267–0056, United States of America, 2 Department of Ophthalmology, University of Cincinnati Medical Center, Cincinnati, Ohio, 45267–0056, United States of America

¤a Current address: Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA
¤b Current address: Department of Molecular Cardiovascular Biology, Cincinnati Children's Hospital, Cincinnati, Ohio 45220, USA
* ying.xia@uc.edu

Abstract

IkB kinase β (IKKβ) is a key signaling kinase for inflammatory responses, but it also plays diverse cell type-specific roles that are not yet fully understood. Here we investigated the role of IKKβ in the cornea using $Ikk\beta^{\Delta CS}$ mice in which the $Ikk\beta$ gene was specifically deleted in the corneal stromal keratocytes. The $Ikk\beta^{\Delta CS}$ corneas had normal morphology, transparency and thickness; however, they did not heal well from mild alkali burn injury. In contrast to the $Ikk\beta^{F/F}$ corneas that restored transparency in 2 weeks after injury, over 50% of the $Ikk\beta^{\Delta CS}$ corneas failed to fully recover. They instead developed recurrent haze with increased stromal thickness, severe inflammation and apoptosis. This pathogenesis correlated with sustained myofibroblast transformation with increased α smooth muscle actin (α-SMA) expression, higher levels of senescence β-Gal activity and scar tissue formation at the late stage of wound healing. In addition, the $Ikk\beta^{\Delta CS}$ corneas displayed elevated expression of hemo-oxygenase-1 (HO-1), a marker of oxidative stress, and activation of stress signaling pathways with increased JNK, c-Jun and SMAD2/3 phosphorylation. These data suggest that IKKβ in keratocytes is required to repress oxidative stress and attenuate fibrogenesis and senescence in corneal wound healing.

Introduction

IκB kinase β (IKKβ) is a key catalytic subunit of the IKK complex [1]. It plays a crucial role in the activation of NF-κB, which is a transcription factor that binds to κB elements in promoters and enhancers of target genes [2]. Stress stimuli can activate the IKKβ-NF-κB cascade, leading to either activation or repression of gene expression in a highly cell type-specific fashion. In immune cells, i.e. neutrophils and macrophages, this cascade leads to induction of genes coding for cytokines, chemokines, enzymes and molecules with microbicidal activity [3]. The immune cell IKKβ, therefore, plays a crucial role in protection against dangerous environmental stimuli. Although IKKβ is ubiquitously expressed in essentially all mammalian organisms, its role in non-immune cells is less well understood.

With the advent of genetic mutant mice in which the *Ikkβ* gene is deleted in specific cell types, it has become evident that IKKβ has diverse roles in the regulation of homeostasis, stress responses, survival and apoptosis [4]. Studies in mutant mice have shown that the IKKβ is required to maintain homeostasis of the immune responses in skin [5,6], to inhibit sensory excitability in neurons [7], to repress proliferation in hepatocytes [8], and to potentiate apoptosis in mammary epithelial cells, leading to mammary gland involution [9]. The physiological effects of IKKβ could be the consequence of modulation of tissue homeostasis and cell-cell interactions. The intestinal epithelial IKKβ, for example, protects the intestinal tract from bacterial infection via the suppression of local inflammation and improvement of epithelial cell survival [10]. Similarly, the hepatocyte IKKβ prevents chemical carcinogenicity by reducing hepatocyte ROS accumulation and apoptosis and alleviating the activation of liver macrophages [11].

The cornea consists of five distinct layers: a stratified non-keratinized epithelial cell layer, the Bowman's membrane, a highly organized collagenous stroma layer interspersed with keratocytes, the Descemet's membrane and a single endothelial cell layer [12]. Previously, we used gene knockout approach and investigated the role of IKKβ in corneal epithelial cells [13]. We showed that IKKβ is dispensable for pre- and post-natal corneal epithelium development, but is required for optimal healing of corneal epithelial debridement wounds. Mechanistically, IKKβ is required for activation of the NF-κB and p38 signaling pathways, which lead to corneal epithelial cell migration for wound healing.

Here we have applied the similar approach to characterize the roles of IKKβ in keratocytes, the residential cells of the corneal stroma. We show that the keratocyte IKKβ is also dispensable for corneal development, but is required for wound healing. In response to mild alkaline burn injury [14], IKKβ-deficient corneas exhibit defective healing associated with excess ROS, stress signaling pathway activation, myofibroblast transformation and senescence. These results suggest that the keratocyte IKKβ modulates multiple stress signaling pathways in corneal wound healing responses.

