Global expression analysis of the fibroblast transcriptional response to TGFβ

H. Gardner, D. Strehlow[1], L. Bradley, R. Widom[1], A. Farina[1], A. de Fougerolles, J. Peyman[2], V. Koteliansky, J.H. Korn[1]

Biogen Inc, Cambridge, Massachusetts; [1]Arthritis Center, Boston University School of Medicine, Boston Massachusetts; [2]Curagen Inc., New Haven, Connecticut, USA.

This work was in part supported by grants AR32343 (USPHS-NIH) (JHK), AR47196 (USPHS-NIH) (DS), and the Scleroderma Foundation (DS and JHK).

Please address correspondence to: Humphrey Gardner, MD, Research Pathology, Biogen Idec, 14 Cambridge Center, Cambridge, MA02142, USA.

Clin Exp Rheumatol 2004; 22 (Suppl. 33): S47-S57.

Key words: Fibroblast, transforming growth factor beta, scleroderma, transcript profile, global expression analysis.

ABSTRACT

Objectives. *Transforming Growth Factor-β (TGFβ) is the predominant cytokine in all forms of fibrotic reactions. As well as being secreted by immune modulators of fibrosis such as macrophages, it is involved in an autocrine feedback loop of fibroblast stimulation whose regulation is still poorly understood. We wished to gain some insight into the mechanisms of the fibroblast response to TGFβ.*

Methods. *We undertook an exhaustive transcript profiling experiment using a widely validated restriction enzyme based method for identifying differentially expressed genes (GeneCalling™). Transcriptional responses throughout a 24-hour time course were examined at multiple time points and classified.*

Results. *By 24 hours of TGF treatment over 1000 bands, representing a large number of transcripts, were down- or upregulated greater than 2-fold. All of the known genes responsive to TGFβ, such as collagen and connective tissue growth factor, were upregulated.*

Conclusions. *This encyclopedic method revealed many unknown transcriptional responses to TGFβ including the upregulation of a variety of less expected cytoskeletal and matrix components, as well as interactions between the TGFβ and tumor necrosis factor (TNF) pathways and alterations in cell death-related pathways. These may in part explain the idiosyncratic responses of mesenchymal cells to TGFβ.*

Introduction

TGFβ plays a key role in a variety of fibrotic reactions including normal wound healing as well as pathologic fibrosis. It is released by a variety of immune cells, including macrophages and T cells, and is also released by platelets at sites of vascular injury. TGFβ is also involved in a fibroblast autocrine feedback loop whose regulation is poorly understood. TGFβ is striking in the apparently opposite effects induced in fibroblast cell types, where it is pro-proliferative and induces apoptosis resistance, and in epithelial cells and leukocytes, where it is growth inhibitory and in some instances promotes apoptosis. TGFβ also causes significant changes in extracellular matrix synthesis by fibroblasts, including upregulation of various collagens, and downregulation of collagenolytic activities.

In order to complete the catalogue of TGFβ induced responses in fibroblasts, with the hope of gaining some insight into the mechanisms of the fibroblast response, we undertook an exhaustive transcript profiling experiment using Gene Calling™ technology (1), in parallel with an identical study on lesional fibroblasts from patients with scleroderma (manuscript in preparation). This study revealed striking changes in fibroblast transcripts, with a dramatic alteration in matrix synthesis, accompanying changes in metabolic pathways, cytokine and receptor transcription, and changes in cell cycle regulators which might account for the proliferative response of fibroblasts to TGFβ.

Methods

Cell culture

Fibroblast cell strains were established from punch biopsies of dermal skin as previously described (2). Cells were used at the 3rd to 6th passage. 15 cm dish cultures from 5 different cell strains were serum starved for 24 hr prior to the addition of TGFβ. Serum deprivation was used to accentuate the TGFβ response, with the inevitable consequence that the effects of serum starvation themselves were overlaid on the

TGFβ response. At 0 (control) 1, 4, 8, and 24 hours, cells were harvested and fibroblasts from the five strains pooled. In order to determine the effects of serum starvation explicitly, cells untreated with TGFβ were retained for the course of the experiment (24 hours) and were also assayed. RNA was recovered with trizol, and subjected to differential gene expression analysis.

Differential gene expression analysis

GeneCalling reactions were performed essentially as described (3). Briefly, following double-stranded cDNA synthesis of polyA+ RNA, cDNA was separated into 96 different pools and each was digested with a different pair of restriction enzymes. The resulting fragments were ligated to adaptors, and amplified by PCR with complementary primers, one labeled with biotin and the other with flouorescamine (FAM). After affinity purification on streptavidin and separation by polyacrylamide gel electrophoresis, the FAM-labeled fragments were detected by laser excitation.

