Essential Roles for Early Growth Response Transcription Factor Egr-1 in Tissue Fibrosis and Wound Healing

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The early growth response gene (Egr-1) codes for a zinc finger transcription factor that has important roles in the regulation of cell growth, differentiation, and survival. Aberrant Egr-1 expression is implicated in carcinogenesis, inflammation, atherosclerosis, and ischemic injury. We reported previously that normal fibroblasts stimulated by transforming growth factor- β showed rapid and transient induction of Egr-1. Moreover, we observed that tissue expression of Egr-1 was elevated in patients with scleroderma, which suggests that Egr-1 may be involved in tissue repair and fibrosis. Here, we investigated matrix remodeling and wound healing in mice harboring gain of function or loss of function mutations of Egr-1. Using the model of bleomycin-induced scleroderma, we found that the early influx of inflammatory cells into the skin and lungs, and the subsequent development of fibrosis in these organs, were markedly attenuated in Egr-1 null mice. Furthermore, full-thickness incisional skin wound healing was impaired, and skin fibroblasts lacking Egr-1 showed reduced migration and myofibroblast transdifferentiation *in vitro*. In contrast, transgenic mice with fibroblast-specific Egr-1 overexpression showed exuberant tissue repair, with enhanced collagen accumulation and increased tensile strength of incisional wounds. Together, these results point to the fundamental role that Egr-1 plays in the regulation of transforming growth factor- β -dependent physiological and pathological matrix

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Scleroderma, also known as systemic sclerosis, is a chronic and progressive disorder of unknown cause associated with visceral organ complications and high mortality. The pathogenesis is poorly understood, and there are currently no effective disease-modifying treatments.¹ Scleroderma (hard skin), the hallmark of systemic sclerosis, is largely due to excessive collagen deposition in the dermis. Epithelial-mesenchymal cell transition and transdifferentiation of resident fibroblasts into activated myofibroblasts are thought to play key roles in the process. Tissue fibrosis affects multiple visceral organs, with progressive interstitial lung disease as a prominent manifestation and leading cause of death.² Although there are no animal models of scleroderma that reproduce all cardinal features of the human disease, subcutaneous injection of bleomycin in mice results in tissue alterations that phenocopy key traits.^{3,4} Bleomycin-induced scleroderma is characterized by dermal sclerosis, s.c. adipose tissue atrophy, and progressive fibrosis in the lung and the kidney, and is well-suited as an animal model for dissecting the roles of individual cell types, molecules, and signaling pathways in the pathogenesis of fibrosis.^{5–7}

Transforming growth factor- β (TGF- β) is a multifunctional cytokine that exerts potent effects on fibroblast proliferation and survival, collagen production and myofibroblast differentiation *in vivo* and *in vitro*, and is a critical mediator of both normal wound healing and pathological fibrosis.⁸ Cellular responses are mediated via the sequential activation of the Type I TGF- β receptor (T β RI), phosphorylation of cytoplasmic Smad2/3 and their heterodimerization with Smad4, and accumulation within the

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nucleus. The nuclear Smad2/3/4 complex interacts directly with consensus Smad-binding element DNA sequences on target genes to activate their transcription. Coactivators such as p300/CBP are recruited to DNA to modulate Smad-dependent transcriptional responses.⁹ Although the Smads are considered as key intracellular mediators for fibrotic responses,^{10–13} Smad-independent alternate signal pathways and transcription factors are also implicated in TGF- β responses.¹⁴ Intracellular signal transduction via mitogen-activated protein kinases, TAK1, c-Abl, and phosphatidylinositol 3-kinase all contribute to translating TGF- β signals into cell type-specific and target gene-specific biological responses, and play distinct roles in the pathogenesis of cancer, autoimmunity, inflammation, and fibrosis.¹⁵

The early growth response-1 (Egr-1) transcription factor (also termed NGF1-A and Krox 24) belongs to a family of zinc finger DNA-binding proteins that include Egr-2 (Krox 20) and Nab2.¹⁶ Normally, Egr-1 shows only low level of tissue expression at baseline, but is rapidly and transiently induced by various stress signals. In turn, Egr-1 plays a key role in orchestrating the tissue response to acute injury by inducing the synthesis of platelet-derived growth factor, connective tissue growth factor (CTGF), vascular endothelial growth factor, plasminogen activator inhibitor-1, and TGF- β .¹⁷ Surprising in light of this broad range of biological responses, mice lacking Egr-1 showed no overt phenotype, with the exception of infertility in the female Egr-1^{-/-} mice.¹⁸ Under physiological conditions, both Egr-1 expression and transcriptional activity are tightly regulated, ensuring rapid extinction of Egr-1-mediated responses after injury. During the course of studies investigating the molecular pathogenesis of fibrosis, we found that TGF- β acted as a potent stimulus for inducing the expression of Egr-1 in normal skin and lung fibroblasts.¹⁹ This Egr-1 response was independent of Smad2/3, and occurred instead through activation of an intracellular signaling cascade involving the mitogen-activated protein kinases, Erk1/2, and the ternary complex factor, Elk-1.²⁰ Furthermore, ectopic Egr-1 was sufficient to induce collagen gene expression in normal fibroblasts *in vitro*, whereas fibroblasts lacking Egr-1 showed diminished responses to TGF- β , implicating Egr-1 as an important downstream mediator for TGF- β signaling in the context of fibrosis. Consistent with a putative role of Egr-1 in regulating fibrogenesis, aberrant Egr-1 expression was detected in fibrotic skin and lung tissues from patients with scleroderma.²⁰

Since Egr-1 can directly stimulate collagen and fibronectin synthesis, enhance epithelial-mesenchymal cell transition and myofibroblast transdifferentiation, and induce the production of growth factors such as platelet-derived growth factor, vascular endothelial growth factor, plasminogen activator inhibitor-1, CTGF, and TGF- β , sustained tissue expression of this normally short-lived early response gene in scleroderma could have an influence on the evolution of pathological fibrosis at multiple levels. To explore this hypothesis, we compared the development of experimentally-induced scleroderma in mice harboring a null mutation of Egr-1 and wild-type mice. The results reveal key roles for Egr-1 in bleomycin-induced

inflammatory responses and in the progression of skin and lung fibrosis, as well as in cutaneous wound healing. Furthermore, transgenic mice with fibroblast-specific Egr-1 overexpression showed exuberant healing of incisional skin wounds. Taken together, these observations confirm the importance of Egr-1 as a novel mediator of physiological and pathological tissue remodeling, and identify this inducible transcription factor as a promising potential target in developing anti-fibrotic therapies for scleroderma.