Materials and Methods

Mouse strains, reagents and antibodies

The *Ikkβ*$^{F/F}$ mice were a gift from Dr. Michael Karin at the University of California at San Diego and the *Kera-Cre* mice were described before [15], The mice (n = 94) used in this work were housed in the Experimental Animal Laboratory at the University of Cincinnati. The procedures carried out in this work are in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol (no. 06-04-19-01) approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati. Euthanasia was done by carbon dioxide (CO_2) to effect followed by cervical dislocation.

The following antibodies were used in the study: anti-p-SMAD 2 (Ser-465, 467), anti-SMAD 3 (Ser-423, 425) and anti-p-Jun (Ser 63, 73) were from Cell Signaling, anti-α-SMA from Abcam, anti-β-actin from Sigma-Aldrich, anti-CD45 and anti-CD11b from Invitrogen, anti-p-JNK (Thr-183, Tyr-185) from Promega, and anti-HO-1 from StressGen Biotechnologies

In vivo alkali burn of the cornea

Alkali burn corneal injuries were done following protocols described before with minor modifications [14]. Briefly, animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (80 mg/kg) and xylazine (10 mg/kg). Ocular surface alkali burns were produced by

placing 3MM chromatography paper (Whatman) cut into 2-mm diameter circles previously soaked in 0.05 N NaOH onto the central cornea for exactly 90 seconds. The eyes were continuously washed with PBS for 1 min, terramycin ophthalmic ointment was topically administered to the eyes, and the animals were placed on a warming pad. At least 8 mice were used under each experimental condition.

Evaluation of corneal opacity, histological and immunohistochemical analysis

Corneal opacity was evaluated by stereoscopic microscopy and slit lamp. The opacity was scored as: 0, completely clear cornea, +1, slight opacity, +2, mild opacity with iris and lens visible, +3, severe opacity with iris and lens invisible, but opacity was limited to the cauterized area, +4, extensive opacity throughout the entire cornea. Cryosections of the eye tissues were subjected to H&E staining following standard procedures. Immunohistochemical analysis was done as described previously [13]. TUNEL was done using the ApopTag Plus In Situ Apoptosis Fluorescein Detection Kit in accordance to the manufacture's instruction (Millipore). The SA-β-Gal activities were measured at the pH 6.0 using Beta-Glo Assay system (Promega). Stained sections were mounted and photographed using an Axio Observer Inverted Microscope (Carl Zeiss).

Statistical analyses

The data were analyzed by either two-tailed student t-test or ANOVA. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ were considered statistically significant.

Results

Generation of $Ikk\beta$ keratocyte knockout mice

The $Kera$-Cre transgenic mice carry Cre recombinase gene controlled by the $Keratocan$ promoter [16]. We crossed $Ikk\beta^{F/F}$ and $Kera$-Cre mice and identified the $Ikk\beta^{F/F}$ and Kera-Cre genes in the offspring by genotyping of tail genomic DNA (Fig 1A). To evaluate the efficiency of $Ikk\beta$ ablation, we isolated corneal stromal cells from adult eyes, extracted genomic DNA, and performed PCR using primers amplifying the $Ikk\beta^F$ allele. While the products of PCR amplification were detected in cells isolated from $Ikk\beta^{F/F}$ corneas, they were absent in cells isolated from $Ikk\beta^{F/F}$/Kera-Cre corneas, though $Gapdh$ used as control was amplified in both cells (Fig 1B). These data confirmed that IKKβ was successfully ablated in the corneal stroma of $Ikk\beta^{F/F}$/Kera-Cre mice, henceforth referred to as $Ikk\beta^{\Delta CS}$.
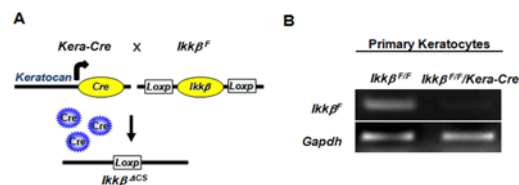
Fig 1. Generation of $Ikk\beta^{\Delta CS}$ mice. (A) Schematic illustration of the strategy for generating corneal stroma-specific $Ikk\beta$ knockout mice, namely $Ikk\beta^{\Delta CS}$ mice. The $Kera$-Cre transgenic mice, in which the Cre recombinase gene was controlled by the $Keratocan$ promoter, were crossed with $Ikk\beta^{F/F}$ mice. In the $Kera$-$Cre/Ikk\beta^F$, i.e. $Ikk\beta^{\Delta CS}$ mice, the $Ikk\beta$ floxed alleles were ablated specifically in the corneal stromal keatocytes. (B) Genomic DNA of the corneal stroma isolated from the $Ikk\beta^F$ and $Ikk\beta^{\Delta CS}$ mice was genotyped by PCR using primers specific for the $Ikk\beta^F$ allele and $Gapdh$.