A composite restriction fragment profile was generated for each sample, based on average peak height and variance of nine separate restriction enzyme digestions (each sample triplicate was subjected to 3 separate restriction enzyme digestions). The restriction fragment profiles of two samples were compared and differentially expressed fragments were identified. Linkage of a differentially expressed cDNA fragment to a gene was made through knowledge of the restriction enzymes used to generate the fragment ends, and hence 6bp of sequence, and the length of the fragment itself. A species-specific query of databases reveals genes that fit these criteria, with gene identification confirmed by competitive PCR using gene-specific oligonucleotides.

Semi-quantative RT-PCR analysis

cDNA samples were prepared according to manufacturer's instructions (Promega). Each sample was tested for genomic DNA contamination by performing a minus reverse transcriptase control with GAPDH primers. PCR amplification from the cDNA was performed on 0.25 µg RNAequivalent, using the Promega Taq system with 1.5 mM $MgCl_2$ and 0.2 mM dNTPs in a total volume of 25 µl. Cycles were as follows: 95ºC, 60 second denaturation, cycles of 95ºC, 20 seconds, 58ºC, 45 seconds, 72ºC, 90 seconds. Primer pairs and optimal cycle numbers for detection within the linear range were as follows: COL11A1; Fw-*tttccccctctccctc - cccaat* Rv-*ggttgttacggtgaaatcccagagc* 208bp, 29 cycles. COMP-1; Fw-*acacg - gacgaggacaagtgg* Rv-*gcatctcccacaaa - gtcgtg* 274bp , 28 cycles. LAMA3 Fw-*atgggatggctgtggatctttgg* Rv-*ccgtccg - gtatacaagcctttatga* 189 bp, 31 cycles. CCR11, Fw-*aggttcagggagcagaggtata - gcc* Rv-*tggggtgttactacgcagatggaaa* 285 bp, 29 cycles. GAPDH; Fw-*accaggtg - gtctcctctgacttcaa* Rv-*tactccttggaggc - catgtggg* 172 bp, 27 cycles. PERIPLAKIN; Fw-*catcgtggacacagaggcc* Rv-*gaagtatggccctgacttcaa* 900bp, 37 cycles. 10 µl of each sample was electrophoresed on a 2% agarose TAE gel. To achieve even and selective staining of the DNA, the gel was washed in 0.1 µg/ml ethidium bromide in 1xTAE, destained in 1xTAE, 30 minutes and then visualised under UV and photographed using a Stratagene eagle eye. Band intensities were quantified using Tiffany software. This quantitation method proved to be directly comparable to radioactive methods.

Results

*Overall changes in gene expression by normal fibroblasts exposed to TGF*β

There was a steady increase in the number of differentially expressed bands detected over the time course of exposure to TGFβ (Fig. 1), reflecting a cascade of signal transduction events downstream of the initial events. Overall more bands were upregulated than downregulated, with 820 bands upregulated and 481 downregulated by greater than two-fold in cells at 24 hours post TGFβ, compared to the untreated cells at the same time. The predominance of upregulation was more striking when looking at bands showing a high level of TGFß regulation: 46 bands were upregulated more than five-fold while 12 were downregulated to the same extent. There were changes in a small number of genes over 24 hours in cells unstimulated by TGFβ (last column).

Early changes in gene expression

Within one hour, there were marked increases in the levels of the transcripts for three transcription regulators: JunB, a component of the AP1 complex; and Id1 and Id3, two dominant negative inhibitors of transcriptional complexes upregulated in B cells, and induced by TGFβ (4, 5) Interestingly, protein tyrosine phosphatase D1, a known activator of Stat3, was also upregulated, and Stat

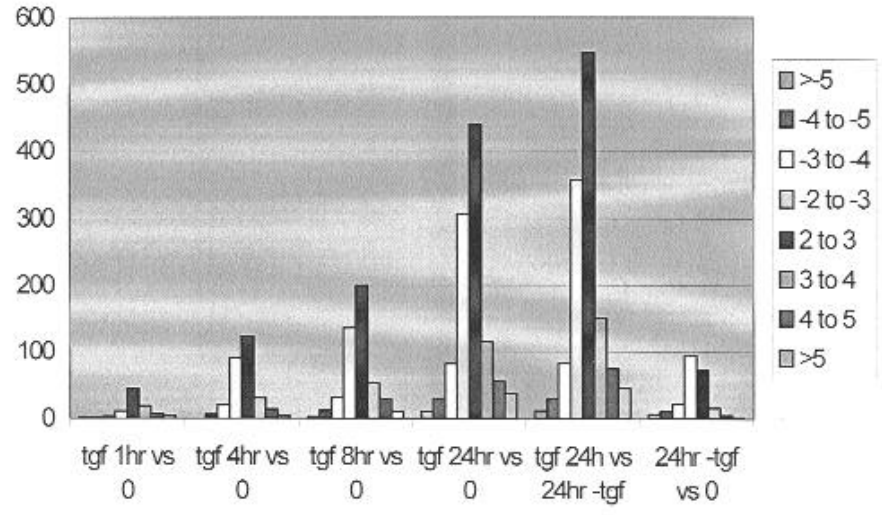


Fig. 1. Overview of bands up and downregulated in response to TGFβ. The total number of called bands and their fold change was quantitated at each time point in the experiment. Many bands may represent one gene. Overall, more bands are upregulated than downregulated.