Materials and Methods

Animals and Experimental Protocols

Homozygous Egr-1 null (Egr-1^{-/-}) mice¹⁸ were backcrossed to C57BL/6 isogenic background, and both female and male Egr-1^{+/+} (wild-type) mice and Egr-1^{-/-} littermates were used in these studies. The results with male and female mice were identical. The mice were provided with Rodent Diet (Harlan, Madison, WI) and water ad lib. Starting at 8 weeks of age, mice were administered bleomycin (1 mg/kg/day) (Mayne Pharma, Paramus, NJ) or PBS by daily s.c. injections. Five or 28 days after the initiation of the injections, mice were sacrificed and the lesional skin was harvested and processed for analysis as described.²⁰ Sections were embedded in paraffin, and consecutive 5- μ m sections were cut, taking care to maintain the same angulation in all samples. In experiments focusing on fibrosis in the lungs, high-dose bleomycin (10 mg/kg/day) was administered by s.c. injections for 14 days, followed by a 14-day period of recovery. For evaluation of the lungs, a 20-gauge angiocath was sutured into the trachea, the lungs were removed with the heart *en bloc*, inflated with 4% paraformaldehyde, and processed for analysis.²¹ Four- μ m-thick sections of paraffin-embedded lung tissue were stained with H&E or Masson's Trichrome, and examined by two experienced anatomical pathologists who were blinded to the experimental conditions. Each treatment group consisted of at least five mice. Experiments were performed according to institutionally approved protocols and in compliance with the animal welfare guidelines of the Northwestern University Animal Care and Use Committee. Genotyping of DNA from tail snips was performed by a PCR procedure.

Generation and Analysis of Col1a2-Egr-1 Transgenic Mice

Transgenic mice were produced by pronuclear injection of DNA into fertilized single cell B6SJL embryos. The mouse Egr-1 cDNA was cloned in the Sall site of pCD3 containing the 6-kb enhancer of the Col1a2 gene (–19.5/–13.5 kb), and the internal ribosome entry site lacZ reporter cDNA downstream of the Sall cloning site.²² The plasmid was linearized with NotI, purified from agarose gel, and microinjected at a concentration of 2 to 4 ng/ml in 10 mmol/L Tris (pH 7.4) and 0.1 mmol/L EDTA. Injected embryos were transferred into pseudopregnant B6SJL

females. Three transgenic founder lines were established and stratified according to the level of transgene expression at embryonic day 14.5, when the Col1a2 promoter is known to be maximally active.²³ A single line of mice that expressed the transgene at high levels during embryogenesis and that showed strongly inducible transgene activity on skin injury was selected for further study. Embryos at embryonic day 14.5 were fixed in lacZ fixative solution containing 0.2% glutaraldehyde, 5 mmol/L EDTA (pH 7.3), and 100 mmol/L MgCl₂ in 0.1 M NaPO₄ (pH 7.3) for 1 hour at room temperature, washed 3 times in lacZ wash buffer containing 0.1 M PBS (pH 7.4), 1 M MgCl₂, 1% sodium deoxycholate, and 2% Nonidet-P40 in 100 mmol/L sodium phosphate (pH 7.3), and stained in X-Gal staining solution (1 mg/ml 5-bromo-4-chloro-3-indolyl-galactoside [X-Gal], 2 mmol/L MgCl₂, 5 mmol/L potassium ferrocyanide, 5 mmol/L potassium ferricyanide, 0.01% sodium deoxycholate, and 0.02% Nonidet-P40) at 37°C overnight. For some experiments, 20- μ m sections from frozen lesional skin were processed for X-Gal staining (Specialty Media, Cell and Molecular Technologies, Phillipsburg, NJ) as previously described.²⁴

Histochemical Studies and Electron Microscopy

To evaluate the severity of skin fibrosis in mice with bleomycin-induced scleroderma, the thickness of the dermis, defined as the distance between the epidermal-dermal junction to the dermal-adipose layer junction, was measured at six randomly selected sites/microscopic fields from two or more microscopic fields from each animal.⁶ To analyze the collagen content and architecture in the lesional skin, deparafinized sections were stained with Picrosirius red and viewed under a polarized light.²⁵ Images representing a standardized area of the lesional dermis were analyzed by using Metamorph software (Micro Software, Inc., Aliso Viejo, CA). Pixels representing red/orange and green birefringence were quantified for each field. The scar index was determined by calculating the ratio of red to green pixels.²⁶

Electron microscopy and analysis of collagen fibril diameter were performed as previously described.²⁷ Briefly, lesional skin tissue from wild-type and Egr-1^{-/-} mice was fixed in 4% glutaraldehyde and after processing, embedded in Spurr's epoxy resin (Electron Microscopy Sciences, Fort Washington, PA). Ultrathin sections (80 nm) were stained with uracyl acetate and lead citrate, and examined with a transmission electron microscope (JEOL 1220, JEOL, Peabody, MA). The diameters of at least 300 individual collagen fibrils per section were measured manually, and histograms of the relative frequencies for each diameter were generated.

Immunohistochemistry and Immunofluorescence

Four- μ m sections from lesional skin and lungs were prepared and processed for immunohistochemistry as described⁶ by using primary antibodies against CTGF, TGF- β 1 and phospho-Smad2/3 (all from Santa Cruz Bio-

technology, Santa Cruz, CA), Type II TGF- β receptor (T β R2) (Upstate, Billerica, MA), phospho-Smad2 (Cell Signaling Technology, Danvers, MA), α -smooth muscle actin (Sigma-Aldrich, St. Louis, MO), or Mac-3 (BD Pharmingen, San Diego, CA). Substitution of the primary antibody with isotype IgG served as negative controls. To avoid observer bias, all specimens were examined blindly. The ratio of clearly immunopositive fibroblasts to total fibroblasts in each microscopic field was calculated. For immunofluorescence analysis, primary cultures of skin fibroblasts explanted from Egr-1^{-/-} mice and wild-type littermates were studied at low passage.²⁰ Briefly, confluent cultures were washed in PBS and fixed in 4% paraformaldehyde and 0.5% Triton X-100 to permeabilize the membranes, followed by incubation with indicated primary antibodies and appropriate fluorescein isothiocyanate-labeled anti-mouse IgG secondary antibodies, or staining with 4'-6-diamidino-2-phenylindole (DAPI) to detect cell nuclei. Cells were then visualized by fluorescence microscopy by using a UV LSM510 META confocal microscope (Zeiss, Oberkochen, Germany). Images were obtained by digital capture.