doi:10.1371/journal.pone.0151869.g001

Normal corneal development of *Ikkβ^ΔCS* mice

To assess if the keratocyte IKKβ might be required for corneal development, we used stereoscopic examination of the eyes of *Ikkβ^F* and *Ikkβ^ΔCS* mice from 1 month- up to 8 months-old. The gross morphology and transparency of the eyes of *Ikkβ^ΔCS* mice were indistinguishable from those of *Ikkβ^F* mice (Fig 2A). *Ikkβ^F* and *Ikkβ^ΔCS* adult eyes had also similar corneal thickness as determined by histological examination after H&E staining (Fig 2B). Neither the *Ikkβ^F* nor the *Ikkβ^ΔCS* corneas had obvious evidence of cell proliferation or apoptosis as shown by PCNA staining and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), respectively. Hence, IKKβ expression in keratocytes is dispensable for corneal development and maintenance.

IKKβ is required for optimal corneal wound healing

To evaluate whether keratocyte IKKβ was required for wound healing after environmental insults [17], we performed mild alkali burn injury on the *Ikkβ^F* and *Ikkβ^ΔCS* corneas and examined healing stereoscopically. Both *Ikkβ^F* and *Ikkβ^ΔCS* eyes displayed haze at 1 day post-injury and gradual resolution of the haze 4–7 days after injury (Fig 3A). However, while all of the *Ikkβ^F* corneas restored transparency at 2 weeks after injury, approximately half of the *Ikkβ^ΔCS* corneas (4 out of 8) had recurrent haze and became cloudy. The opacity score was high at day 0–1 and reduced at 4–14 days after injury in all of the eyes examined. It remained low in *Ikkβ^F*, but became significantly higher in *Ikkβ^ΔCS* corneas at 4 weeks after injury (Fig 3B). Histological examination showed that the *Ikkβ^F* and *Ikkβ^ΔCS* corneas were not much different at 1 and 4 days after injury, but at 28 days, though the *Ikkβ^F* corneas showed normal morphology (data not shown), the cloudy *Ikkβ^ΔCS* corneas were swollen with increased stroma thickness (Fig 3C). These *Ikkβ^ΔCS* corneas also exhibited severe epithelium disruption, epithelial cell protrusion into stroma and scar tissue formation.

## Inflammatory and stress responses in *Ikkβ^ΔCS* corneas

Alkali burn injury of the cornea evokes inflammatory responses and cellular stress. Inflammatory responses facilitate tissue remodeling essential for wound healing, but if excessive, they
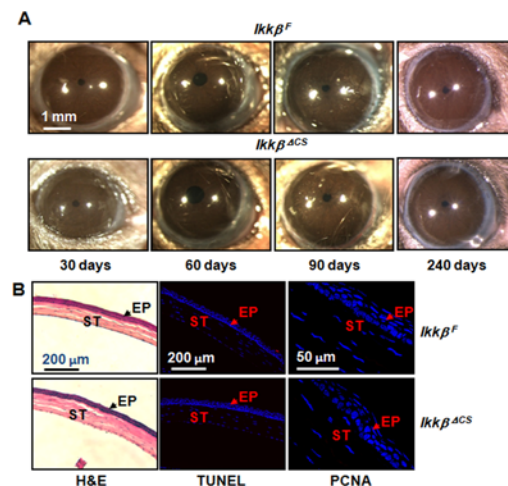
Fig 2. Role of IKKβ in corneal development and maintenance. (A) The eyes of *Ikkβ^F* and *Ikkβ^ΔCS* mice were examined under a stereoscope and photographed. (B) The *Ikkβ^F* and *Ikkβ^ΔCS* eyes were analyzed by H&E staining, TUNEL assay and immunostaining using anti-PCNA. Blue: Hoechst for nuclei. Red: TUNEL and PCNA positive signals, which were absent in the cornea of adult mice. ST: corneal stroma, EP, corneal epithelium, labeled with arrows. Pictures represent results from at least 3 mouse corneas of each genotype.