3 is known to cause the upregulation of Id1 levels (6). At the same time, upregulation of two antiproliferative signals – insulin-like growth factor binding protein 6, and the cell cycle inhibitor CDKN2C – occurred. Accompanying this antiproliferative chorus was upregulation of the transcript for the long form of Bcl-X, an inhibitor of apoptosis. This upregulation marks a difference between the fibroblast response to TGFβ and the epithelial response, as Hep3B cells downregulate Bcl-XL in response to TGFβ (7), on the way to their death.

In the cytoskeleton, the non-muscle tropomyosin, tropomyosin 3, showed a rapid and sustained downregulation first evident at 1 hour, while there was a reciprocal upregulation of the skeletal muscle alpha-tropomyosin 1 by 4 hours. This may represent the first sign of acquisition of the myofibroblast phenotype in response to TGFβ. Lastly, upregulation of two well known targets of TGFβ stimulation, plasminogen activator inhibitor-1 and collagen I, was already evident at 1 hour.

Extracellular matrix

Changes in extracellular matrix component transcription were extensive. Early events included the upregulation of collagen I, with other collagens following suit, including fibrillar collagens V and XI, basement membrane collagens IVand VIII, and the facit collagen XVI. The transmembrane collagen XIII was significantly downregulated, and the endostatin precursor collagen XVIII was slightly so. Parallel to the increase in collagens was upregulation of the collagen modifying enzymes prolyl hydroxylase and lysyl oxidase. Concomitant with upregulated collagen I was an increase in expression of fibrillin, as well as the matrix regulatory proteins thrombospondin I (but a downregulation of its relative punctin), tenascin C, and SPARC, and the proteoglycans versican and perlecan. In contrast, decorin, which has been observed to be associated with antifibrotic events (8), was not detected. One remarkable finding, consistent with previous observations (9) was a striking and sustained upregulation of Cartilage Oligomeric Matrix Protein (COMP), a pentameric extracellular protein whose intracellular accumulation is associated with chondrocyte malfunction (10), but whose normal function in cartilage has not been clearly elucidated. Recent data suggests that COMP may be an accessory component involved in accretion of collagen monomers to promote fibrogenesis (K. Rosenberg, personal communication), and thus an upregulation induced by TGFβ, in parallel with increased collagen synthesis, would be a logical outcome.

Proteases

In general, protease changes were consistent with a profibrotic condition. The collagenase MMP1 was downregulated, consistent with previous observations (11), as well as the gelatinase MMP9 and the inhibitor/coactivator TIMP2. The most striking upregulation in this compartment was PAI-1, which has been recently implicated in fibrosis through a variety of mechanisms, and whose absence, in the PAI1 knockout mouse, confers fibrosis resistance (12).

Intercellular signalling

As would be expected, TGFβ stimulation resulted in a sustained increase in connective tissue growth factor (CTGF) expression, along with autocrine upregulation of TGFβ itself. Another CTGF family member, WISP2, was downregulated. It is noteworthy that WISP2 appears to be highest in adult dermal fibroblasts and lowest in fetal isolates (13) and thus has a reciprocal expression pattern to CTGF.

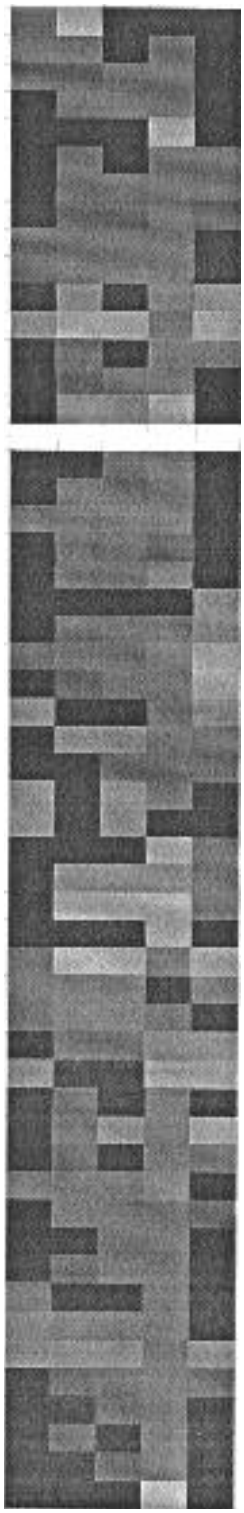
Other growth factor changes included a reduction in Keratinocyte Growth Factor and an upregulation of vascular endothelial growth factor (VEGF), suggesting that the TGFβ response includes support for angiogenesis in the mesenchyme. After a transient increase, VEGF receptor expression was markedly reduced; the role for VEGF receptor in the fibroblasts themselves is not clear but may relate to its function as neuropilin, a semaphorin receptor (see below).

