Regenerative healing immune response after corneal chemical burn

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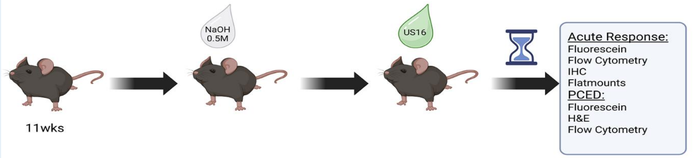
Introduction

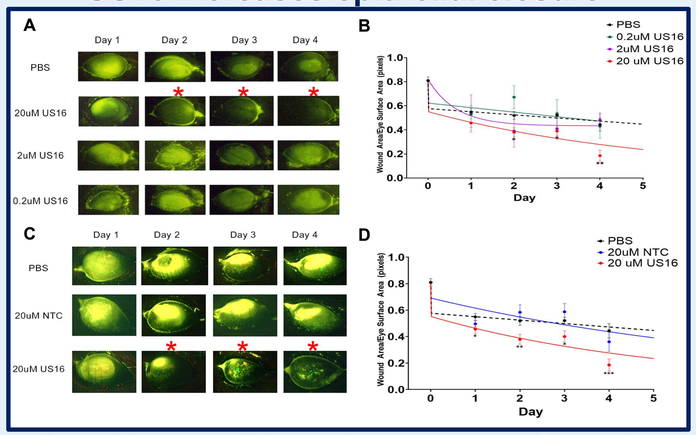
Scarring is driven by the persistence of myofibroblasts and inflammation in healing tissue. After wounding, the gene expression of the deubiquitinase (DUB) USP10 is upregulated. Knockdown of USP10 in rabbit cornea reduced scarring, fibrotic markers, and CD45+ cells. Using an alkaline wound model in mice, we investigated the effects of USP10 knockdown on early immune cell response and corneal scarring using a specialized mouse, USP10-targeting siRNA therapeutic called US16.