doi:10.1371/journal.pone.0151869.g002
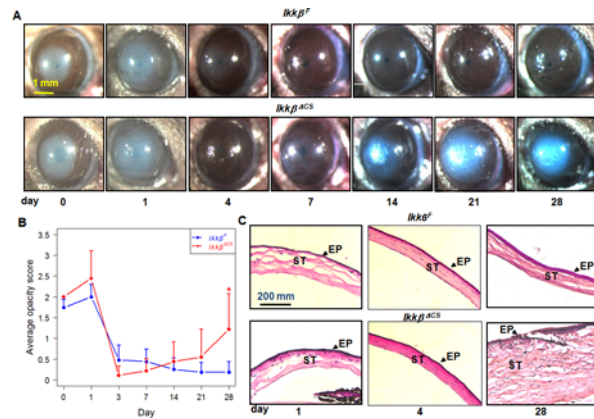
Fig 3. Corneal wound healing in *Ikkβ^F* and *Ikkβ^{ΔCS}* mice. The *Ikkβ^F* and *Ikkβ^{ΔCS}* mice were subjected to mild alkali burn corneal injury, and the eyes were examined under a stereoscope and slit lamp. (A) Representative photos of the eyes at different days after injury, and (B) the average opacity score are presented as mean±SEM of at least 8 eyes examined under each genotype/experimental conditions. Significant differences between groups were calculated using 2-way repeated measures ANOVA followed by post hoc multiple comparisons of means (Tukey method), and *$p < 0.05$ is considered statistically significant between the genotypes on the given days of injury. (C) The wounded eyes were harvested at different days after injury and examined by H&E. ST: corneal stroma, EP, corneal epithelium, labeled with arrows.

doi:10.1371/journal.pone.0151869.g003

obstruct healing and cause severe damage [18]. Given IKKβ's role in inflammatory signaling, we hypothesized that its ablation in keratocytes would perturb corneal inflammation. The injured corneas were examined by immunohistochemistry using anti-CD11b that detects macrophages and anti-CD45 that detects neutrophils. At the early phase of wound healing, the *Ikkβ^F* and *Ikkβ^{ΔCS}* corneas were similar, with massive macrophages and neutrophils at 1 day, but no inflammatory cells detected at 4 days after injury (Fig 4A–4C). At the late phase, i.e. 28 days, the *Ikkβ^F* corneas (eye 1 and eye 2) had a few detectable inflammatory cells; however, the opaque *Ikkβ^{ΔCS}* corneas (eye 1) were filled with macrophages and neutrophils (Fig 4D). On the
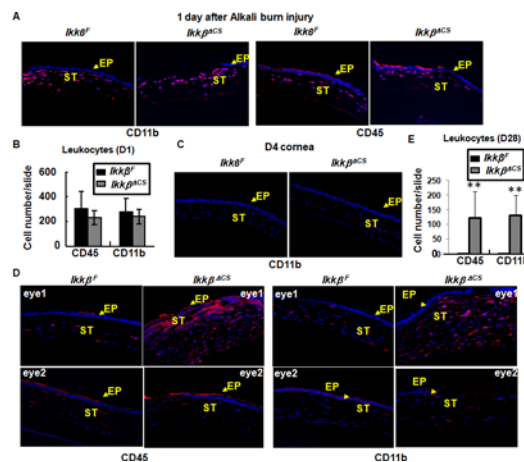
Fig 4. Inflammation in the injured cornea. The *Ikkβ^F* and *Ikkβ^{ΔCS}* eyes were harvested at (A and B) 1 day, (C) 4 days, and (D and E) 28 days after alkali burn injury. The tissues were processed and used for immunohistochemistry using anti-CD11b and anti-CD45 for infiltrated leukocytes. Blue: DAPI (nuclei), Red: leukocytes. (D) Of the two *Ikkβ^{ΔCS}* eyes examined, only eye1 was opaque. The number of leukocytes in the eyes at (B) 1 day and (E) 28 days after injury was quantified. Data represent average values from at least 5 slides/eye and 3 injured eyes examined. ST: corneal stroma, EP, corneal epithelium, labeled with arrows. **$p<0.01$ was considered significantly different between *Ikkβ^F* and *Ikkβ^{ΔCS}* eyes.

doi:10.1371/journal.pone.0151869.g004
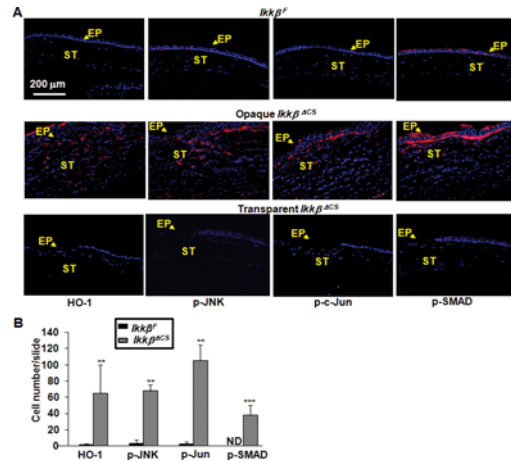
Fig 5. Oxidative stress and stress signaling in the injured cornea. The *Ikkβ^F* and *Ikkβ^ΔCS* eyes were harvested at 28 days after alkali burn injury. The tissues were processed and used for immunohistochemistry using anti-HO-1, a marker of oxidative stress, anti-pJNK and p-c-Jun, markers for the stress-activated JNK pathway, and anti-p-SMAD, a marker for active TGFβ signaling. Blue: DAPI (nuclei), Red: leukocytes. ST: corneal stroma, EP, corneal epithelium, labeled with arrows. (B) The number of positive cells was quantified and **p<0.01 and ***p<0.001 was considered significantly different between *Ikkβ^F* and *Ikkβ^ΔCS* eyes. Results represent at least 5 slides/eye and 3 injured eyes examined.