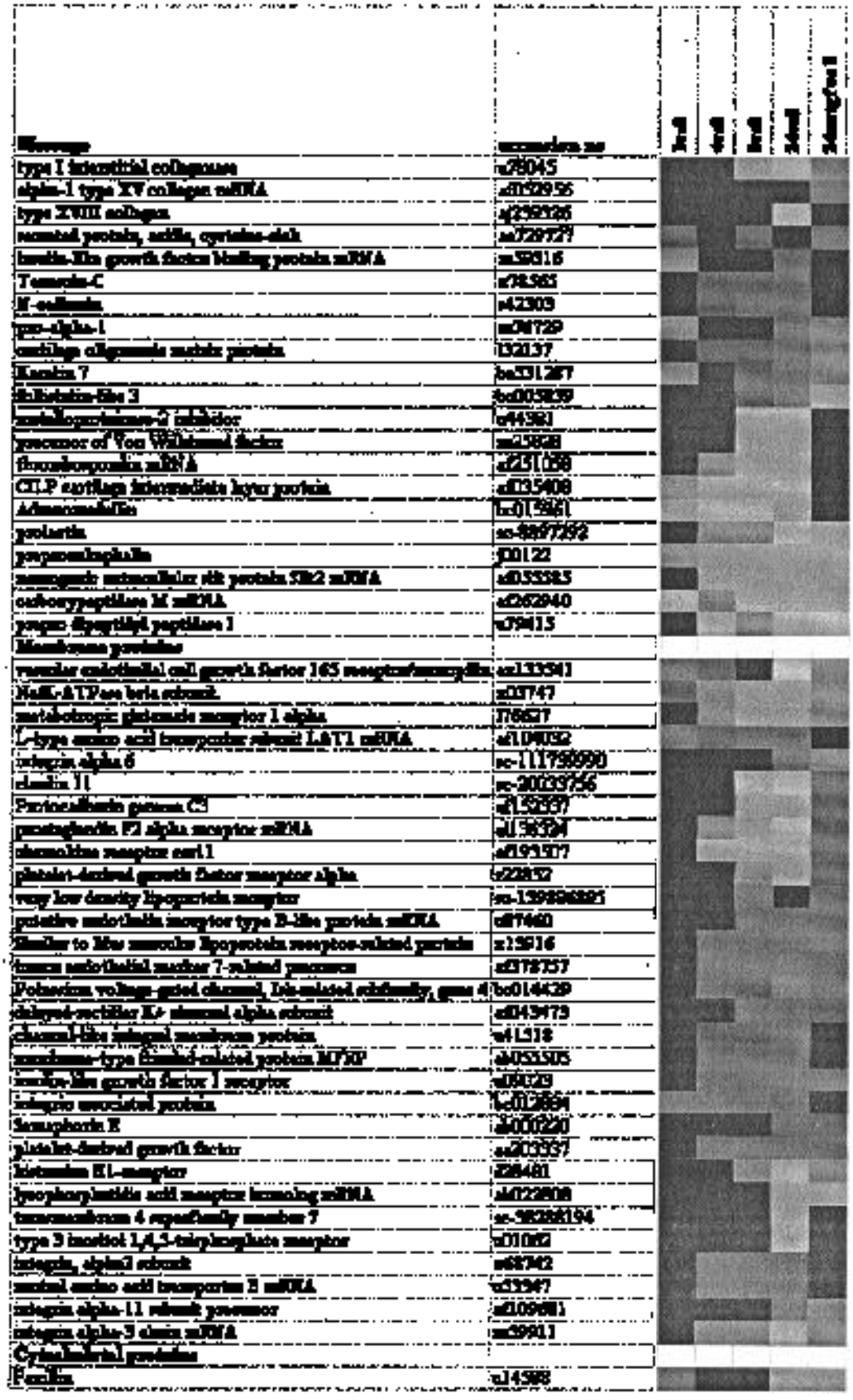
There was also an early (4 hour) downregulation of the tumor necrosis factor-α (TNFα) target, TNFα inducible protein A20, as well as a small downregulation of a TNF receptor, TNFR1α. TNFα is known to inhibit the TGFβ pathway by the partial blockade of Smad-mediated transcription (14). Strands of evidence suggest that TGFβ can reduce TNFα-induced fibroblast death (15), and we have previously shown TGFβ exposure induces resistance to apoptosis (2), but the direct downregulation of components of the TNFα pathway was not previously implicated in the process. Given a reduction of these components at the mRNA level, it is possible that the cells are primed to ignore the TNFα pathway.

