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Inducible Rosa, but not αSMA or Scx, Cre driven excision achieves substantial *Col12a1* knockdown in tendon healing

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Introduction

Tendon healing follows a typical wound healing process involving transient and heterogeneous cell populations. Collagen XII, a fibril-associated collagen, regulates tendon cell and matrix organization,[1] and *Col12a1* expression increases post-injury. Tamoxifen-inducible Cre mouse models permit spatial and temporal knockdown, and these models are advantageous for investigating the specific role of collagen XII in de novo tissue formation following injury. However, these models may also target other intrinsic or extrinsic cell populations that do not contribute to the healing response, such as vascular cells and cells in the adjacent tissue, and the efficiency of different spatial Cre drivers for collagen XII knockdown are unknown. Therefore, the objective of this study was to evaluate the efficiency of *Col12a1* knockdown in the healing tissue versus native tendon using three tamoxifen-inducible Cre mouse models (1) Rosa-CreERT2 model to ubiquitously target cells contributing to the healing response; 2) αSMA-CreER^{T2} model to target peritenon-derived progenitor cells that infiltrate into the injury; and 3) Scx-CreER^{T2} to target tendon-derived cells) and two tamoxifen dosage protocols (short- and long-dose). We hypothesized that 1) the Rosa-CreER^{T2} model would result in the greatest knockdown independent of region, 2) the αSMA-CreER^{T2} model would exhibit increased knockdown in the healing tissue compared to the native tissue, and 3) the Scx-CreER^{T2} model would show greater knockdown in the healing tissue with the long-dose tamoxifen protocol.

Methods

Male Rosa-CreER^{T2};*Col12a1*$^{flox/flox}$ (RosaKO), αSMA-CreER^{T2};*Col12a1*$^{flox/flox}$ (αSMAKO), and Scx-CreER^{T2};*Col12a1*$^{flox/flox}$ (ScxKO) mice with their respective Cre- littermate controls were used (n = 8/group, IACUC approved). At 90 days old, mice underwent bilateral patellar tendon injury surgery as described,[2] and Cre excision of the conditional alleles was induced via four IP injections of tamoxifen (100mg/kg body weight). The short-dose group received tamoxifen at days −1, 0, 1, and 2 days post-injury, and the long-dose group received tamoxifen at −3, 0, 3, and 6 days post-injury, where day 0 is the day of surgery. Mice were sacrificed two weeks later, and left knees were fixed for three hours in 4% paraformaldehyde prior to cryo-embedding. Injured patellar tendons were sectioned axially at a thickness of 40μm, and sections were microdissected using a 25G needle to ensure isolation of the healing tissue and the adjacent, native tendon struts. The tissue was digested, RNA was isolated as described,[3] and qPCR was performed for *Col12a1* expression. ΔCt values were normalized to the housekeeper gene, *Abl1*.

Statistics

Two-way ANOVAs for genotype and tamoxifen dosage protocol were conducted within each region, and paired t-tests were conducted to compare *Col12a1* expression in the native and healing regions. Significance was set at $p<0.05$.

Results

Supporting our hypothesis, the RosaKO model demonstrated the highest knockdown compared to control regardless of region and tamoxifen dosage protocol with substantial decreases in *Col12a1* expression (~117-2,200-fold decrease, Figure 1A). The short-dose protocol in RosaKO mice also resulted in greater knockdown compared to the long-dose protocol in both the native (~22-fold decrease) and healing (~5.5-fold decrease) regions. Contrary to our hypothesis, *Col12a1* expression surprisingly increased in the healing region of αSMAKO mice with the short dose protocol (~2.5-fold, Figure 1B), and no differences between ScxKO and control mice were observed in any region or tamoxifen dosage protocol (Figure 1C). However, expression was increased in the long dose group in the healing region of ScxKO compared to the short dose group. Finally, *Col12a1* expression was elevated across several groups in the healing region compared to its respective native region.