doi:10.1371/journal.pone.0151869.g005

other hand, if the *Ikkβ^ΔCS* corneas were transparent (eye 2) at 28 days, they were similar to the *Ikkβ^F* eyes with little, if any, detectable inflammatory cells. The number of inflammatory cells in *Ikkβ^ΔCS* corneas was more abundant than that in *Ikkβ^F* corneas (Fig 4E). These observations suggest that IKKβ may prevent corneal opacity by facilitating resolution of inflammation at the late phase of wound healing.

Besides its role in inflammatory signaling, the IKKβ is known to be involved in the modulation of redox homeostasis; its ablation has been linked to severe oxidative stress and tissue injury, which may in turn potentiate inflammation and cytotoxicity [11,19,20]. We therefore examined whether IKKβ ablation affected the stress responses by measuring the expression of hemeoxygenase-1 (HO-1), encoded by an oxidative stress-inducible gene. In contrast to the *Ikkβ^F* corneas, which did not have any detectable HO-1, the opaque, but not transparent, *Ikkβ^ΔCS* corneas had abundant HO-1 expression at 28 days of injury (Fig 5A and 5B). It is known that elevated oxidative stress can lead to the activation of stress-induced signaling pathways, such as the JNK-c-Jun cascade [21]. Indeed, the expression of HO-1 is accompanied by the activation of stress markers, e. g., p-JNK, p-C-Jun (Fig 5). In addition, the opaque *Ikkβ^ΔCS* corneas had increased phosphorylation of SMAD, markers of TGFβ signaling [22–24]. The *Ikkβ^F* and transparent *Ikkβ^ΔCS* corneas, in contrast, did not have any detectable p-SMAD. Taken together, the aberrant wound healing responses in the *Ikkβ^ΔCS* corneas correspond to sustained inflammation with concurrent increase of oxidative stress and activation of the stress signaling pathways.

Cellular activities affected by IKKβ ablation

TGFβ promotes myofibroblast transformation in corneal wound healing [25,26]. The finding that TGFβ signaling was upregulated in the *Ikkβ^ΔCS* corneas prompted us to examine the expression of α smooth muscle actin (α-SMA), a marker of myofibroblasts. There was indeed abundant α-SMA expression in the opaque *Ikkβ^ΔCS*, but not in the *Ikkβ^F* and transparent *Ikkβ^ΔCS* corneas at 28 days after injury (Fig 6A and 6B).

Both TGFβ activation and sustained oxidative stress can induce, stabilize and amplify senescence, leading to the detrimental effects of cell death [27,28]. The expression of senescence-
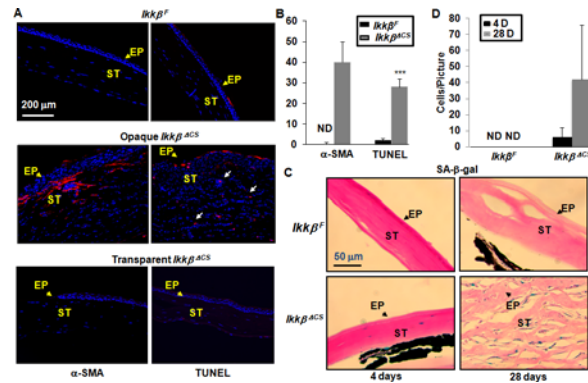
Fig 6. Myofibroblast transformation, senescence and apoptosis of the injured cornea. (A) The *Ikkβ*^F
and *Ikkβ*^{ΔCS} eyes were harvested at 28 days after alkali burn injury. The tissues were processed and used for
immunohistochemistry using anti-α-SMA, a marker for myofibroblast and TUNEL assays for the detection of
apoptotic cells. Blue: DAPI (nuclei), Red: leukocytes. (B) The *Ikkβ*^F and *Ikkβ*^{ΔCS} eyes were harvested at 4
days and 28 days after alkali burn injury. The tissue sections were examined by SA-β-Gal staining. The SA-β-
Gal positive cells are stained with blue color. ST: corneal stroma, EP, corneal epithelium, labeled with arrows.
(B and D) The number of staining positive cells was quantified and **p<0.01 and ***p<0.001 was
considered significantly different between *Ikkβ*^F and *Ikkβ*^{ΔCS} eyes. Data represent at least at least 5 slides/
eye and 3 injured eyes examined.