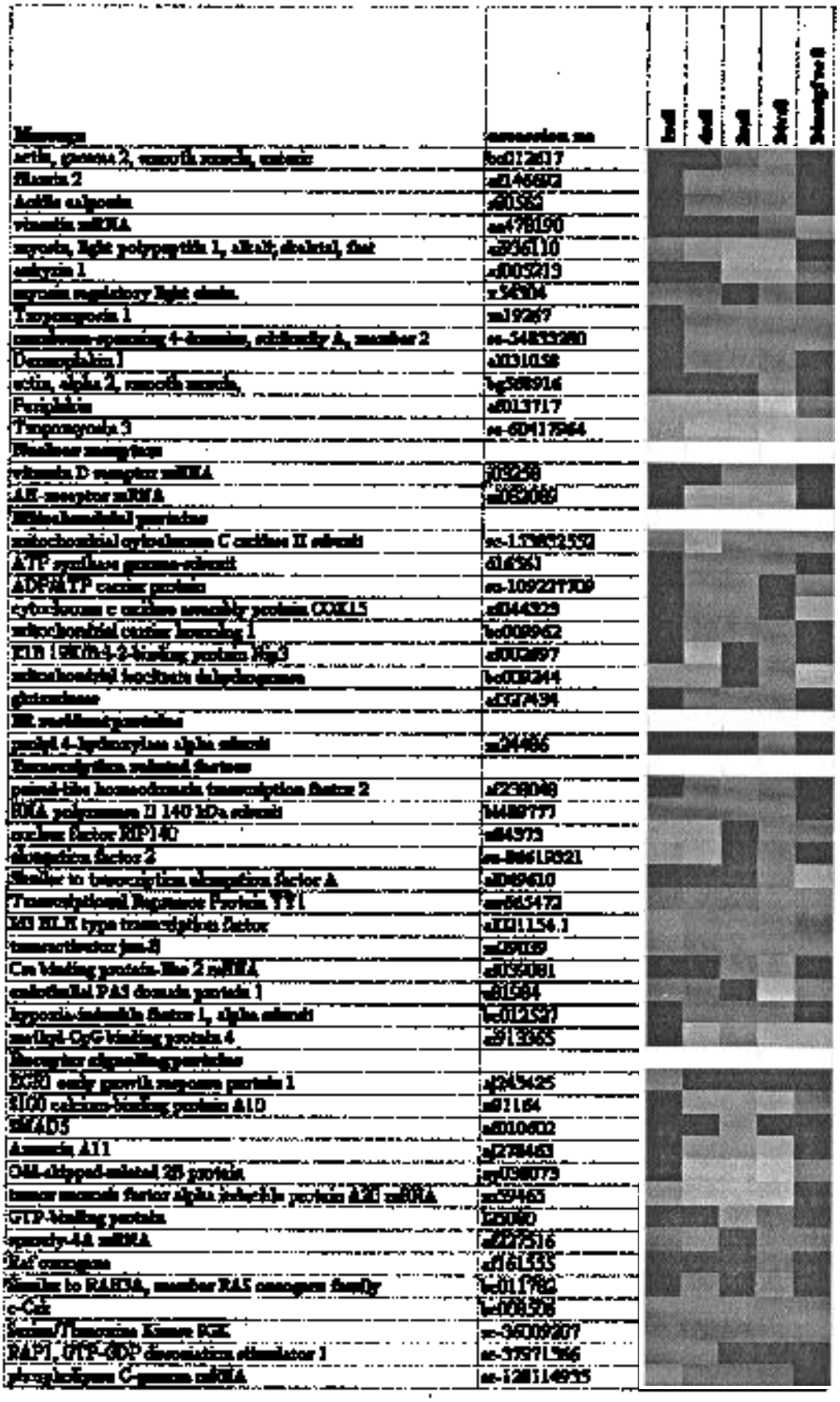
Cell surface molecules

There was a striking downregulation in the chemokine receptor CCR11 (presumably expressed only in a subset of fibroblast samples, see below), the receptor for TECK. Semaphorin E, best known for a role in neuronal guidance, was upregulated, whereas neuropilin, the VEGF/semaphorin 3 receptor found on neurons, endothelium, and myoepithelial cells, was downregulated. Other surface molecules previously associated with neurons were downregulated, including the intracellular adhesive protocadherin gamma C3 and the myelin-associated tight junction protein Claudin 11. Despite cytoskeletal changes associated with the myofibroblast transition, prostaglandin F2 receptor, involved in uterine contraction, was downregulated. Interestingly, platelet-derived growth factor receptor-α was downregulated while the β form was upregulated. This has also been observed at the protein level (16), and raises unanswered questions about the different roles of these two receptors.

