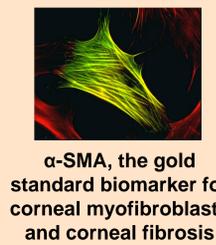
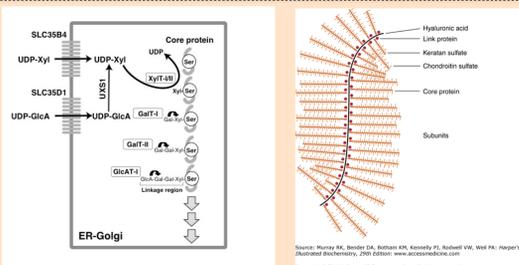


Background and Rationale

Importance

- Corneal scarring is the 3rd leading cause of blindness globally and affects 1.3 million Americans each year.
- Accidental and surgical injury alike can cause irreversible loss of vision due to corneal scarring.
- 6% of all combat casualties in Operation Iraqi Freedom have had injuries sustained to the eye.
- 2-5% of patients have corneal scarring after visual corrective procedures, especially photorefractive keratectomy.
- Due to the lack of effective and safe drugs many cases of corneal scarring require transplantation, which poses the challenges of postsurgical complications and limited availability of high quality donor corneas.

Role of xylosyltransferase-1 (as the rate limiting step) in glycosaminoglycan addition to the proteoglycan backbone present in the ECM during wound healing and fibrosis.



Hypothesis and Objectives

The specific aims were: 1) to characterize XYLT1 expression in naïve and wounded human and rabbit corneas, 2) investigate its role in corneal wound healing, and 3) determine whether XYLT1 can serve as a biomarker for corneal fibrosis.

Materials and Methods

Materials used included normal human corneal fibroblasts (HCFs) from human donors, naïve and injured (alkaline treated and PRK treated) rabbit cornea tissue, naïve and injured (viral keratitis) human cornea tissue. Methods used included a TGF- β 1 treated *in vitro* corneal fibrosis model, PCR gel electrophoresis, qRT-PCR, Western Blot, and immunofluorescence

Results

In vitro

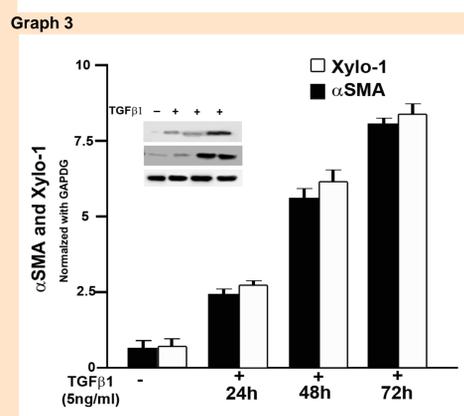
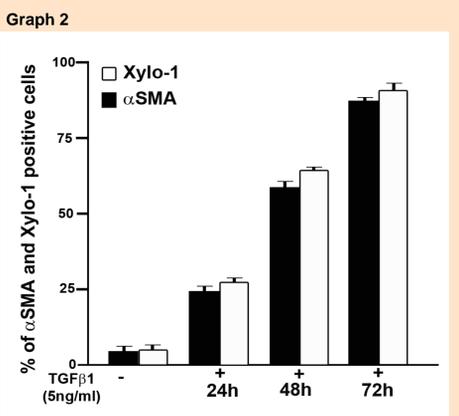
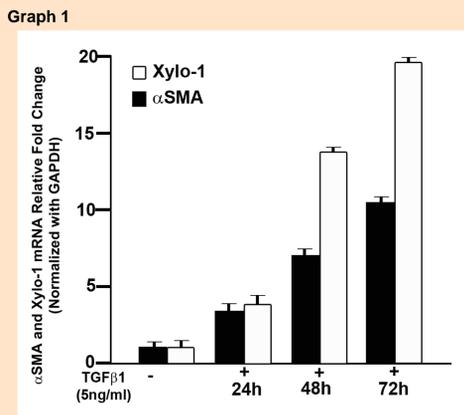
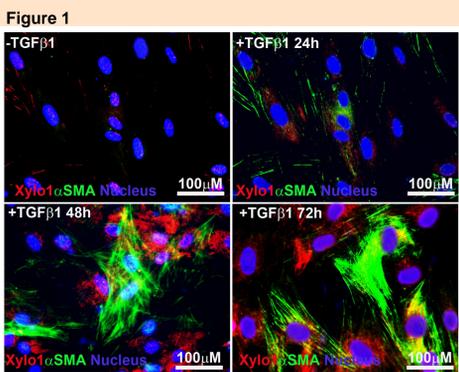