doi:10.1371/journal.pone.0151869.g006

associated β-Galactosidase (SA-β-Gal) [29], although completely undetectable in the *Ikkβ*^F cor-
neas, was detected in a few cells in the *Ikkβ*^{ΔCS} corneas at 4 days after injury (Fig 6C). While the
SA-β-Gal activity remained undetectable in the *Ikkβ*^F corneas at 28 days after injury, it was
markedly amplified in the opaque *Ikkβ*^{ΔCS} corneas (Fig 6C and 6D). Concurrently, the opaque,
but not transparent *Ikkβ*^{ΔCS} corneas displayed increased apoptosis, detected by TUNEL stain-
ing (Fig 6A and 6B). Our data suggest that IKKβ expression in keratocytes is required for the
repression of fibrogenesis, senescence and apoptosis in corneal wound healing.

Discussion

In the present work, we show that IKKβ expression in keratocyte is dispensable for corneal
development, but required for optimal wound healing. We and others have previously shown
that IKKβ expression in fibroblasts is essential to maintain redox homeostasis, and it does so
through NF-κB, which regulates anti-oxidant gene expression [20,30–32]. Data presented here
suggest that IKKβ has a similar role in keratocytes, the cornea-specific fibroblasts [17]. *Ikkβ*^{ΔCS}
corneas exhibit elevated oxidative stress and activation of stress signaling pathways after stro-
mal injury. In contrast to wild type corneas, which eventually recover from mild alkaline burn
injury, many of the *Ikkβ*^{ΔCS} corneas become cloudy and swollen with scar formation. Our data
are consistent with the notion that excessive oxidative stress impede the healing of corneal stro-
mal wounds [33,34].

The corneal keratocytes are quiescent in the absence of external insults, but enter cell cycle
and become active under pathologic conditions [35,36]. In response to injury, the keratocytes
differentiate to myofibroblasts essential for contraction and wound closure; excessive myofi-
broblast transformation, on the other hand, will result in fibrosis and scars [37,38]. The *Ikkβ*^{ΔCS}
corneas have sustained myofibroblast activation defined by the expression of α-SMA, and cor-
respondingly, they exhibit strong activation of the TGFβ pathway, a potent inducer of myofi-
broblast differentiation [39]. Interestingly, *Ikkβ*^{-/-} fibroblasts display similar phenotype (Chen,
et. al., data not shown). Studies in fibroblasts have shown that loss of IKKβ leads to oxidative
stress, which induces c-Jun binding and activation of the *Tgfβ* promoter and gene expression;

TGFβ in turn potentiates myofibroblast transformation and senescence (Chen, et. al., data not shown). It is possible that oxidative stress also serves as a molecular link between IKKβ and TGFβ signaling in the $Ikk\beta^{\Delta CS}$ corneas.

Corneal wound healing involves an early inflammatory phase followed by a late remodeling phase. In the early phase, tissue damage triggers neutrophil infiltration and macrophage invasion. These inflammatory cells produce cytokines, chemokines and molecules with microbicidal activity important for protecting the cornea from infection and environmental insults. Previous studies by Saika, et. al. have shown that activation of the IKK-NF-κB pathways in the neutrophil and macrophage makes the major contribution to the inflammatory responses in corneal wound healing [40]. Consistent with this notion, we show that IKKβ in keratocytes is not required for early phase inflammatory responses, but instead seems to be involved in the maintenance of tissue homeostasis. In fact, IKKβ exhibits similar functions in other non-immune cells [41], such as hepatocytes [11], keratinocytes [5] and intestinal epithelial cells [42].

The mechanisms through which IKKβ regulates redox homeostasis and TGFβ signaling have been investigated in fibroblasts. In essence, IKKβ is required for optimal expression of redox scavengers, and IKKβ-null cells have decreased capacity to counteract oxidative stress elicited by environmental insults. When oxidative stress increases to a threshold level, it activates the JNK-c-Jun pathway, which induces TGFβ expression and activity; TGFβ in turn can act through NADH oxidase to further potentiate oxidative stress. Due to activation of the autocrine amplification of the ROS-TGFβ-NOX loop, IKKβ ablation in fibroblasts leads to a progressive increase of oxidative stress and TGFβ signaling, and a gradual myofibroblast transformation and premature senescence (Chen, et. al. unpublished data). It is possible that IKKβ also regulates redox homeostasis in keratocytes, where the activation of the ROS-TGFβ-NOX loop leads to the more severe wound healing defects observed in the $Ikk\beta^{\Delta CS}$ corneas.