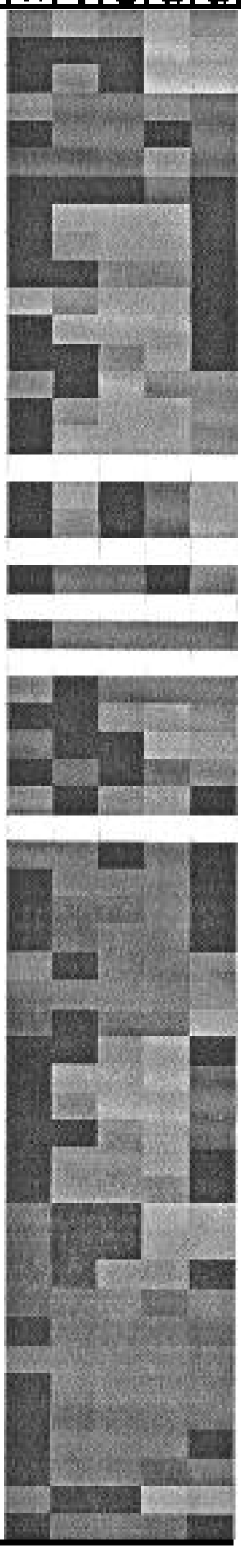
Several integrin changes were noted, including the upregulation of α6, a laminin receptor component, with reciprocal downregulation of α3, an alternative integrin β1 partner and laminin receptor component. Integrin α11, a recently identified β1 partner and collagen receptor (17), along with the major collagen binding integrin subunit α2 (18), were also upregulated. Many other integrin alpha subunits are known to be upregulated in surface expression in







Message	accession no
guanylate binding protein isoform I	nc-66383644
MAP kinase kinase 3b	af87116
novel protein incoding Smad2 gene	nc-145174390
Inositol 1,4,5-trisphosphate 3-kinase isoform B	nj242780
PX and FX domain-containing protein SH3PX1	af131214
PTPL1-associated RhoGAP mRNA	nc-175163717
calmodulin	d45887
Calcium/calmodulin-dependent protein kinase II delta 2-subunit	af095149
guanine nucleotide binding protein	bc010448
ras GTPase-activating-like protein	l33075
receptor-type protein tyrosine phosphatase gamma	u46089
protein phosphatase 2A regulatory subunit A beta isoform	af163473
PAK1 superfamily serine/threonine kinase	af00198
p75NTR-associated cell death executor; ovarian granulosa cell	af00994
Beta catenin-like	af157510
PI3-kinase associated p85 mRNA sequence	ae61906
Translation initiation factors	
eukaryotic translation initiation factor 4E binding protein 1	bc006198
PKKC16 eukaryotic translation initiation factor 4E binding p	nc-20823967
Splicing factors	
splicing factor SC35	af90104
RNA localization machinery	
exportin 5 mRNA	af298880
Golgi and other organelles	
lysosomal acid phosphatase	bc003160
golgi membrane protein GP73	bc0017
vesicle soluble NSF attachment protein receptor VTI2	nc-112285061
lysyl hydroxylase isoform 2	nc-31095551
Amphiphysin	af1438
Cell-cycle/apoptosis machinery	
NIMA	bc000101
TAG1/PMEPA1	nc-109096298
histone deacetylase 8	aj277724
K-ras oncogene protein mRNA	bc010502
M3	bc012420
CYR61	y11307
myeloid cell leukemia sequence 1	l08246
Max-associated protein	af082740
Gas1	l13698
cyclin G1	x77794
RFP2	nc-53209250
cyclin G2 mRNA	u47414
BCL2-antagonist of cell death	u66879
Protein regulator of cytokinesis 1	bc003138
cyclin A	x68303
BCL7A protein	nc-145095861
retinoblastoma binding protein 2	af087482
death associated transcription factor 1	bc000770
cyclin-dependent kinase inhibitor 2B	bc014469
v-abl A related ras-3 gene	af462029
Oxygen-regulated protein ORP150	u65785
B-cell lymphoma 3-encoded protein	af01732
bcl-xL mRNA	nc-164453144
cyclin-dependent kinase inhibitor	af084716
Notch3	u97669



