Absence of Myofibroblasts in Subsynovial Connective Tissues in Human and Rabbit Models of Carpal Tunnel Syndrome

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INTRODUCTION:
The most common histological finding of carpal tunnel syndrome (CTS) is non-inflammatory fibrosis of the subsynovial connective tissue (SSCT) in the carpal tunnel.1,2 The etiology of this finding and its relationship to the development of CTS remain poorly understood. Myofibroblasts, contractile fibroblasts which express alpha-smooth muscle actin (α-SMA), have a key role in wound healing and many other pathological conditions characterized by fibrosis.3 The purpose of this study was to determine if myofibroblasts were associated with SSCT fibrosis in CTS patients or in a rabbit model of SSCT fibrosis and CTS.

METHODS:
Idiopathic CTS Patients
We obtained SSCT specimens from around the flexor digitorum superficialis tendon of the middle finger from 10 patients (mean 62yrs, range, 42-75) with idiopathic CTS during carpal tunnel release, and compared these specimens to SSCT from a control group of 10 fresh frozen cadavers (mean 76yrs, range, 56-95) which did not have an antemortem diagnosis of CTS, and from whom the SSCT was similarly obtained. This study was approved by our institutional review board (IRB).

Rabbit CTS Model (SSCT Stretch Rabbit)
32 female New Zealand white rabbits were used for this study. Two volar incisions were made at proximal and distal of the carpal tunnel of a forepaw of rabbit, and the flexor digitorum superficialis (FDS) tendon of the middle digit was exposed and cut at the muscle-tendon junction. Two marks, 5 mm apart, were made on the middle digit FDS tendon and then the marks were brought together by a suture, in effect, distally shifting the middle digit FDS tendon by 5 mm, and thereby stretching the surrounding SSCT by that amount (Figure 1, pilot studies had shown that this amount was sufficient to damage the SSCT). 8 rabbits were sacrificed at 6, 12, and 24 weeks after surgery. 8 other rabbits were used as normal controls.

After specimen harvest, both human and rabbit SSCT were formalin-fixed, paraffin embedded and 4-μm-thick sections were made. The sections were processed for hematoxylin and eosin (HE) staining and immunohistochemistry for anti α-SMA, and hematoxylin or methyl green by standard methods (Abcam, Cambridge, MA). The percentage of α-SMA positive cells and the cell density were measured in three randomly selected areas by immunohistochemistry and HE staining, respectively. A two-sample t-test was used to compare the α-SMA positive cells and the total cells density of CTS patients, and one way ANOVA and post hoc Tukey test was used to compare that of CTS rabbit model to controls. Any p value less than 0.05 was considered significant.

RESULTS
There were few α-SMA positive cells in CTS patients, in the rabbit model, or in the controls (Figure 1). Most of these α-SMA positive cells were vascular smooth muscle cells and not myofibroblasts (Figure 2E and 2F). The percentage of α-SMA positive cells within the SSCT but out of vessels was not different between CTS patients and controls, or between the rabbit model and controls at any time point. However, the cell density in the human CTS patients (p<0.01), and in the 6 (p<0.01) rabbit model was significantly larger than that in their respective controls (Figure 3).

DISCUSSION:
We demonstrated an absence of α-SMA positive fibroblasts in the SSCT of CTS patients and in a CTS relevant rabbit model of SSCT fibrosis, despite increased cell density in both CTS patients and in the rabbit model. The presence of non-fibroblast α-SMA positive cells demonstrates successful staining, suggesting that the absence of myofibroblasts is not an artifact, and suggests that the mechanism of fibrosis in CTS differs from that associated with wound contraction or proliferative fibroblastic diseases such as Dupuytren’s contracture.

This study has several limitations. First, the rabbit model of SSCT fibrosis might not truly mimic human CTS. Second, the age of the human controls and CTS subjects was different, and this may have affected the result. Third, it is possible that myofibroblasts may play a role at earlier time points in the evolution of SSCT fibrosis, both in humans and in the rabbit model.

SIGNIFICANCE:
By demonstrating that myofibroblasts are absent in the SSCT of patients with CTS we have narrowed somewhat the variety of possible pathways of SSCT fibrosis that might be implicated in CTS, and thus have added some insight to the pathogenesis of CTS.

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REFERENCES:

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