Supporting Information

S1 File. Supplemental file for reviewers.
(PDF)

Acknowledgments

Author Contributions

Conceived and designed the experiments: YX. Performed the experiments: LC MM QM. Analyzed the data: LC YX. Contributed reagents/materials/analysis tools: WK QW. Wrote the paper: LC YX.

References

1. Hacker H, Karin M (2006) Regulation and function of IKK and IKK-related kinases. Sci STKE 2006: re13. PMID: 17047224

2. Perkins ND (2007) Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 8: 49–62. PMID: 17183360

3. Vallabhapurapu S, Karin M (2009) Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol 27: 693–733. doi: 10.1146/annurev.immunol.021908.132641 PMID: 19302050

4. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, et al. (1999) The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J Exp Med 189: 1839–1845. PMID: 10359587

5. Pasparakis M, Courtois G, Hafner M, Schmidt-Supprian M, Nenci A, Toksoy A, et al. (2002) TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature 417: 861–866. PMID: 12075355

6. Stratis A, Pasparakis M, Rupec RA, Markur D, Hartmann K, Scharffetter-Kochanek K, et al. (2006) Pathogenic role for skin macrophages in a mouse model of keratinocyte-induced psoriasis-like skin inflammation. J Clin Invest 116: 2094–2104. PMID: 16886058

7. Bockhart V, Constantin CE, Haussler A, Wijnvoord N, Kanngiesser M, Myrczek T, et al. (2009) Inhibitor kappaB Kinase beta deficiency in primary nociceptive neurons increases TRP channel sensitivity. J Neurosci 29: 12919–12929. doi: 10.1523/JNEUROSCI.1496-09.2009 PMID: 19828806

8. Malato Y, Sander LE, Liedtke C, Al-Masaoudi M, Tacke F, Trautwein C, et al. (2008) Hepatocyte-specific inhibitor-of-kappaB-kinase deletion triggers the innate immune response and promotes earlier cell proliferation during liver regeneration. Hepatology 47: 2036–2050. doi: 10.1002/hep.22264 PMID: 18393321

9. Baxter FO, Came PJ, Abell K, Kedjouar B, Huth M, Rajewsky K, et al. (2006) IKKbeta/2 induces TWEAK and apoptosis in mammary epithelial cells. Development 133: 3485–3494. PMID: 16887827

10. Chae S, Eckmann L, Miyamoto Y, Pothoulakis C, Karin M, Kagnoff MF (2006) Epithelial cell I kappa B-kinase beta has an important protective role in Clostridium difficile toxin A-induced mucosal injury. J Immunol 177: 1214–1220. PMID: 16818780

11. Maeda S, Kamata H, Luo JL, Leffert H, Karin M (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121: 977–990. PMID: 15989949

12. Zieske JD (2004) Corneal development associated with eyelid opening. Int J Dev Biol 48: 903–911. PMID: 15558481

13. Chen L, Meng Q, Kao W, Xia Y (2011) IkappaB kinase beta regulates epithelium migration during corneal wound healing. PLoS ONE 6: e16132. doi: 10.1371/journal.pone.0016132 PMID: 21264230

14. Miyazaki K, Okada Y, Yamanaka O, Kitano A, Ikeda K, Kon S, et al. (2008) Corneal wound healing in an osteopontin-deficient mouse. Invest Ophthalmol Vis Sci 49: 1367–1375. doi: 10.1167/iovs.07-1007 PMID: 18385052

15. Liu H, Zhang J, Liu CY, Hayashi Y, Kao WW (2012) Bone marrow mesenchymal stem cells can differentiate and assume corneal keratocyte phenotype. J Cell Mol Med 16: 1114–1124. doi: 10.1111/j.1582-4934.2011.01418.x PMID: 21883890

16. Liu C, Arar H, Kao C, Kao WW (2000) Identification of a 3.2 kb 5'-flanking region of the murine keratocan gene that directs beta-galactosidase expression in the adult corneal stroma of transgenic mice. Gene 250: 85–96. PMID: 10854782

17. Graw J (2009) Genetics of crystallins: cataract and beyond. Exp Eye Res 88: 173–189. doi: 10.1016/j.exer.2008.10.011 PMID: 19007775

18. Pfister RR, Haddox JL, Sommers CI (1998) Injection of chemoattractants into normal cornea: a model of inflammation after alkali injury. Invest Ophthalmol Vis Sci 39: 1744–1750. PMID: 9699566

19. Tanaka M, Fuentes ME, Yamaguchi K, Durnin MH, Dalrymple SA, Hardy KL, et al. (1999) Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. Immunity 10: 421–429. PMID: 10229185