Effect of Mechanical Strain on Fibroblasts Cultured in Collagen Gels

Fibroblasts explanted from the dorsal skin of Egr-1^{-/-} mice and wild-type littermates were suspended (0.5×10^6 cells/ml) in a solution of rat tail collagen (2.5 mg/ml). The collagen was allowed to gel in specially-designed frames to which dynamic linear compressive strain can be applied by using a computer-controlled mechanical device in standard incubator conditions.²⁸ Three-dimensional gels containing the embedded fibroblasts were submerged in Dulbecco's modified Eagle's medium under static conditions for 24 hours. The gels were then subjected to 25% compressive strain at 1 Hz, or maintained under static culture conditions for a further 48 hours. Gels were then fixed in 4% paraformaldehyde, and incubated with anti-human α -smooth muscle actin monoclonal antibody (R&D Systems, Minneapolis, MN) followed by Alexa Fluor 488 goat anti-mouse IgG, and counterstained with DAPI. Gels were then examined by confocal immunofluorescence microscopy.

Determination of mRNA Levels by Quantitative Real-Time PCR

Tissue levels of mRNAs were determined by real-time quantitative PCR.²⁰ Total RNA was isolated from frozen skin or lung tissue by using Trizol Reagent (Invitrogen, Carlsbad, CA), purified and reverse transcribed, followed by real-time quantitative PCR by using the oligonucleotide primers listed in Table 1 and SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on the ABI 7500 Prism Sequence Detection System machine. GAPDH or 18S RNA was used for normalizing between individual samples. The results were expressed as mRNA

Table 1. Primers Used for Quantitative Real-Time PCR

Gene	Primers
COL1A1	Forward: 5'-CCTGAGTCAGCAGATTGAGAA-3' Reverse: 5'-ACTGAACTTGACCGTACACCAGTACTCTCCGCTCTTCAA-3'
COL1A2	Forward: 5'-CCGTGCTTCTCAGAACATCA-3' Reverse: 5'-CTTGCCCCATTCATTGTCT-3'
18s rRNA	Forward: 5'-TTCGAACGCTCTGCCCTATCA-3' Reverse: 5'-ATGGTAGGCACGGCGACTA-3'
Glyceraldehyde-3-phosphate dehydrogenase	Forward: 5'-GTCGTGGATCTGACGTGCC-3' Reverse: 5'-GATGCCTGCTTCACCACCTT-3'

rRNA, ribosomal RNA.

levels corrected for 18S RNA or GAPDH levels in each sample.

Quantification of Tissue Collagen

The collagen content of the lungs was determined by colorimetric assays.²¹ Briefly, tissue specimens were homogenized in 0.5M glacial acetic acid and after centrifugation at 10,000 rpm for 15 minutes, supernatant were assayed for soluble collagen by using Sircol Collagen Assay kits (Biocolor, Newtown Abbey, Northern Ireland).

Bone Marrow-Derived Macrophage Cultures

Bone marrow macrophages were obtained from femurs and tibias of 12-week-old wild-type and *Egr-1*^{-/-} mice.²⁹ Cells were centrifuged at 1500 rpm for 5 minutes, resuspended in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum, penicillin and streptomycin, and 50 ng/ml of mouse macrophage colony-stimulating factor, and seeded in 6-well plates. Five days later, cells were reseeded at a density of 4×10^5 cells/ml in 6-well plates, and 4 days later, fresh media with 10 ng/ml TGF- β or 100 ng/ml lipopolysaccharide was added. After an additional 48 hours incubation, supernatants were harvested and the levels of TGF- β 1 and tumor necrosis factor- α (TNF- α) secreted in the conditioned media were determined by enzyme-linked immunosorbent assay (R&D Systems).

Skin Wound Healing

The effect of *Egr-1* loss-of-function or gain-of-function mutations on cutaneous wound healing was investigated in an *in vivo* model.³⁰ Four full-thickness 3-cm incisional dorsal wounds were created along the paramedian skin of 14-week-old wild-type and *Egr-1*^{-/-} mice. The wounds were closed with continuous 6-0 nylon suture and covered with semiocclusive dressing. Seven or 14 days later, mice were sacrificed and wounds were harvested for histology or for mechanical testing. H&E, Picrosirius Red staining and immunohistochemistry were performed as described above. The expression of LacZ in the wounds at 24 hours and 14 days was detected in frozen sections as described above. Peak wound breaking strength of 1 cm strips of the harvested skin oriented perpendicular to the incisional line was determined by using a commercial

digital tensiometer (Instron 4442; Universal Testing System; Instron, Norwood, MA) as described.³¹ One-cm test chambers were secured to the wounds (8 to 16 wounds/group), and while measuring, increasing negative pressure was applied at a rate 5 mmHg/second until bursting.

Fibroblast Cultures and Cell Migration Assays

Fibroblasts were explanted from the dorsal skin of 6-week-old wild-type and *Egr-1*^{-/-} mice and studied in parallel. Low passage³⁻⁴ fibroblasts were seeded in 6-well plates (10^5 cells/well) and incubated in serum-free Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin in the presence or absence of TGF- β 1 (Genzyme, Framingham, MA) for up to 48 hours. Myofibroblast differentiation was evaluated by immunofluorescence microscopy by using antibodies against α -smooth muscle actin.³² Cell migration was analyzed by *in vitro* wound-closure assays.³³ Briefly, confluent monolayers of dermal fibroblasts were incubated in serum-free media with 10 μ g/ml mitomycin C to block proliferation. Mechanical injury was then performed with a single scratch across the monolayers by using sterile p-1000 plastic pipette tips. The wells were then washed to remove detached cells and incubated in serum-free media containing 0.1% bovine serum albumin and TGF- β for up to 24 hours. Cultures were examined by phase contrast microscopy, and the width of the gaps was measured at six different sites at each time point.

Statistical Analysis

Results are expressed as the means $\pm$ SD or $\pm$ SEM. Mann-Whitney's *U*-test (*in vivo* studies) or Student's *t*-test (*in vitro* studies) was used for comparison between two groups. *P* values <0.05 were considered statistically significant.