Fig 1 and graph 1. HCFs treated for differing times with TGF- β 1. Cells immunocytochemically stained for both XYLT & α -SMA. Levels of expression were calculated & graphed. No treatment yielded very little expression of both proteins, whereas increasing levels of treatment lead to increasing levels of protein expression of both XYLT1 and α -SMA, roughly 5-15 fold from 24h to 72 h ($P < 0.0013$). Graph 2. mRNA extracted from treated HCFs, qRT-PCR run, and results graphed. The data shows an increase in mRNA expression as the time exposed to treatment increased. This true for both XYLT1, 4-20 fold increase from 24 to 72h, and α -SMA, 3-10 fold increase from 24 to 72h ($P < 0.001$). Graph 3. Protein isolated from HCFs, Western blot performed, protein expression quantified and graphed. No treatment yielded very little expression of both proteins, whereas increasing levels of treatment lead to increasing levels of protein expression of both XYLT1 and α -SMA, roughly 5-15 fold from 24 to 72 h ($P < 0.003$).

In vivo

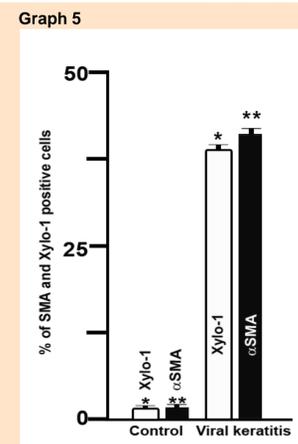
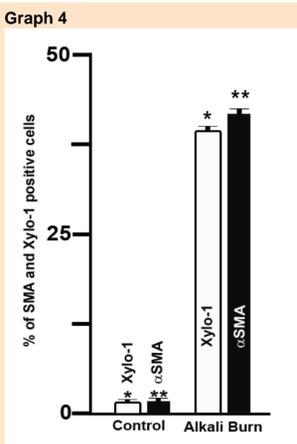
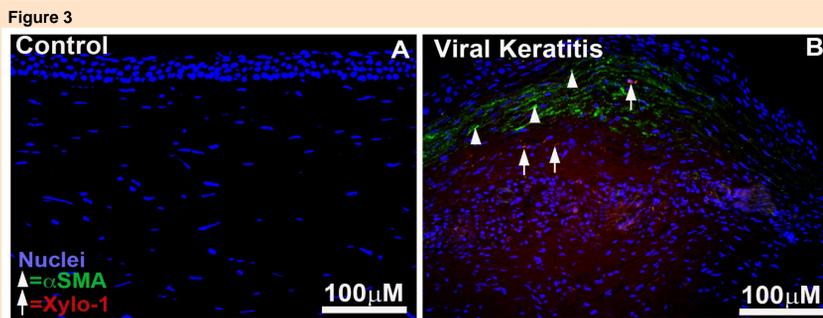
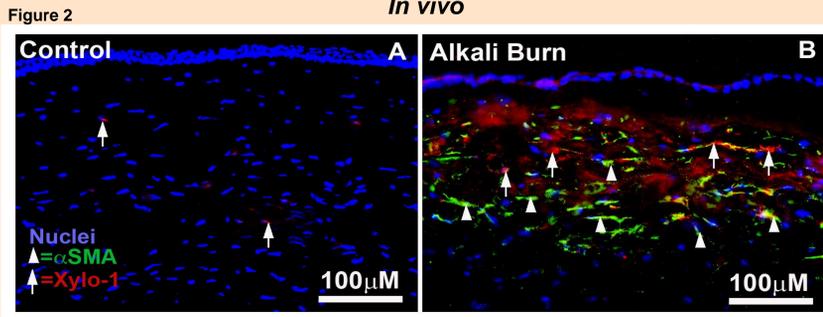


Fig 2 & graph 4. Naïve rabbit tissue immunohistochemically stained showed low levels of both XYLT & α -SMA, while the injured alkali burn tissue showed a roughly 20 fold increase in both proteins ($P < 0.0014$). Fig 3 & graph 5. Naïve human tissue immunohistochemically stained showed low levels of both XYLT & α -SMA, while the injured viral keratitis tissue showed a nearly 35 fold increase in both proteins ($P < 0.001$).

Conclusions

- XYLT1 and α -SMA are present at low levels in naïve tissue
- XYLT1 and α -SMA are present at significantly increased levels in injured tissue and tissue that is exposed to profibrotic cytokines.
- Based on its expression in damaged tissues and similar expression to α -SMA, XYLT1 can serve as a biomarker for corneal fibrosis.

Next Steps

- Measurements of the activity of XYLT1
- Inhibition of XYLT1 in naïve and injured tissue to determine its effects in both and to better characterize its pathway.
- Combination treatments with inhibition of XYLT1 and induction of anti-fibrotic pathways.

Acknowledgements

- Primary: Ruth Kraeuchi Missouri Endowed Chair Ophthalmology Fund, University of Missouri
- Partial: Veteran Health Affairs Merit (1101BX000357), Washington DC (RRM), National Eye Institute (RO1EY17294), NIH, Bethesda MD (RRM)