20. Peng Z, Peng L, Fan Y, Zandi E, Shertzer HG, Xia Y (2007) A critical role for IkappaB kinase beta in metallothionein-1 expression and protection against arsenic toxicity. J Biol Chem 282: 21487–21496. PMID: 17526490

21. Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M (2005) Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120: 649–661. PMID: 15766528

22. Fleckenstein K, Zgonjanin L, Chen L, Rabbani Z, Jackson IL, Thrasher B, et al. (2007) Temporal onset of hypoxia and oxidative stress after pulmonary irradiation. Int J Radiat Oncol Biol Phys 68: 196–204. PMID: 17448873

23. Roy S, Dontamalla SK, Mondru AK, Sannigrahi S, Veerareddy PR (2011) Downregulation of apoptosis and modulation of TGF-beta1 by sodium selenate prevents streptozotocin-induced diabetic rat renal impairment. Biol Trace Elem Res 139: 55–71. doi: 10.1007/s12011-010-8635-z PMID: 20174975

24. Bataller R, Schwabe RF, Choi YH, Yang L, Paik YH, Lindquist J, et al. (2003) NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. J Clin Invest 112: 1383–1394. PMID: 14597764

25. Datto MB, Frederick JP, Pan L, Borton AJ, Zhuang Y, Wang XF (1999) Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. Mol Cell Biol 19: 2495–2504. PMID: 10082515

26. Phan SH (2002) The myofibroblast in pulmonary fibrosis. Chest 122: 286S–289S. PMID: 12475801

27. Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, Martin-Ruiz C, et al. (2012) A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11: 345–349. doi: 10.1111/j.1474-9726.2012.00795.x PMID: 22321662

28. Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, et al. (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol Syst Biol 6: 347. doi: 10.1038/msb.2010.5 PMID: 20160708

29. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 92: 9363–9367. PMID: 7568133

30. Chen F, Castranova V, Li Z, Karin M, Shi X (2003) Inhibitor of nuclear factor kappaB kinase deficiency enhances oxidative stress and prolongs c-Jun NH2-terminal kinase activation induced by arsenic. Cancer Res 63: 7689–7693. PMID: 14633691

31. Peng Z, Geh E, Chen L, Meng Q, Fan Y, Sartor M, et al. (2010) I{kappa}B kinase {beta} regulates redox homeostasis by controlling the constitutive levels of glutathione. Mol Pharmacol.

32. Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, et al. (2003) NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. EMBO J 22: 3898–3909. PMID: 12881424

33. Sarchahi AA, Maimandi A, Tafti AK, Amani M (2008) Effects of acetylcysteine and dexamethasone on experimental corneal wounds in rabbits. Ophthalmic Res 40: 41–48. PMID: 18025839

34. Kubota M, Shimmura S, Kubota S, Miyashita H, Kato N, Noda K, et al. (2011) Hydrogen and N-acetyl-L-cysteine rescue oxidative stress-induced angiogenesis in a mouse corneal alkali-burn model. Invest Ophthalmol Vis Sci 52: 427–433. doi: 10.1167/iovs.10-6167 PMID: 20847117

35. West-Mays JA, Dwivedi DJ (2006) The keratocyte: corneal stromal cell with variable repair phenotypes. Int J Biochem Cell Biol 38: 1625–1631. PMID: 16675284

36. Klenkler B, Sheardown H (2004) Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. Exp Eye Res 79: 677–688. PMID: 15500826

37. Wilson SE (2012) Corneal myofibroblast biology and pathobiology: generation, persistence, and transparency. Exp Eye Res 99: 78–88. doi: 10.1016/j.exer.2012.03.018 PMID: 22542905

38. Myrna KE, Pot SA, Murphy CJ (2009) Meet the corneal myofibroblast: the role of myofibroblast transformation in corneal wound healing and pathology. Vet Ophthalmol 12 Suppl 1: 25–27.

39. Funderburgh JL, Funderburgh ML, Mann MM, Corpuz L, Roth MR (2001) Proteoglycan expression during transforming growth factor beta -induced keratocyte-myofibroblast transdifferentiation. J Biol Chem 276: 44173–44178. PMID: 11555658

40. Saika S, Ikeda K, Yamanaka O, Flanders KC, Okada Y, Miyamoto T, et al. (2006) Loss of tumor necrosis factor alpha potentiates transforming growth factor beta-mediated pathogenic tissue response during wound healing. Am J Pathol 168: 1848–1860. PMID: 16723700

41. Gerondakis S, Grumont R, Gugasyan R, Wong L, Isomura I, Ho W, et al. (2006) Unravelling the complexities of the NF-kappaB signalling pathway using mouse knockout and transgenic models. Oncogene 25: 6781–6799. PMID: 17072328

42. Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, et al. (2007) Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. Nature 446: 552–556. PMID: 17322906