Results

Attenuated Inflammatory Responses in *Egr-1*^{-/-} Mice

Subcutaneous bleomycin injection triggered a robust early influx of mononuclear cells and neutrophils into the lesional dermis. After 4 days of daily bleomycin injec-

Figure 1. Attenuated skin inflammation and reduced TNF- α production in *Egr-1*^{-/-} mice. **A:** *Egr-1*^{-/-} mice and wild-type littermates in parallel received s.c. bleomycin or PBS injections daily. At day 5, lesional skin was harvested and stained with H&E (left panels). Original magnification, $\times 100$ (a, e, c, g) or $\times 400$ (b, f, d, h). Leukocytes infiltrating the dermis were quantitated (right panels). Bars represent the means $\pm$ SD of triplicate determinations. Closed bars represent mononuclear cells and open bars represent neutrophils. $*P < 0.05$. **B:** Mice received a single s.c. injection of TGF- β 1 or PBS and lesional skin was harvested 24 hours later and stained with H&E. Representative images. Original magnification, $\times 100$ (a, c, e, g) or $\times 400$ (b, d, f, h). **C:** Macrophages were isolated from bone marrow as described under *Materials and Methods* and incubated in serum-free media with 10 ng/ml TGF- β 2 for indicated periods or 100 ng/ml lipopolysaccharide for 24 hours. Culture supernatants were harvested and assayed for secreted TNF- α or TGF- β 1 by enzyme-linked immunosorbent assay. Results are the means $\pm$ SD of triplicate determinations from a representative experiment. Closed bars represent wild-type macrophages; open bars represent *Egr-1*^{-/-} macrophages. $*P < 0.05$.

tions, the period of the maximal response, a significantly lower number of inflammatory cells infiltrating the dermis was seen in *Egr-1*^{-/-} mice (Figure 1A). The numbers of both mononuclear infiltrating cells (presumably macrophages and T lymphocytes) and of neutrophils were reduced. Skin inflammation induced by a single s.c. injection of TGF- β was also attenuated in *Egr-1*^{-/-} mutant mice (Figure 1B). Next, the ex vivo TGF- β response of bone marrow-derived macrophages was examined. Stimulation of the secretion of TGF- β 1 and TNF- α by TGF- β 2 was attenuated in *Egr-1*-null macrophages compared with wild-type macrophages, whereas the lipopolysaccharide-induced responses were similar (Figure 1C). Together, these findings demonstrate that the absence of *Egr-1* is associated with markedly impaired *in vivo* and *in vitro* inflammatory responses elicited by bleomycin or by TGF- β .

Attenuated Dermal Fibrosis in *Egr-1*^{-/-} Mice

To investigate the role of *Egr-1* in tissue fibrosis, lesional skin harvested after 28 days of bleomycin injections was examined. Compared with wild-type mice that demon-

strated a >twofold increase in dermal thickness ($P < 0.05$), *Egr-1*^{-/-} mice showed only a modest (<40%) increase in thickness, and the dermis appeared less sclerotic (Figure 2). Picrosirius Red staining revealed reduced accumulation of strongly red birefringent collagen fibers in the lesional dermis of *Egr-1*^{-/-} mice (Figure 2B panel d versus panel h) compared with wild-type mice (panel b versus panel f). The scar index in wild-type mice treated with bleomycin was fivefold higher than in PBS-treated mice, whereas only an approximate twofold increase was observed in *Egr-1*^{-/-} mice (Figure 2B, right panel). Levels of collagen mRNA (Figure 2C) and local accumulation of α -smooth muscle actin-positive myofibroblasts (Figure 2D) were also significantly lower in the lesional dermis in *Egr-1*^{-/-} mice. Wild-type control mice had a thick layer of s.c. adipose fat subjacent to the dermis, and the development of skin fibrosis was accompanied by progressive obliteration of the s.c. adipose layer (Figure 2A, compare panels b and f). In contrast, the s.c. adipose layer was relatively preserved in *Egr-1*^{-/-} mice treated with bleomycin (Figure 2A, compare panels d and h).

Figure 2. Reduced skin fibrosis in Egr-1^{-/-} mice. Egr-1^{-/-} mice and wild-type littermates received daily s.c. injections of PBS or bleomycin for 28 days and lesional skin was examined. **A:** H&E stain. Original magnification, $\times 100$ (a, c, e, g) or $\times 400$ (b, d, f, h); scale bar = 100 μ m and 50 μ m. The thickness of the dermis was quantitated (right panel). The results represent the means $\pm$ SD from five mice/group. Open bars represent PBS; closed bars represent bleomycin. * $P < 0.05$. **B:** Picrosirius Red stain, viewed by polarized light microscopy. Original magnification, $\times 100$ (dermis: a, c, e, g); Original magnification, $\times 400$ (s.c. adipose layer: b, d, f, h). Right panel shows the scar index determined as described under Methods. Results represent the means $\pm$ SD from five mice/group. Open bars represent PBS; closed bars represent bleomycin. * $P < 0.05$. **C:** Real-time quantitative PCR analysis. Total RNA was isolated from lesional skin. The results, normalized for 18S RNA, represent the means $\pm$ SD of duplicate determinations from three mice/group; * $P < 0.05$. **D:** Myofibroblasts were detected by immunohistochemistry. The proportion of α -smooth muscle actin-positive fibroblasts was quantitated (right panel). The results indicate the means $\pm$ SD of triplicate determinations in at least six microscopic fields from four mice/group. Open bars represent PBS; closed bars represent bleomycin. * $P < 0.01$.

Figure 3. Altered skin collagen fibril ultrastructure in *Egr-1*^{-/-} mice. **A:** Mice received s.c. injections of PBS (**a,c**) or bleomycin (**b,d**) for 28 days. Lesional skin was harvested and transverse sections were examined by transmission electron microscopy. Scale bar = 250 nm. **B:** Collagen fibril diameters were measured. At least 300 individual fibrils were measured in six different areas in each sample from three mice/group. The frequencies of collagen fibrils with a given diameter are shown. Closed bars represent bleomycin; open bars represent PBS.

Lesional skin was further examined by electron microscopy for changes in the collagen fibril ultrastructure. In PBS-treated *Egr-1*^{-/-} mice, dermal collagen appeared less tightly packed than in wild-type mice of the same age, and individual collagen fibrils were smaller and exhibited a somewhat irregular outline in cross-sections when compared with the regular circular outline in wild-type mice (Figure 3A). The development of dermal fibrosis after 4 weeks of daily bleomycin injections in wild-type mice was associated with a significant decrease in the average diameter of collagen fibrils (from a mean $\pm$ SEM 110 ± 26 nm in PBS-treated mice to 82 ± 27 nm in bleomycin-treated mice) (Figure 3B). The reduction of collagen fibril diameter is considered to reflect active fibrosis and rapid rate of collagen synthesis, and has been previously described in the fibrotic response.³⁴ In contrast to the wild-type mice, the dermis of *Egr-1*^{-/-} mice contained collagen fibrils of smaller average diameter at baseline and showed no significant change on bleomycin treatment (mean 92 ± 25 nm versus 85 ± 21 nm; NS).

