Decorin modulation of corneal wound healing through autophagy

Veterinary Research Scholars Program
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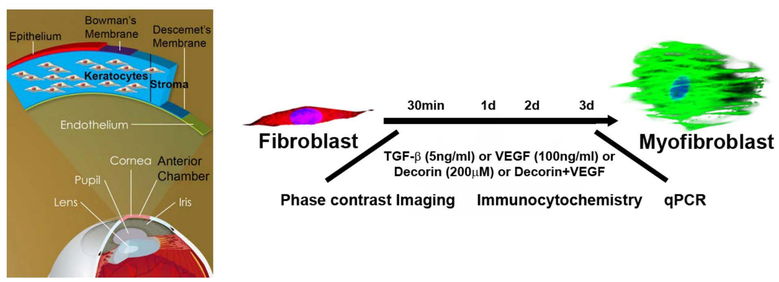
Background and Rationale

- Autophagy regulates cellular homeostasis and pathology.
- The cornea is vulnerable to trauma, injury, or infection resulting in a complex wound healing process.
- The role of autophagy in corneal wound healing is still unknown.
- Decorin, a key extracellular matrix protein, has been found to modulate autophagic signaling pathways in non-ocular tissues.
- Decorin has been shown to modulate TGF-β- and VEGF-induced cellular proliferation and differentiation in corneal wound healing.
- RNA expression of autophagic genes, such as LC3 and Beclin1, has been detected in human corneal epithelial, stromal, and endothelial cells.

Hypothesis and Objective

- We hypothesize that decorin regulates LC3 and Beclin1 gene function in response to VEGF during corneal wound healing.
- The objective of this study was to determine decorin's role in the modulation of corneal wound healing through autophagy.

Strategy



Results

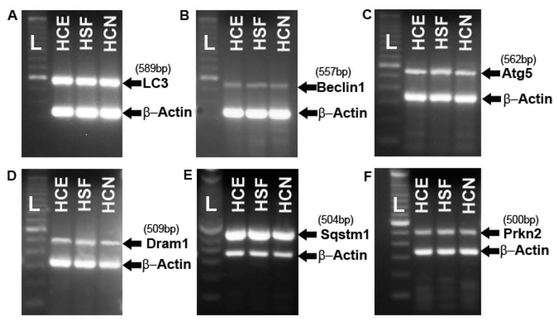


Fig. 1 PCR shows expression of LC3, Beclin1, Atg-5, Dram1, Sqstm-1, and Prkn2 in human corneal epithelial, stromal, and endothelial cells. From these results, LC3 and Beclin1 were selected for further study.

