TGFβ signaling promotes matrix assembly during mechanosensitive embryonic salivary gland restoration

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Abstract

Mechanical properties of the microenvironment regulate cell morphology and differentiation within complex organs. However, methods to restore morphogenesis and differentiation in organs in which compliance is suboptimal are poorly understood. We used mechanosensitive mouse salivary gland organ explants grown at different compliance levels together with deoxycholate extraction and immunocytochemistry of the intact, assembled matrices to examine the compliance-dependent assembly and distribution of the extracellular matrix and basement membrane in explants grown at permissive or non-permissive compliance. Extracellular matrix and basement membrane assembly were disrupted in the glands grown at low compliance compared to those grown at high compliance, correlating with defective morphogenesis and decreased myoepithelial cell differentiation. Extracellular matrix and basement membrane assembly as well as myoepithelial differentiation were restored by addition of TGFβ1 and by mechanical rescue, and mechanical rescue was prevented by inhibition of TGFβ signaling during the rescue. We detected a basal accumulation of active integrin β1 in the differentiating myoepithelial cells that formed a continuous peripheral localization around the proacini and in clefts within active sites of morphogenesis in explants that were grown at high compliance. The pattern and levels of integrin β1 activation together with myoepithelial differentiation were interrupted in explants grown at low compliance but were restored upon mechanical rescue or with application of exogenous TGFβ1. These data suggest that therapeutic application of TGFβ1 to tissues disrupted by mechanical signaling should be examined as a method to promote organ remodeling and regeneration.

Introduction

Cell and organ development are mechanosensitive [1–5], yet the cellular and molecular mechanisms through which mechanical signaling is transmitted throughout organs and sensed by the cells are not well understood. The majority of mechanobiology research has utilized isolated cell lines, which has set a foundation to investigate the mechanically regulated pathways. Although these studies have provided insight into cellular signals that may play a larger role in tissue development, they do not address complex multicellular and tissue-level responses to compliance changes. Many pathologic conditions, such as specific solid tumors and fibrosis, are characterized by high stiffness (low compliance), due to excess deposition and assembly of the extracellular matrix [6,7]. Therapeutic options are needed to restore normal tissue structure in such situations in which compliance has been disrupted. Since the developing embryonic mouse submandibular salivary gland (mSMG) is mechanosensitive [8–10], it is a useful model system for investigating mechanical signaling in the context of a 3D organ. We previously demonstrated that polyacrylamide (PA) gels of a high compliance that is similar to embryonic in vivo

tissue (Young's modulus of approximately 0.5 kPa), are permissive for branching morphogenesis and epithelial differentiation of embryonic mouse submandibular salivary glands (mSMG) organ explants [9,11], whereas low-compliance PA gels more similar to pathological stiffness (20 kPa) are non-permissive for

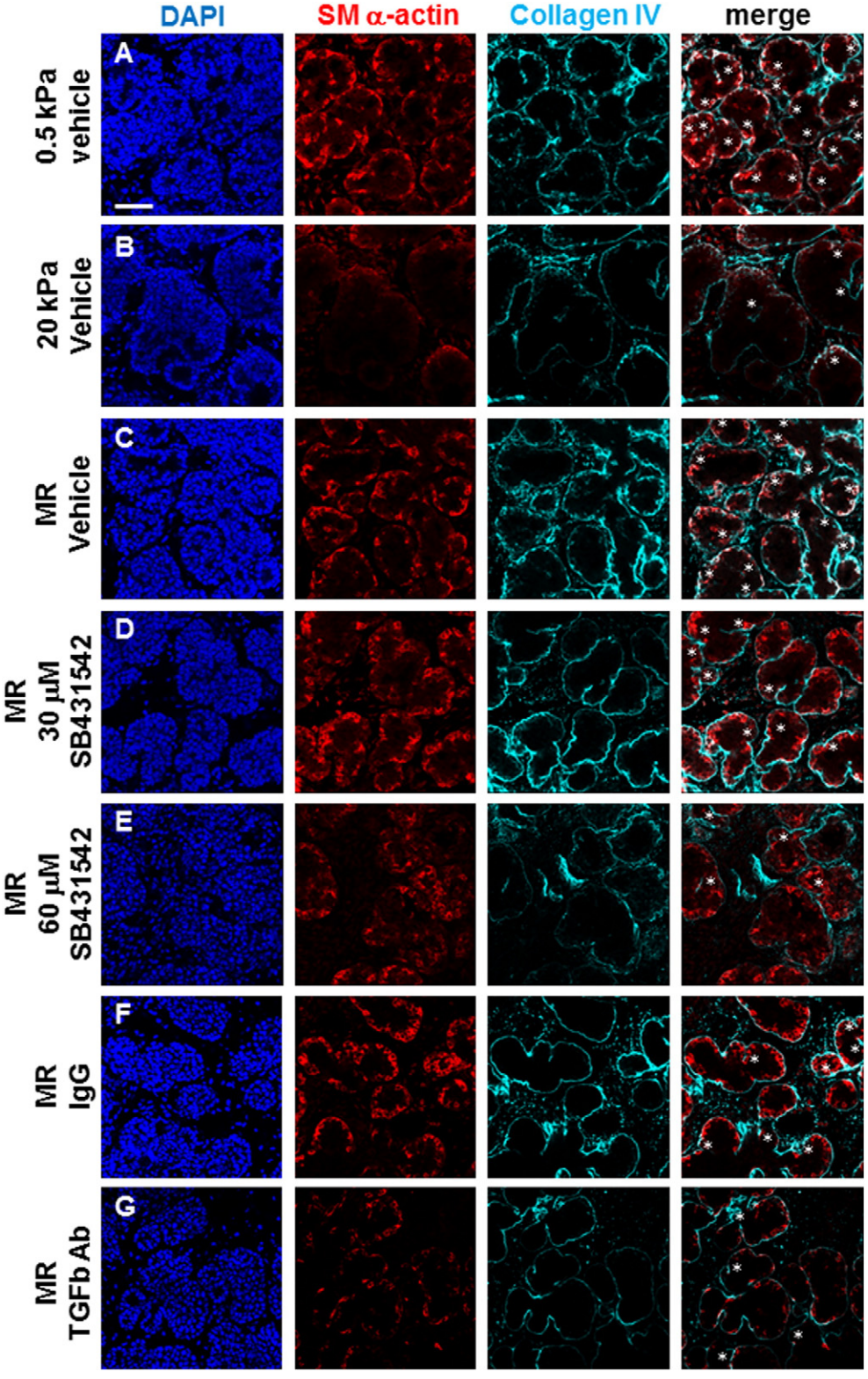

Fig. 1 (*legend on next page*)