Attenuated TGF- β Expression and Signaling in the Skin in *Egr-1*^{-/-} Mice

The development of bleomycin-induced scleroderma is accompanied by evidence of *in situ* activation of TGF- β

signaling.²⁵ To characterize the role that Egr-1 might play in this process, the mediators of the TGF- β response were examined in the lesional skin by immunohistochemistry. We found that the induction of both TGF- β 1 and T β R2, as well as levels of phospho-Smad2, a sensitive *in situ* marker of TGF- β activity, were markedly attenuated in fibroblastic cells in *Egr-1*^{-/-} dermis (Figure 4, A and B). Expression of CTGF, a TGF- β -inducible growth factor implicated as an important mediator for profibrotic TGF- β responses, was also significantly lower in *Egr-1*^{-/-} mice (Figure 4C). When TGF- β was administered as a single s.c. injection, a rapid increase in local phospho-Smad2 accumulation was seen in the dermis, with a predominantly nuclear pattern of immunolocalization (Figure 4D). The early increase in the tissue levels of phospho-Smad2 induced by TGF- β was comparable in wild-type and *Egr-1*^{-/-} mice, as expected, since Smad2/3 activation by TGF- β occurs independent from Egr-1.¹⁹ In contrast, however, at later time points after TGF- β injection, substantially reduced accumulation of phospho-Smad2 was seen in *Egr-1*^{-/-} mice. Since sustained Smad activation depends on continuous TGF- β receptor stimulation by the ligand,³⁵ defective TGF- β autoinduction provides a likely explanation for attenuated Smad activation noted in *Egr-1*^{-/-} mice at 48 hours. These results, therefore, suggest that Egr-1 was required for endogenous TGF- β production and resultant persistence of profibrotic signaling in the lesional dermis.

Attenuated Lung Fibrosis in *Egr-1*^{-/-} Mice

Intratracheal bleomycin administration is widely used to model injury-associated fibrosis.³⁶ During the course of the present studies, we observed that administration of bleomycin by the s.c. route was also accompanied by fibrotic changes in the lungs. In preliminary studies, we found that optimal induction of lung fibrosis required 200 μ g/day of bleomycin given over a period of 14 days, followed by a 14-day period of recovery. The changes in the lungs were characterized by an influx of macrophages, patchy areas of alveolar septal thickening, collagen-rich fibrotic foci seen predominantly in subpleural and peribronchial locations, and increased septal collagen deposition (Figure 5A and data not shown). These changes were distributed in a pattern distinct from the bronchiolocentric pattern that is typically associated with intratracheal administration of bleomycin and were more reminiscent of the nonspecific interstitial pneumonitis pattern characteristic of scleroderma-associated pulmonary fibrosis.³⁷ The severity of bleomycin-induced lung pathology was attenuated in *Egr-1*^{-/-} mice (Figure 5D). These mice showed reduced infiltration of Mac3-positive macrophages in the lungs. Furthermore, the number and size of subpleural and interstitial fibrotic foci, and especially the intensity of collagen deposition as seen in trichome staining, were markedly reduced in *Egr-1*^{-/-} mice (Figure 5A). Diminished collagen accumulation and Col1a2 mRNA expression in the lungs were demonstrated by SIRCOL assays and by real-time quantitative PCR (Figure 5, B and C).

Figure 4. Reduced TGF- β signaling in Egr-1^{-/-} mice. Mice were sacrificed on day 28 after starting bleomycin or PBS injections (A–C) or 24 hours after a single injection of 250 ng TGF- β (D). The expression of TGF- β , T β R2, phospho-Smad2, and CTGF in the lesional skin was examined by immunohistochemistry. Representative images. **Right panels** show the proportions of immunopositive fibroblasts. The results indicate the means $\pm$ SD of triplicate determinations. In **B** and **C**, open bars represent PBS; closed bars represent bleomycin. In **D**, open bars represent Egr-1^{-/-} mice; closed bars represent wild-type mice. * $P < 0.05$.

Egr-1 is Required for Optimal Wound Healing

Acute tissue injury is associated with rapid and transient up-regulation of Egr-1 expression localized to the site of the injury.³⁸ To investigate the functional role that Egr-1 may have in cutaneous repair, full-thickness incisional wounds were made in the dorsal skin of Egr-1^{-/-} mice and wild-type controls, and wounds were examined 7 or 14 days later. Histological analysis indicated that Egr-1^{-/-} mice had a moderately reduced rate of wound contraction (data not shown). Moreover, Picrosirius Red staining showed that day 7 wounds had reduced collagen deposition in the wound bed (Figure 6A), with a 40% lower scar index (Figure 6B). Accumulation of α smooth muscle cell-positive myofibroblasts was also reduced in the wounds from Egr-1^{-/-} mice (Figure 6C). The maximal breaking strength of healing incisional wounds was significantly lower in Egr-1^{-/-} mice than in identically-treated wild-type controls (Figure 6D).

To investigate the mechanistic basis underlying the contribution of Egr-1 to wound repair, the regulation of *in vitro* cell migration and differentiation were investigated. For this purpose, skin fibroblasts explanted from the back of 6-week-old wild-type and Egr-1^{-/-} mice were used to establish primary cultures. Genotypes were confirmed by RT-PCR (data not shown). When the monolayers reached confluence, a linear scratch was made by using a pipette tip and fibroblasts migration into the wounded area was measured. At 24 hours after mechanical injury, TGF- β -induced migration was >75% lower in Egr-1^{-/-} fibroblasts ($P < 0.05$) (Figure 7A). In addition, TGF- β

stimulation of α -smooth muscle actin, a marker of myofibroblast differentiation, was markedly attenuated in Egr-1^{-/-} fibroblasts (Figure 7B), indicating that this process is also Egr-1-dependent. Likewise, myofibroblast differentiation of fibroblasts subjected to cyclic compressive mechanical stress in 3-D collagen gels, demonstrated previously,²⁸ was blocked in Egr-1^{-/-} cells (Figure 7C).