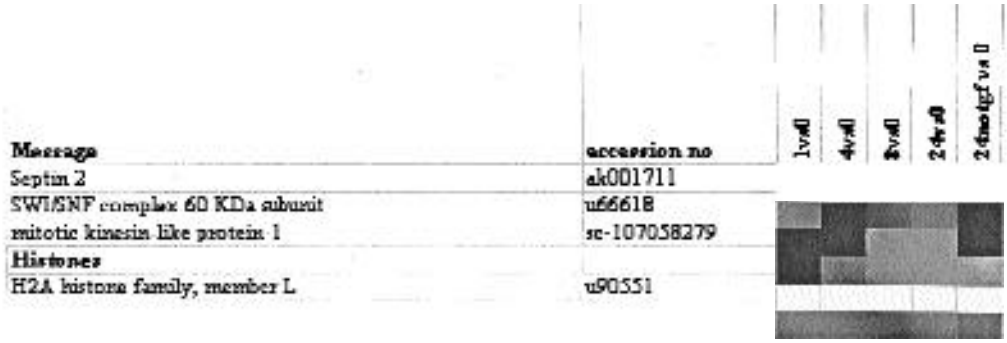


Fig. 2. Expression changes in annotated genes in response to TGFβ. Genes are classified according to percieved cellular function. Genes in italics are also listed in (14). Expression changes at 1, 4, 8 ,and 24 hours +TGFβ versus time = 0 are indicated at the right, followed by 24hr-TGFβ versus time = 0. The latter is presented as a control for the effects of serum starvation and cell culture. Values are shown as a heat map, from bright red indicating more than 4.5-fold upregulation to bright green indicating more than 4.5-fold downregulation.

response to TGFβ (19), including α subunits 1 through 5. The absence of calls from these molecules in the data set may reflect relatively low levels, in general, of integrin message. Indeed, they are notoriously difficult to detect by Northern analysis.

Intracellular signalling

Many of the key steps in intracellular signalling are phosphorylations, and therefore the direct interpretation of intracellular events by transcript profiling is impossible. Nonetheless, it is possible that the determination of which components of the signalling machinery are transcribed may be a guide to the pathways favored. There was a striking downregulation, by 24 hours, of MAPkinase kinase 3b, which is responsible for activating the p38 pathway. As p38 activation is a consequence of integrin alpha2 activation and collaborates with TGFβ in inducing collagen synthesis (20), it is likely that this downregulation is, like the even more dramatic downregulation of SMAD2, a consequence of negative feedback.

Our finding of SMAD2 downregulation contrasts recent studies in hepatic stellate cells, where the message was unchanged by TGFβ at 24 hours (21). It has been suggested that SMAD3, whose message was not changed in our study, is primarily responsible for fibrosis (22), whereas in some systems SMAD2 is more responsible for epithelial cell death (23) [and its loss is associated with tumor survival (24)]. It is possible therefore that the downregulation of SMAD2 but maintenance of SMAD3 reflects the death resistance of fibroblasts in response to TGFβ. No transcriptional upregulation of the inhibitory SMADs 7 and 8 was seen in the fibroblasts, in contrast to some epithelial cell types where their upregulation presumably contributes to a regulatory negative feedback loop (25). Another notable finding was the upregulation of Sprouty 4, an inhibitor of ras signalling that is upregulated in activated smooth muscle (26).

Metabolic pathway changes

A variety of metabolic enzymes were upregulated, undoubtedly reflecting the increased synthetic activity of the cells. One of the most interesting changes was in the ratio of transcript for the glutaminyl and methionyl tRNA synthetases, where the former was two-fold downregulated and the latter was two-fold upregulated. The average ratio of methionine to glutamine in proteins is approximately 0.5 (27), while actins, strongly upregulated in the contractile TGFβ treated fibroblast, have a met/glu ratio of ~1.5. Other metabolic changes included the upregulation of N-acetylglucosamide-(beta 1-4)-galactosyltransferase, presumably reflecting increased proteoglycan synthesis, and members of the pyruvate dehydrogenase complex. In concert with this were increases in expression of the water channel aquaporin 1, known to be induced during hypertonic stress (28), and the VLDLreceptor, which is capable of internalizing UPA-PAI-1 complexes (29).

Cytoskeletal changes

The fibrotic pathway proceeds along an axis which includes, in some (usually pathological) circumstances, a transition in the fibroblast phenotype into a more contractile, smooth muscle-like cell, the myofibroblast. Consistent with this was a striking upregulation in tropomysin 1 with a reciprocal downregulation of tropomyosin 3, as well as the upregulation of smooth muscle actin and filamin 2. Paxillin was notably downregulated, suggesting a reduction in cell motility (30) reciprocal to the increased contractility of the myofibroblast state. There was a striking downregulation of the protein periplakin. This protein appears to be predominantly an epidermal component, and may connect keratin filaments to the hemidesmosomal component collagen XVII, although it is known to be expressed at low levels in fibroblasts. Desmoplakin, usually regarded as a desmosomal component, was by contrast upregulated, as was the simple epithelial keratin, keratin 7. To our knowledge, expression of keratins has not been previously noted in fibroblasts. However, the canonical distributions of intermediate filaments have been challenged by “unexpected” observations; in particular, vimentin is expressed by epithelial cells in some inflammatory conditions (31).

