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Original Article

Cartilage Intermediate Layer Protein 1 Suppresses TGF-β Signaling in Cardiac Fibroblasts☆

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SUMMARY

Background: Since transforming growth factor (TGF)-β1-induced cardiac fibrosis following myocardial infarction (MI) leads to heart failure and poor clinical prognosis, we aimed to identify a novel and unknown target for cardiac fibrosis related to the TGF-β signaling.

Method and result: We performed and investigated RNA-Seq using infarcted mouse hearts, culminating in cartilage intermediate layer protein 1 (CILP1). Interestingly, *Cilp1* expression was increased along with TGF-β1 expression in infarcted hearts, and was also upregulated after TGF-β1 stimulation in cardiac fibroblasts *in vitro*. Histological analysis revealed that Cilp1 was localized at the fibrotic regions of infarcted hearts. Full length CILP1 (F-CILP1) was cleaved into both N-terminal CILP1 (N-CILP1) and C-terminal CILP1 at the furin cleavage site, and both F-CILP1 and N-CILP1 were extracellularly secreted. We further found that CILP1 bound to TGF-β1 via thrombospondin type 1 domain, and suppressed both smad3 phosphorylation and fibroblasts differentiation to myofibroblasts induced by TGF-β1.

Conclusion: We identified CILP1 as a potential regulator of cardiac fibrosis by inhibiting TGF-β signaling, and these results suggest the promise of CILP1 as a novel therapeutic target for preventing cardiac fibrosis and heart failure in MI patients.

1. Introduction

Although fibrosis is one of the most essential causes of cardiac remodeling, its precise mechanism remains unclear. In cases of myocardial infarction (MI), cardiac fibrosis causes left ventricular (LV) remodeling, resulting in cardiac systolic and diastolic dysfunction and thus heart failure[1]. Importantly, LV remodeling is an independent prognostic risk factor for fatal arrhythmia in MI patients[2]. The renin–angiotensin–aldosterone and sympathetic nervous systems, oxidative stress, and inflammatory cytokines are involved in LV remodeling[3], which is currently treated with angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and β-blockers. Transforming growth factor-β (TGF-β) signaling has recently been focused on for developing new treatments for suppressing cardiac fibrosis. Indeed, TGF-β expression levels increase immediately after MI onset[4] and causes increased collagen production as it directly acts on cardiac fibroblasts, inducing their differentiation to myofibroblasts. TGF-β also regulates the extracellular matrix by inhibiting matrix destruction via protease inhibitors in the infarcted area[1]. Thus, TGF-β signaling is an important factor controlling cardiac fibrosis, and new approaches targeting TGF-β signaling are anticipated to decrease cardiac remodeling associated with cardiac fibrosis.

☆ Conflict of interest: All contributing authors declare that they have no conflicts of interest.

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Gene symbol	Fold change	FDR p-value
Postn	86.96	<0.01
Fn1	68.43	<0.01
Col1a1	61.99	<0.01
Col1a2	45.53	<0.01
Mfap4	42.62	<0.01
Mpeg1	39.44	<0.01
Nppa	39.04	<0.01
Col5a2	32.29	<0.01
Ctss	31.80	<0.01
Col3a1	30.89	<0.01
Eln	29.87	<0.01
Wisp2	27.33	<0.01
Col8a1	26.59	<0.01
Mmp14	26.47	<0.01
Cilp1	23.00	<0.01

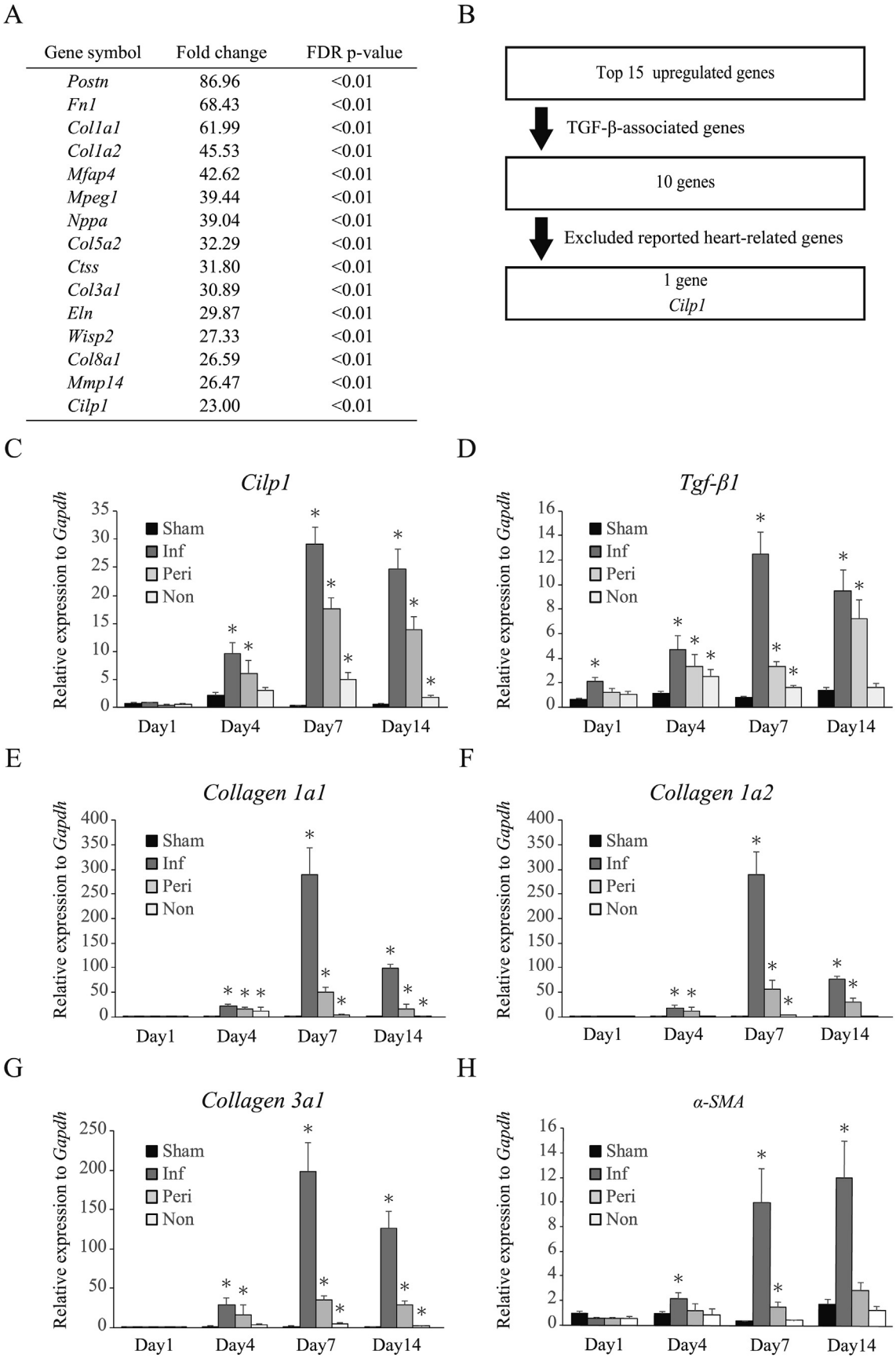

Fig. 1. RNA-Seq analysis of the infarcted area in the MI group. A. The top 15 genes that exhibited large changes in expression in infarcted areas in the MI group, compared with those in the sham group (FDR; $P < 0.01$). B. The search for cardiac fibrosis-related candidate genes was performed. First of all, the genes reportedly related to TGF-β were identified,