Introduction

Flexor tendon injuries caused by deep lacerations to the hands are a challenging problem for tissue engineers. Such injuries often result in debilitating adhesions, scar tissue that prevents the normal gliding motion of the flexor tendons through their lubricating synovial sheaths, preventing the hands from opening and closing. Evidence exists that tendon adhesions as well as scarring throughout the body are largely mediated by the pleiotropic growth factor, TGF-β1 [1], the effects of which are poorly understood in tendon. Using an in vitro model of tendon healing, we previously found that TGF-β1 causes gene expression changes in tenocytes that are consistent with scar tissue and adhesion formation [2], and upregulates the anti-fibrinolytic protein, PAI-1. Therefore, we hypothesized that TGF-β1 contributes to scarring and adhesions by reducing MMP activity through suppression of plasminogen-mediated activation by PAI-1. In this study, we used our in vitro tendon-healing model to examine the effects of TGF-β1 on the expression and activation of gelatinases and their activity regulators.

Materials and Methods

Tissue Harvest & Cell Culture: All animals were cared for in compliance with regulations of the University Committee on Animal Research. Flexor digitorum longus tendons were obtained from five freshly sacrificed, 7 month old C57BL/6 mice, and processed for tendon cell (i.e. tenocyte) culture as described previously [2].

Experimental Model: 6-well plates were pre-coated with 50 µg/cm² of rat tail tendon collagen I (BD Biosciences). Tenocytes were seeded onto the plates (70,000 cells/well) and incubated overnight in control media (MEM α and 1% FBS). The next day, time 0 samples were collected, and the remaining samples were treated with fresh control media with or without 10 ng/mL of TGF-β1, 20 µg/mL of Plasminogen, and 50 ng/mL of tPA. Cell and media samples were collected and processed after 48 h to assess gene expression with RT-PCR, PAI-1 protein levels with ELISA, plasmin activity with a fluorogenic substrate assay, and MMP activity with gelatin zymography. Experiments were performed twice (n=3 for each experiment).

Statistical Analysis: Gelatin zymograms were quantified in ImageJ, and band densities were analyzed, along with gene expression and protein levels, using 1-way ANOVA and Bonferroni post-tests in GraphPad Prism 4. Statistical significance was defined as p<0.05.

Results

Gene Expression: Treatment with TGF-β1 in the absence of plasminogen or tPA significantly reduced Mmp2 and Plau (uPA) expression by 35% and 57%, respectively, but had no effect on their expression in the presence of plasminogen or tPA. TGF-β1 treatment significantly decreased Plat (tPA) in all groups by 47-89%. TGF-β1 also increased Serpine1 (PAI-1) expression by 2-4 fold in all groups except those treated with tPA, which increased Serpine1 expression by 45% but did not reach significance. PAI-1 Protein Levels: TGF-β1 stimulated significant, 14-40 fold increases in PAI-1 protein in the culture media collected from all samples treated, independent of the presence of plasminogen or tPA. Plasmin Activity: TGF-β1 treatment significantly reduced plasmin activity by 36% in samples treated with a combination of plasminogen and tPA. MMP Activity: Active MMP-2 levels (55-58 kDa) as assessed with gelatin zymography were reduced 75% by treatment with TGF-β1 in the presence of plasminogen and tPA, and 87% in the presence of plasminogen only, but significance was only achieved in the presence of tPA (Figure 1).

Discussion and Conclusions

In this study, we demonstrated that flexor tendon tenocytes treated with the pro-scarring growth factor, TGF-β1, had significantly reduced levels of active MMP-2 in the presence of plasminogen and tPA. The reduction in active MMP-2 was associated with reduced Plasmin activity, and an increase in the gene expression and protein levels of PAI-1. These findings suggest that TGF-β1 reduces MMP activity by suppressing plasminogen-mediated activation of MMPs via PAI-1 upregulation. Taken together, this data provides evidence that PAI-1 may be a novel therapeutic target for preventing debilitating tendon adhesions and mediating a scarless, regenerative repair of flexor tendon injuries.

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References