Alpha-SMA Expression in Wound Closure of Older Rats

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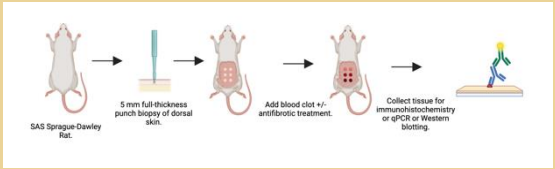
INTRODUCTION

- Dermal fibroblasts maintain collagen and extracellular matrix components in the skin.
- Following injury or the secretion of pro-fibrotic factors like transforming growth factor-beta (TGF-beta), fibroblasts can become activated and differentiate into myofibroblasts, which express alpha-smooth muscle actin (α-SMA).[1] α -SMA allows for the contraction of granulation tissue and wound closure.
- α -SMA presents as a potential marker to monitor differences in scarring between different anti-fibrotic treatments.
- In young rats, α -SMA expression peaks at day 5 post-injury.[2]
- In both human and rat skin, aging results in alterations in myofibroblasts, and the differences in healing markers in aged skin remain under-characterized.
- This study investigates α -SMA as a potential marker for comparing the effectiveness of antifibrotic treatments in aged skin. We hypothesize that α -SMA levels will differ between treatments.

METHODS



- Full-thickness wounds were created on the dorsal skin of three female (285 +/- 12 day) aged rats (Sprague Dawley)
- Treatments of blood clot (BC), resveratrol (RSV) + BC, antifibrotic drug pirfenidone (PFD) + BC were applied to respective wounds.
- α -SMA expression was evaluated at 3, 5, and 7 days with rabbit-derived monoclonal antibody. Samples of each wound were stained with H&E and trichrome for comparison.

RESULTS

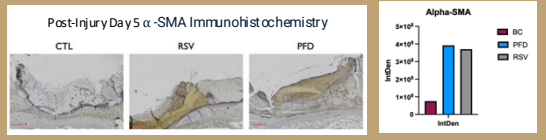


Figure 1: α -SMA expression detected by immunohistochemistry (left) and quantification of integrated density (right) at 5-days post injury in wounds treated with blood clot (control), resveratrol (RSV), and pirfenidone (PFD).

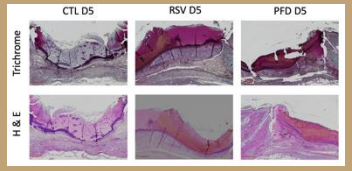


Figure 2: Trichrome and H&E staining of wounds from Figure 1 showing areas of collagen deposition in blue.

Figure 3: Absence of α -SMA detection in wounds 7 days post-injury.

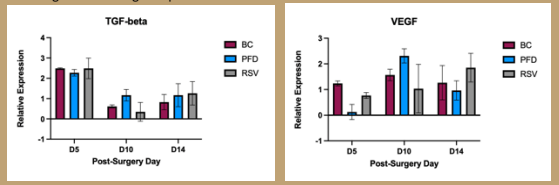


Figure 4: Transforming growth factor beta (TGF-beta) detection by qPCR on left with highest expression in all treatment groups on day 5. Vascular endothelial growth factor (VEGF) expression on right.

CONCLUSIONS

- Detection of α -SMA in skin of 9-month-old rats treated with RSV and PFD suggests that α -SMA expression is conserved in aged rats and present at the site of wounds.
- Increased α -SMA expression at 5 days and lack of expression at 10 days post-injury is consistent with previous literature, which finds α -SMA expression peaks at 5 days post-injury.
- The lack of correlation between α -SMA levels and TGF-beta make the mechanism behind alpha-SMA production unclear.
- Increased expression of α -SMA in wounds treated with anti-inflammatory drug (RSV) and antifibrotic drug (PFD) indicates that α -SMA may be a marker of wound response to treatment.
- Rats studied in this experiment featured more fragile skin compared to younger rats but maintained the ability to undergo re-epithelialization and cell migration to close wounds, indicating that although wound healing is delayed with age in both rats and humans, the healing mechanism remains intact.
- Further studies will compare the α -SMA levels at more timepoints, confirm TGF-beta expression, and compare α -SMA with other markers of healing in aged skin.

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