**Laser Capture Microdisection (LCM):** OCT-embedded skin samples (4mm punch biopsies) were sectioned (10μm) onto PEN-membrane (2.0μm) slides (MicroDissect GmbH, Herborn, Germany). Prior to LCM, consecutive sections were immunostained (see below for details) with antibodies to CD31 (PharMingen, San Diego, CA), an endothelial cell marker, CD45 (PharMingen, San Diego, CA), a bone marrow-derived cell marker, and type I procollagen (Millipore Corp, Burlington, MA) or HSP47 (Assay Designs, Ann Arbor, MI), fiboblast marker. Fibroblasts were identified based on 1) morphology, 2) interstitial location within the dermis, 3) negative for endothelial cell marker CD31 immunostaining, and 4) negative for bone marrow-derived cell marker CD45 immunostaining. Approximately 200 dermal fibroblasts were obtained by LCM (Leica ASLMD system; Leica Microsystems, Wetzlar, Germany), from one section. Total RNA was prepared from LCM-captured dermal fibroblasts using a commercial kit (RNeasy micro kit; Qiagen, Chatsworth, CA), and was used for amplification of mRNA using the RiboAmp HS RNA amplification kit (Acturus). The quality and quantity of amplified mRNA were determined with the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA). Quantitation of transcript levels of several different genes in samples of total RNA and amplified mRNA yield essentially identical results.

**TABLE I** Primers and FAM-labeled probes for real-time PCR

| Gene | Strand | Sequence |
|---|---|---|
| TGF-β1 | Sense | 5’-CATTCAGTCACCATAGCAACACTCT-3’ |
| | Anti-sense | 5’-CCTTAACCTCTCTGGGCTTGTTT-3’ |
| | Probe | 5’-TGGCAGGGACTCTGATAACACCCATTTTAA-3’ |
| TGF-β2 | Sense | 5’-CCATTAAGTGGAGTTGCTGTACGT-3’ |
| | Anti-sense | 5’-TGCCTATTGCATAGCAATACAGAAA-3’ |
| | Probe | 5’-CCGTTCCTATCCCGCGCCTCA-3’. |
| TGF-β3 | Sense | 5’-GACCCTGTGTTCATTTGGTGTTC-3’ |
| | Anti-sense | 5’-GTGTGGCACATGTCGAGCTT-3’ |
| | Probe | 5’-TGCGACAACATGTGAGGCATTCGG-3’ |

**Immunohistology:** OCT-embedded skin cryosections were sectioned (7μm) onto positively charged glass slides (Superfrost/Plus (Fisher Scientific, Itasca, IL), and fixed in 2% paraformaldehyde. Sections were treated with Peroxide Block (BioGenex Laboratories Inc, San Ramon, CA) for 10 minutes and Protein Block (BioGenex) for 20 minutes. Subsequently, the slides were incubated for 1 hour at room temperature with Rat anti-Procollagen 1 (Millipore, Billerica, MA), Rabbit anti-TGF -β1 (detects active TGF-β1, Santa Cruz Biotech, Santa Cruz, CA) or Rabbit anti-CTGF (Santa Cruz Biotech) followed by incubation with Super Sensitive MultiLink (BioGenex Laboratories Inc) for 10 minutes and BioGenex Super Sensitive Label for 10 minutes. Slides were developed with BioGenex One Step AEC Solution for 3 minutes and counterstained with hematoxylin (BioCare, Concord, CA) for 20 seconds and mounted with BioGenex Supermount. Control staining was performed with corresponding rabbit immunoglobulin and confirmed no immunoreactivity (data not shown).

**Western Blots:** Cells were washed twice with PBS, covered with 150μl of fresh whole cell extraction buffer (25mM HEPES [pH 7.7], 0.3 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 mM DTT, 20 mM β-glycerolphosphate, 0.1 mM $Na_3VO_4$, 2 μg/ml leupeptin, and 100 μg/ml PMSF) for 5 minutes, scraped from tissue culture plates with Cell Lifter Polyethyene (Coster®, Fisher Scientific), and stored at -80°C. Concentrations of proteins were measured by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) using BSA as a standard. Proteins were resolved on 12% SDS-PAGE, transferred to PVDF membrane, and reacted with primary antibodies, described in Methods and Materials section. Blots were visualized and quantified with enhanced chemifluorescence (ECF) (Vistra ECF Western Blotting System, GE Healthcare, Piscataway, NJ) following the manufacturer's protocol. The intensities of each band were quantified and normalized using β-actin as loading control by STORM PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Table II** siRNA Sequences

| Gene | Sequence (Sense) |
|---|---|
| CTGF | 5'-GTACCAGTGCACGTGCCTG-3' |
| TGF-β1 | 5'-AACAATTCCTGGCGATACCTC-3' |
| TGF-β2 | 5'-AAAATAGACATGCCGCCCTTA-3' |
| TGF-β3 | 5'-AACAGCGCTATATCGGTGGC-3' |

**Enzyme-linked immunosorbent assay (ELISA) for secreted type I procollagen and antibody neutralization of TGF-β activity.** Secreted type I procollagen was determined from fibroblast culture fluids using enzyme-linked immunosorbent assay kit (Takara Mirus Bio USA, Madison, WI.) according to manufacturer's instructions. The impact of neutralization of TGF-β on type I procollagen synthesis was tested by the addition of a neutralizing rabbit pan-specific TGF-β antibody (AB-100-NA, R&D System) that neutralizes the biological activity of TGF-β1, TGF-β2, and TGF-β3. Dermal fibroblasts were treated with indicated amounts of the neutralizing antibody for the indicated times. To exclude nonspecific inhibitory effects of immunoglobulins, cells were treated with rabbit IgG (60μg) instead of neutralizing rabbit pan-specific TGF-β antibody. At the end of the each time point, the culture fluid was collected and assayed for type I procollagen by enzyme-linked immunosorbent.