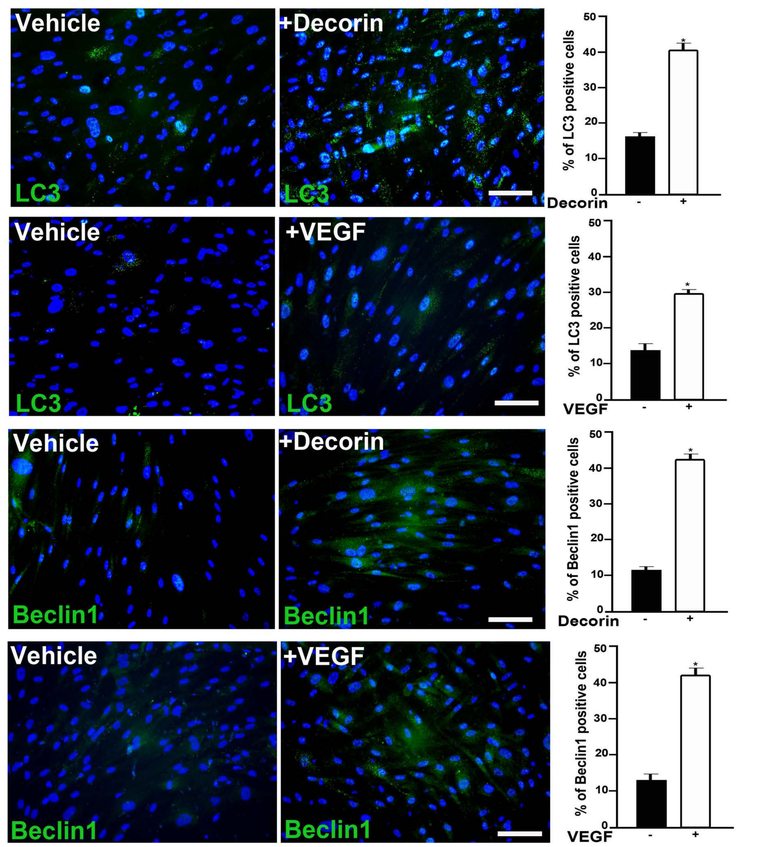


Fig. 2 Immunocytochemistry shows the distribution of LC3 and Beclin1 protein after 30 minutes of treatment with decorin/VEGF. (Scale bar= 100μm.) (Green=LC3 & Beclin1; Blue=DAPI.) Bar graphs show significantly increased levels of LC3 and Beclin1 after treatment with decorin/VEGF.

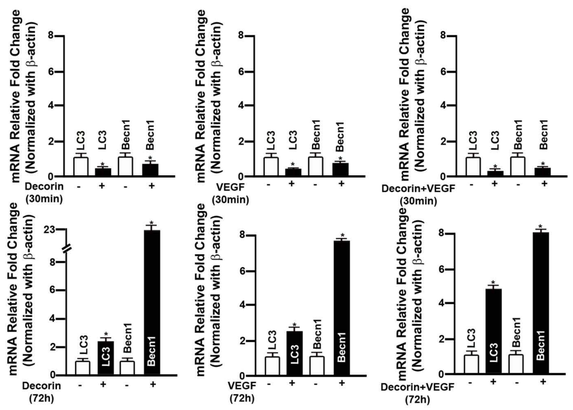


Fig. 3 qPCR quantification shows expression of LC3 and Beclin1 mRNA after 30 minutes and 72 hours of treatment with decorin/VEGF and decorin+VEGF. LC3 and Beclin1 mRNA expression increased significantly after 72 hours of treatment.

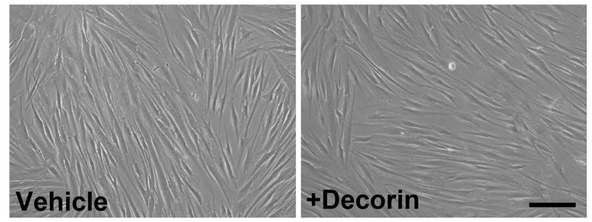


Fig. 4 Phase contrast photomicrographs allow visualization of the effect of decorin treatment (72 hours) on human stromal fibroblast cells. (Scale bar=100μm.)

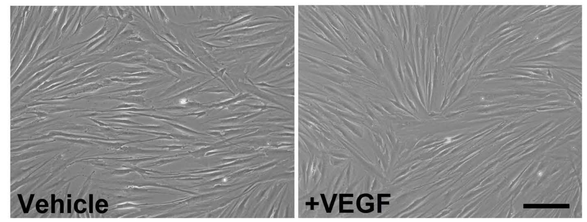


Fig. 5 Phase contrast photomicrographs allow visualization of the effect of VEGF treatment (72 hours) on human stromal fibroblast cells. (Scale bar=100μm.)

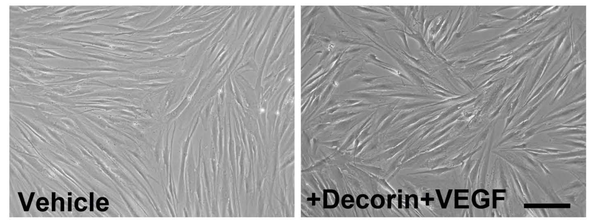


Fig. 6 Phase contrast photomicrographs allow visualization of the effect of treatment with decorin+VEGF (72 hours) on human stromal fibroblast cells. (Scale bar= 100μm.)

Key Findings and Conclusions

- LC3, Beclin1, Atg5, DRAM1, Sqstm1, and Prkn2 are genes related to autophagy which are present in the human cornea.
- Decorin modulates the expression of LC3 and Beclin1 protein and RNA in a time-dependent manner and in response to VEGF.
- The activities of decorin and VEGF appear to compete with one another in this study.
- Decorin may be critical in the corneal wound healing process via autophagy.
- Further analysis of pending studies is warranted.

Acknowledgments

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