Col1a2-Egr-1 Transgenic Mouse Shows Enhanced Wound Healing

To explore further the contribution of Egr-1 in tissue remodeling, we generated a line of transgenic mice in which Egr-1 overexpression was targeted to fibroblasts. As illustrated in Figure 8, A and B, fibroblast-specific Egr-1 expression was achieved by using a 6-kb enhancer from the pro α 2(I) chain of the mouse type I collagen (Col1a2) gene.²³ In addition, a lacZ cassette was cloned downstream of the Egr-1 cDNA by using an internal ribosome entry site sequence to generate a bicistronic message that allows evaluation of transgene expression *in vivo* by using lacZ histochemistry (Figure 8A). Analyses were performed with mice that were hemizygous for the Egr-1 transgene (Tg+) and wild-type (Tg-) littermates as controls (Figure 8B). Strong transgene expression throughout the dermis was observed in embryos at embryonic day 14.5, a time point when the Col1a2 promoter is known to be maximally active (Figure 8C). At 12 weeks of age, Col1a2-Egr-1 transgenic mice appeared grossly

Figure 5. Reduced lung inflammation and fibrosis in *Egr-1*^{-/-} mice. *Egr-1*^{-/-} mice and wild-type littermates received daily s.c. injections of bleomycin (200 μg) or PBS for 14 days. Fourteen days after the last injections, the lungs were harvested and processed as described under Methods. **A:** Light microscopic appearance of the lungs from *Egr-1*^{+/+} mice (**a–e**) or *Egr-1*^{-/-} mice (**f–j**) injected with PBS (**a,f**) or bleomycin (**b–e, g–j**). Masson's trichrome stain, original magnification ×25 (**a,b,f,g**) or ×100. Representative images illustrating subpleural fibrosis (**c**), peribronchovascular fibrosis (**h**), or interstitial fibrosis (**d,e,i,j**). **B:** Collagen content of the lungs was determined by Sircol assays. Results represent the means ± SD of multiple determinations from five mice/group. Open bars represent PBS; closed bars represent bleomycin. **P* < 0.05. **C:** Total RNA was isolated and subjected to real-time quantitative PCR. The results, normalized for glyceraldehyde-3-phosphate dehydrogenase, represent the means ± SD of duplicate determinations from 3 to 5 mice/group. Open bars represent PBS; closed bars represent bleomycin. **P* < 0.05. **D:** Immunohistochemistry with Mac-3 antibody. Original magnification, ×400. Mac-3-positive cells were quantitated (**right panel**). The results represent the means ± SD from five mice/group. Open bars represent PBS; closed bars represent bleomycin. **P* < 0.05.

Figure 6. Impaired healing of cutaneous wounds in Egr-1^{-/-} mice. Full-thickness incisional wounds were made in the dorsal skin, and 7 or 14 (**D**) days later mice were sacrificed and wounds were examined. **A:** Picrosirius Red staining at day 14 viewed under polarized light. Original magnification, $\times 100$ (**a, c**) or $\times 400$ (**b, d**). **B:** Scar index. The scar index of each wound was determined by calculating the ratio of red to green pixels as described under Methods. Closed bars represent wild-type mice; open bars represent Egr-1^{-/-} mice. The results represent the means $\pm$ SD of triplicate determinations from eight separate wounds. $*P < 0.05$. **C:** Immunohistochemistry with antibody against α -smooth muscle actin. Original magnification, $\times 100$ (**a, c**) or $\times 400$ (**b, d**). **D:** Wound breaking strength. Peak load at specimen break of standardized wound strips. Results are as means $\pm$ SEM of duplicate determinations from four different mice; $*P < 0.05$.

indistinguishable from wild-type controls (data not shown). Previous results have demonstrated that the Col1a2 promoter activity in the skin of transgenic mice is maximal at embryonic day 15.5 and declines progressively thereafter, with undetectable level by 12 weeks of age.²² Consistent with these previous results, the Egr-1 transgene expression was maximally active at embryonic day 14.5 and down-regulated by birth in unwounded skin (Figure 8D). However, the expression of the transgene was strongly induced after injury when Col1a2 is activated during fibroblast activation and injury repair (Figure 8D).

To examine the effect of Egr-1 overexpression on tissue remodeling, Col1a2-Egr-1 transgene expression in 12-week-old mice was stimulated by creating cutaneous wounds to induce Col1a2 promoter activity. Incisional wounding of the skin was shown to cause re-activation of the Col1a2 promoter in dermal fibroblasts.²² At 7 days after injury, when they are maximally activated during the proliferative phase of healing, fibroblasts in the wound bed in Tg- control mice showed increased Egr-1 staining compared with unwounded skin fibroblasts (Figure 9A). Identically-treated Tg+ mice showed significantly greater Egr-1 staining in the wound bed. Picrosirius red staining of the wound beds revealed marked accumulation of

densely packed sclerotic bright red birefringent collagen fibers in Col1a2-Egr-1 Tg mice compared with wild-type littermates treated in an identical manner (Figure 9B). The maximal breaking strength of healing incisional wounds was significantly greater in Col1a2-Egr-1 transgenic mice than in identically-treated wild-type controls (Figure 9C). To confirm the activation of Col1a2-Egr-1 transgene expression in the skin during wound healing, LacZ staining was performed. Prominent LacZ staining of the wound area was observed in dermal fibroblasts from Tg+ mice for up to 14 days after injury, indicating active transgene expression in the skin (Figure 9D), whereas no LacZ staining could be detected in wild-type mice (data not shown). Taken together, these results indicate that induction of fibroblast-specific Egr-1 overexpression in the skin in Tg+ mice was associated with accentuated cutaneous healing response with enhanced collagen deposition in the wound bed and greater tensile strength of the healing wounds.

Discussion

Subcutaneous bleomycin injection induces an early and short-lived inflammatory response in mice that is followed by fibrosis in the skin and lungs. The prominent histological findings, such as expansion of the dermis due to collagen accumulation and s.c. adipose tissue atrophy, and progressive parenchymal lung fibrosis, resemble the skin and lung changes characteristic of diffuse cutaneous systemic sclerosis.⁴ As in human scleroderma, activation of TGF- β signaling in the local tissue milieu plays an important role in bleomycin-induced fibrosis.²⁵ To gain a better understanding of the cellular and molecular processes underlying the pathogenesis of scleroderma, we took advantage of the availability of mice harboring a null mutation of Egr-1. The present results demonstrate that Egr-1^{-/-} mice had reduced influx of inflammatory cells in the dermis and the lungs and fibrosis in these organs was attenuated. Moreover, Egr-1^{-/-} mice demonstrated impaired healing of cutaneous wounds. Explanated Egr-1-null fibroblasts showed defective profibrotic responses to TGF- β . Fibroblast-specific Egr-1 overexpression in transgenic mice resulted in robust healing of incisional wounds. Together, these findings provide compelling evidence for critical and nonredundant roles for Egr-1 in the regulation of physiological and pathological tissue remodeling.