Methods



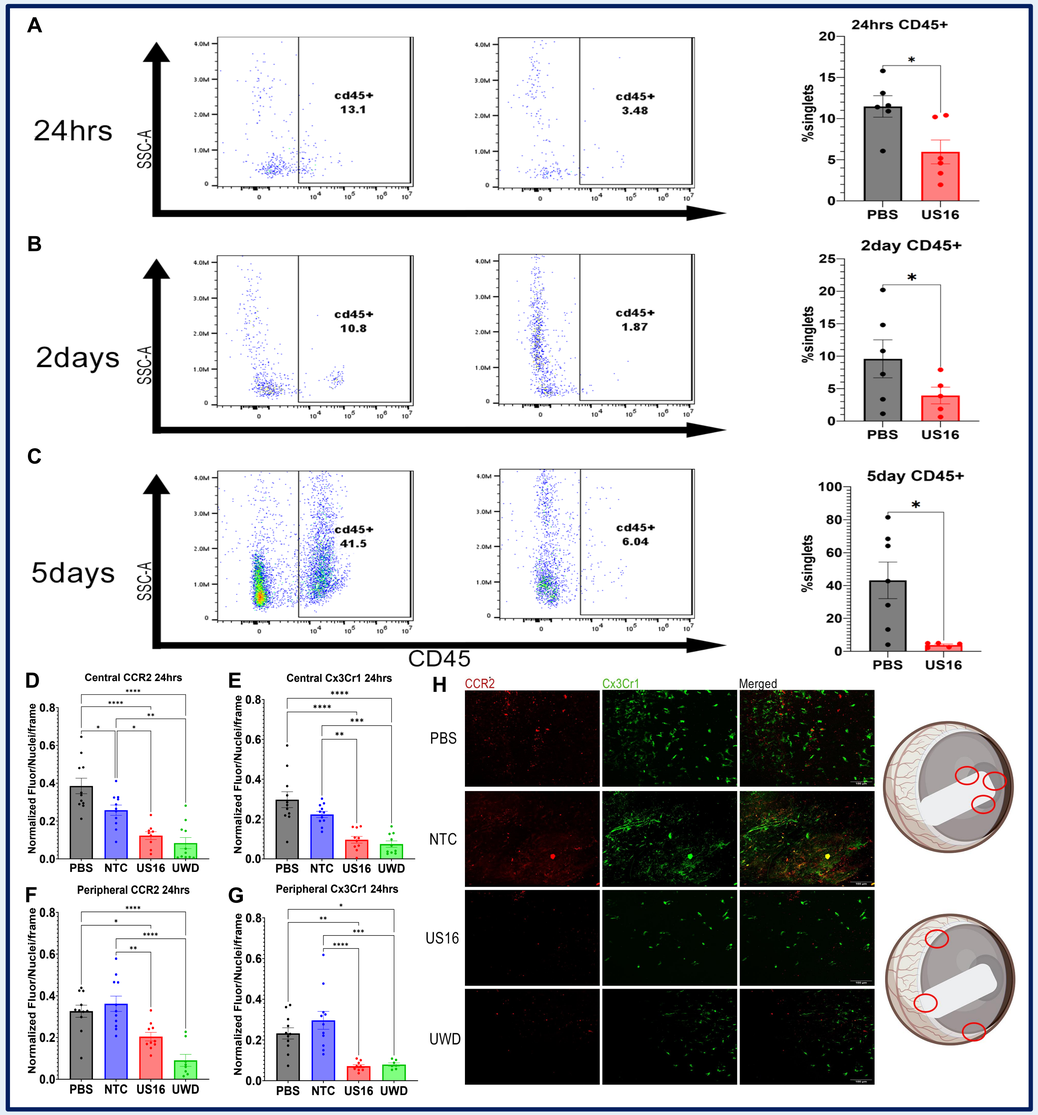
Results

US16 increases epithelial closure



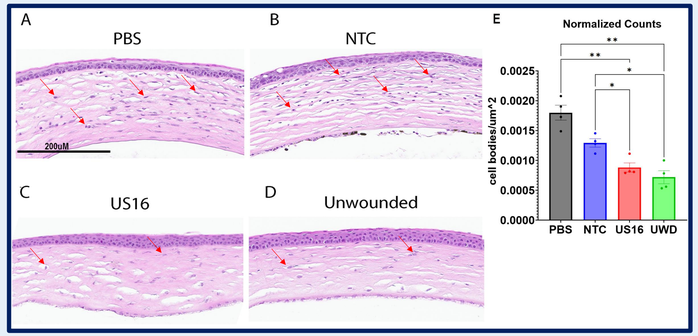
A. Representative images up to 4days post alkaline burn showing difference between US16 (USP10 KD) drug dilution results. B. 20uM dosage of US16 results in visibly increased rate of epithelial closure by day 2 post alkaline burn. C. USP10 KD improves epithelial closure compared to vehicle and platform controls. D. Dose curve of results between controls and US16 showing improved epithelial closure
* = p < 0.05, ** = p< 0.01, *** = p <0.0001.

US16 impedes immune cell infiltration



A.-C. Flow cytometry plots with quantification of comparison for three time points post alkaline burn: 24hrs, 2days, and 5days. D. Quantification of normalized Ccr2-RFP signal 24hrs post alkaline burn of the central, corneal apex. E. Quantification of the Cx3Cr1-GFP signal 24hrs post alkaline burn of the central, corneal apex. F. Quantification of normalized Ccr2-RFP signal 24hrs post alkaline burn of the peripheral rim of the cornea, limbus excluded. G. Quantification of the Cx3Cr1-GFP signal 24hrs post alkaline burn of the periphery of the cornea, limbus excluded. H. Representative images of central cornea 24hrs post burn with Ccr2-RFP and Cx3Cr1-GFP markers. I. Demonstration of location images were taken on the cornea on the central cornea (top) and the periphery of the cornea (bottom).
* = p < 0.05, ** = p< 0.01, *** = p <0.001, **** = p<0.0001

US16 results in prolonged anti-fibrotic tissue recovery



A-D. Central corneas 14days after alkaline burn. Wounded controls, PBS and NTC strongly resemble each other while US16 treatment resembles the Unwounded control. E. Quantification of the remaining number of cell bodies in the stroma. US16 corneas had stromal cell bodies at equivalent levels to the Unwounded corneas.
* = p < 0.05, ** = p< 0.01

Conclusions

Our data suggest that USP10 knockdown is a novel method to improve wound closure, decrease scarring, and alter CD45+ populations. Furthermore, that a decline in early apoptotic events post-injury is protective to the cornea and may reduce the influx of immune cells after wounding, ultimately leading to improved healing.

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