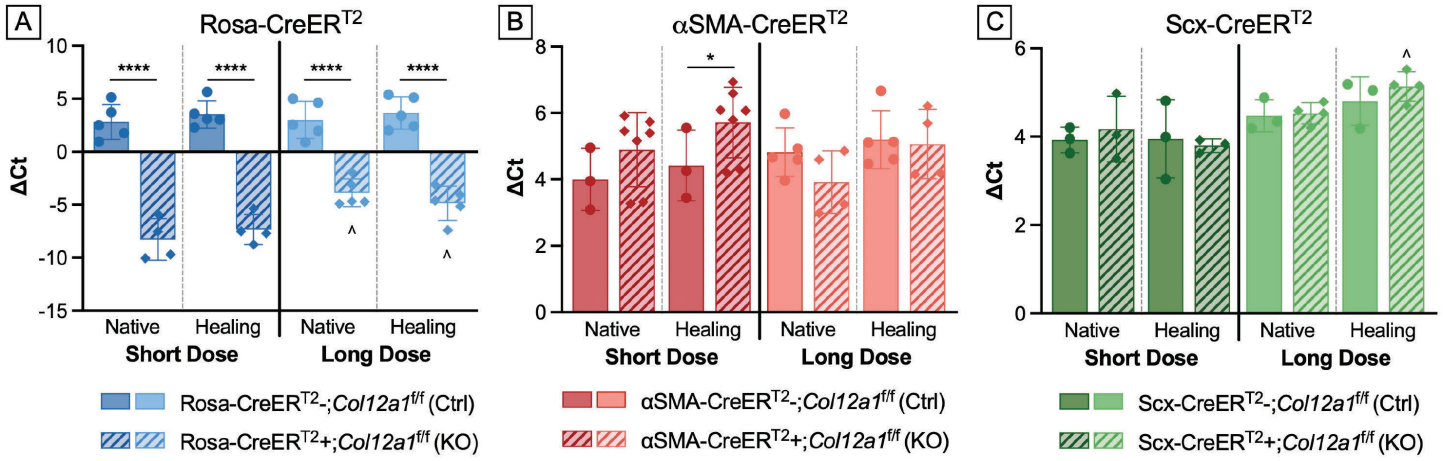

Figure 1. (A) The Rosa model achieved dramatically reduced expression of *Col12a1* expression in both the native and healing regions in mice administered the short and long dose tamoxifen protocols. The short dose groups also demonstrated greater knockdown compared to their respective long dose group; **(B)** In the αSMA model, *Col12a1* expression was surprisingly increased in the short dose, healing region of KO mice, while no other differences were observed; **(C)** In the Scx model, *Col12a1* expression was higher in the long dose, healing region of KO mice compared to its respective short dose group. (*$p < 0.05$, ****$p < 0.0001$, ^$p < 0.05$ compared to short dose protocol)

Discussion

During patellar tendon healing, infiltrating peritenon-derived αSMA+ cells are the primary contributors to the healing response prior to differentiating into Scx+ cells by two weeks post-injury.[4] Our previous data showed that *Col12a1* expression is increased one-week post-injury and returns to uninjured levels by six-weeks post-injury. Therefore, we expected that targeting *Col12a1* knockdown to αSMA+ cells during early healing would result in knockdown within the healing, de novo tissue. However, no knockdown was demonstrated in the αSMA model, even when tamoxifen was administered through 6 days post-injury, and this suggests that this Cre driver may not be sufficient in this model. Similarly, while no knockdown in the healing region of the ScxKO model may be attributed to the timing of Scx expression during tendon healing, there was no significant knockdown in the native region either, and this model also may not be effective in *Col12a1* knockdown. Additionally, both short and long-dose protocols were tested to consider the metabolism rate of tamoxifen in maximizing knockdown to the healing tissue, and in the RosaKO model, greater *Col12a1* knockdown with the short dose protocol indicates that administering tamoxifen on consecutive days before and after injury is more efficient. Finally, as expected, we observed that *Col12a1* expression was generally greater in the healing tissue than the native region, suggesting that collagen XII plays a critical role in tendon healing. Future studies will investigate this role by inducing knockdown of *Col12a1* at the time of tendon injury in a Rosa-CreER^{T2} model.

Significance

Compared to αSMA-CreER^{T2} and Scx-CreER^{T2}, the Rosa-CreER^{T2} model achieves substantial *Col12a1* knockdown when induced at time of tendon injury. Our results highlight the importance of carefully considering an appropriate tamoxifen-inducible Cre model when targeting specific genes.

Acknowledgements

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References

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