Acute injury and ischemia cause rapid and transient induction of Egr-1 expression, which then functions as a master switch to trigger mediators of inflammation, coagulation, and vascular permeability.³⁹ Recent studies point to an additional important function for Egr-1 in modulating the fibrotic process. We have shown that Egr-1 expression is induced by TGF- β in normal fibroblasts.¹⁹ Stimulation of Egr-1 expression involved an ERK1/2-Elk-1 signal transduction pathway, and Egr-1 was required for mediating TGF- β -induced collagen up-regulation.¹⁹ Expression of Egr-1 was found to be elevated in fibrotic tissue from mice with bleomycin-induced scleroderma.²⁰ Moreover, lesional skin and lungs from patients with sys-

Figure 7. Impaired fibrotic responses in explanted Egr-1^{-/-} fibroblasts. **A:** Cell migration was evaluated by scratch wound assays. Following mechanical injury, confluent monolayers of wild-type and Egr-1^{-/-} dermal fibroblasts were incubated with indicated concentrations of TGF-β1 for 24 hours. Representative phase contrast micrographs. The width of the repopulated area was measured. The results, shown as the means ± SD from six different sites, are representative of three independent experiments. Closed circles represent wild-type fibroblasts; open squares represent Egr-1^{-/-} fibroblasts; **P* < 0.05. **B:** Fibroblasts were incubated with 10 ng/ml TGF-β1 for 24 hours were stained with α-smooth muscle actin antibody or DAPI. Three independent experiments were performed in duplicates. Representative images. **C:** Fibroblasts cultured in 3-D gels were subjected to 25% compressive strain at 1 Hz or maintained under static culture conditions. After 48 hours cultures were stained with α-smooth muscle actin antibody and DAPI. Original magnification, ×400. Representative images.

temic sclerosis also showed up-regulated Egr-1 expression.²⁰ The association of elevated Egr-1 levels in tissues with excessive collagen accumulation appears to be a broad phenomenon. Studies with atherosclerotic plaques found a strong correlation between local Egr-1 expres-

sion and collagen accumulation.⁴⁰ The expression of Egr-1 can be induced by a variety of stimuli. Induction is generally transient, with rapid extinction of the effect due to down-regulation of Egr-1 transcription and activity by endogenous inhibitors such as Nab2.¹⁶ Aberrant persistence of Egr-1 tissue expression appears to be a hallmark of pathological fibrosis in murine models of scleroderma and renal fibrosis^{20,41–43}; and in vascular plaques and in lesional tissues from patients with systemic sclerosis.^{20,41} Importantly, in the present studies we observed that skin fibrosis and collagen accumulation were attenuated in Egr-1^{-/-} mice. Furthermore, Egr-1^{-/-} mice were also partially protected from the obliteration of s.c. adipose tissue that typically accompanies dermal fibrosis in the bleomycin model.²⁰ These observations are consistent with a fundamental role of Egr-1 in modulation of profibrotic TGF-β signaling, as well as the regulation of adipocyte differentiation.

Figure 8. Generation of Col1a2-Egr1 transgenic (Tg) mice. **A:** Schematic representation of the transgenic construct in which the coding sequence for rat Egr-1 is driven by a fibroblast-specific 6 kb enhancer and minimal promoter of the Col1a2 gene, linked to the Lac-Z reporter gene by using an internal ribosome entry site sequence to generate a bicistronic message that generates both Egr-1 and β-gal protein. **B:** Mice carrying the transgene (Tg+) and wild type (Tg-) littermate controls are identified by PCR analysis of genomic DNA derived from tail biopsies. **C:** At a developmental timepoint when the Col1a2 promoter is known to be active, embryonic day 14.5, Tg+ embryos showed strong widespread LacZ staining that was not present in Tg- embryos. **D:** At birth, when the transgene is developmentally down-regulated, it is again reactivated after dermal injury. Note the blue lacZ reaction product that is present around the area of skin incision in the Tg+ mouse that is not present in the Tg- mouse.

Studies with animal models have provided compelling evidence for a functional role for Egr-1 in tissue injury and fibrosis. Transgenic mice overexpressing active TGF-β targeted to lung epithelial cells by using the CC10 promoter showed an early wave of epithelial cell apoptosis, followed by progressive lung fibrosis most prominent near the airways, and late-stage alveolar remodeling.⁴² The expression of Egr-1 was markedly elevated in the injured lungs, and an Egr-1 null mutation was associated with amelioration of pulmonary pathology.⁴² Other studies showed that IL-13 transgenic mice had similarly elevated Egr-1 expression and developed lung fibrosis.⁴³ Null mutation of Egr-1 markedly reduced pulmonary in-

flammation and fibrosis, attributed in part to reduced TGF- β activation in the absence of Egr-1. Gut fibrosis in a mouse model of Crohn's disease was similarly linked to IL-13-induced fibrotic responses mediated through cellular Egr-1.⁴⁴ Together, these results highlight the importance of Egr-1 as a critical intracellular mediator of fibrotic responses involving TGF- β or IL-13. In contrast, lung fibrosis induced by transgenic TGF- α appeared to be exacerbated, rather than prevented, in mice lacking Egr-1.⁴⁵ The specific role of Egr-1 in promoting or attenuating lung fibrosis may be determined by the nature of the injury, the extracellular stimuli involved, and the cell types that are critical effectors of the process.

A role for Egr-1 in inflammation has been previously proposed. Egr-1 has been shown to regulate the expression of some chemokines and adhesion molecules involved in leukocyte migration.¹⁶ While earlier reports provided evidence that Egr-1 was required for macrophage differentiation,^{46,47} subsequent studies found that Egr-1 was redundant⁴⁸ or was not involved at all in regulation of macrophage function.⁴⁹ In the present studies, we found that bleomycin-induced inflammatory cell infiltration was reduced in Egr-1 null mice. Furthermore, bone-marrow-derived macrophages from Egr-1 null mice showed reduced TNF- α secretion when stimulated with TGF- β , consistent with a distinct functional role of Egr-1 in mediating TGF- β -induced immune activation events. Furthermore, the autoinduction of TGF- β was also diminished in Egr-1-null macrophages, consistent with a role of Egr-1 in mediating this TGF- β response. Taken together, these observations suggest that an attenuated inflammatory response and leukocyte activation in Egr-1 null mice may have a significant contribution to the reduced fibrosis with bleomycin treatment.

Loss of Egr-1 was associated with impaired healing of cutaneous wounds. Furthermore, the diameter of the dermal collagen fibrils was reduced in Egr-1 null mice, suggesting a possible role of Egr-1 in regulating fibrillogenesis. The expression of Egr-1 is rapidly (within 30 minutes) induced at the skin wound area.³⁸ In contrast to embryonic mouse that shows a self-limited Egr-1 response, normal adult mice show a more sustained elevation, with Egr-1 detectable even 24 hours after injury.³⁸ Persistence of Egr-1 may contribute to the inability of adult wounds, in contrast to embryonic wounds, to heal without scarring. Direct stimulation of collagen and fibronectin synthesis, profibrotic cytokine and growth factor production, TGF- β receptor expression, and activation of latent TGF- β are all potential mechanisms to explain the repair-promoting effects of Egr-1. Consistent with the important role of Egr-1 in autocrine TGF- β production and signaling, we found evidence that local TGF- β activity, as

Figure 9. Hyperplastic wound repair in Col1a2-Egr1 transgenic mice. Bilateral incisional wounds were made through the dorsal skin of 12-week-old mice and wounds were examined 7 days (**A–C**) or 24 hours and 14 days (**D**) later. **A:** Egr-1 immunohistochemistry. Original magnification, $\times 100$; inset, $\times 400$. **B:** Picrosirius Red stain viewed under polarized light. Original magnification, $\times 100$ (upper panels) and $\times 400$ (lower panels). **C:** Wound breaking strength. Peak load at specimen break of standardized wound strips. Results represent the means $\pm$ SEM from four different mice. * $P < 0.05$. **D:** LacZ staining. Blue staining indicates transgene expression. Scale bar = 1 mm.

indicated by phospho-Smad2 expression, was reduced in the skin of bleomycin-treated Egr-1-null mice. Furthermore, in explanted Egr-1 null skin fibroblasts, TGF- β stimulation of collagen synthesis, cell migration, and myofibroblast transdifferentiation were impaired. Myofibroblast differentiation induced by cyclic mechanical stress was also reduced.^{50,51}

Bleomycin, administered as a single intratracheal bolus, has been widely used to model lung injury and fibrosis. Although in its early stages, bleomycin-induced lung damage is associated with biochemical and histological changes (inflammatory cell infiltration and increased collagen) that resemble idiopathic pulmonary fibrosis,⁵² the model does not fully mimic the histological pattern or natural history of either human idiopathic pulmonary fibrosis or scleroderma-associated interstitial lung disease.⁵² Lung fibrosis and inflammation in the mouse model are predominantly peribronchial in distribution and in the later stages are associated with emphysematous changes resembling chronic obstructive pulmonary disease.⁵² Moreover, in contrast to human idiopathic pulmonary fibrosis, which typically shows insidious evolution and progression, the bleomycin mouse model is characterized by initially rapid progression of fibrosis, followed later by improvement in the severity of histological changes and biochemical markers. In the present studies, bleomycin given by repeated s.c. administrations, rather than direct intrapulmonary delivery, was associated with a histological pattern of lung damage that was distinct from that described for intratracheal bleomycin. Consistent with an earlier study with s.c. implanted osmotic minipumps,⁵³ there was no prominent bronchiolo-centric distribution of fibrosis.

We found increased Egr-1 expression in the wound area during the proliferative phase of skin repair. A variety of mechanisms may account for Egr-1 induction during tissue injury, including mechanical damage and the enhanced secretion of cytokines such as platelet-derived growth factor. Previous studies have shown that Egr-1 rapidly accumulates in the skin wound in both embryonic and adult mice, with adults showing a more sustained response.³⁸ We found that the ability of Egr-1-null mice to repair cutaneous injury was reduced. A previous study that examined mouse embryos at 24 hours after injury, before the proliferative phase of wound healing, found no difference in wound healing between wild-type and Egr-1-null mice.³⁸ We speculate that in contrast to embryos where Egr-1 and Egr-2 might play redundant roles in repair, adult mice have a nonredundant requirement for Egr-1. Alternately, it is possible that whereas Egr-1 is dispensable for the initial phase of wound healing despite high level expression at 24 hours in the embryo, it is required for optimal completion of the fibroproliferative stages. To further determine the role of Egr-1 in fibroblast function, we generated a transgenic mouse strain in which Egr-1 is expressed in fibroblasts under the control of the Col1a2 promoter.²³ While these transgenic mice showed little spontaneous Egr-1 expression in the skin postnatally, consistent with the marked down-regulation of Col1a2 promoter activity in adult skin fibroblasts,²² they displayed hyperplastic wound healing and in-

creased collagen accumulation on induction of the transgene, providing further evidence of the importance of Egr-1 in regulating fibroblast function during wound repair. Overexpression of Egr-1 in wound area fibroblasts induces the synthesis of a large array of target genes that are important in wound healing (S. Bhattacharyya, J. Varga, manuscript in preparation), accounting for the more robust repair process in the Egr-1 transgenic mice.

In summary, the present results show that the early-immediate gene product Egr-1, induced rapidly by TGF- β and accumulating in the lesional tissues in a bleomycin-induced model of scleroderma, plays essential roles in fibrosis. Egr-1-null mice showed attenuation of the acute inflammatory response elicited by bleomycin and subsequent reduction of fibrosis in the skin and the lungs, as well as defective healing of incisional skin wounds. The activation of TGF- β signaling on bleomycin treatment was reduced in the lesional skin in Egr-1-null mice. Egr-1-null fibroblasts showed reduced stimulation of collagen gene expression and myofibroblast differentiation and migration *in vitro*. Transgenic expression of Egr-1 in fibroblastic cells was associated with hyperplastic wound healing, further attesting to the pivotal role of Egr-1 in regulating tissue repair. These results suggest that sustained up-regulation or aberrant activity of Egr-1 might represent a potential target in the treatment of fibrosis. Pharmacological agents such as the PPAR- γ ligand rosiglitazone, currently used in the treatment of type 2 diabetes mellitus, mycophenolate, an anti-inflammatory agent, and imatinib mesylate, used for chronic myelogenous leukemia, block aberrant Egr-1 activity.^{20,33,54} Therefore, these and similar drugs may have utility as novel anti-fibrotic agents for the treatment of scleroderma.

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