(a)

Gene Id	Gene definition	GeneCalling result Five pooled samples	RT-PCR result Sample 1	Sample 2	Mean
gbh_l32137	Human cartilage oligomeric matrix protein (COMP) mRNA	2.68	2.83	2.57	2.7
gbh_af101079	*Homo sapiens* collagen type XI alpha-1 (COL11A1) gene	1.83	1.63	1.57	1.6
gbh_x77598	*Homo sapiens* LAMA3 mRNA	-2.08	2.33	2.33	2.33
gbh_af193507	*Homo sapiens* chemokine receptor (CCR11) gene	-4.07	nd	-5	-5
gbh_af013717	*Homo sapiens* periplakin (PPL) mRNA, partial cds	-9.12	-1.43	-3.125	-2

(b)

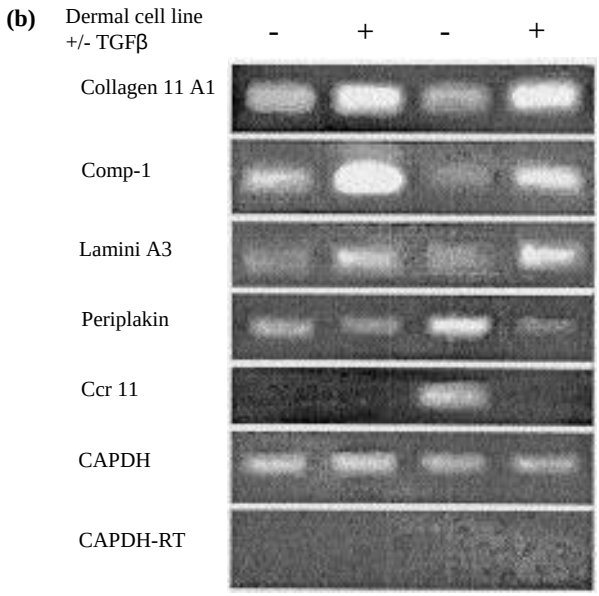


Fig. 3. Validation by RT-PCR. (**A**) Comparison of GeneCalling and RT-PCR data. Fold changes are expressed as log(2)[level treated/level untreated]. (**B**) Photograph of representative RT-PCR experiment, ethidium stained gel.

Transcriptional regulation

As described above the immediate-early transcription factor junB was rapidly upregulated and detected within 1 hour of TGFβ stimulation, as were ID1 and ID3, two anti-differentiation factors best characterized in the hematopoietic system. ID1 has been recently shown to be upregulated in TGFβ-treated embryonic fibroblasts, as well as in fibrotic myofibroblasts in experimental lung injury (32), and ID3 is known to be upregulated by TGFβ and is involved in the apoptotic pathway in lymphocytes (4). Alterations of Ets-1 mRNA [a known transcriptional activator of collagen synthesis (33)], were not detected.

Death, shock, and proliferation

A constellation of transcriptional events appeared to conspire to protect the fibroblasts from cell death. These included upregulation of the cytoprotective hsp70 family member ORP150; upregulation of the survival factor Bcl-XL and downregulation of BAD (which is pro-apoptotic by displacing bax from bcl-x), and upregulation of the survival factor and NFkappaB antagonist Bcl3 (34). One exception was the transient upregulation of DATF1, which has been shown to be death-inducing in mouse tissues.

On the other hand, a variety of transcripts were altered in a fashion consistent with cell cycle arrest, including the upregulation of cyclin-dependent kinase inhibitor 2B, a known TGFβ target, and downregulations of cyclins G and A. Thus, the proproliferative effect of TGFβ on fibroblasts cannot be accounted for by the data seen here.

Confirmation of results using semiquantitative RT-PCR

In general, the effects of TGFβ on fibroblasts that were highly consistent with those previously noted in the literature, with the upregulation of collagen I, connective tissue growth factor, and accessory matrix proteins. This gave us confidence in the less anticipated results. To augment our confidence further we performed RT-PCR quantitation for five of the unanticipated results on two independent fibroblast isolates from different individuals, treated with or without TGFβ for 24 hours (Fig. 3). Of the five, four were replicated with RT-PCR. The fifth, laminin A3, appeared to be upregulated by RT-PCR, but downregulated by gene calling. We ensured that the primers chosen were unique for laminin A3. The RT-PCR result seen with CCR11, where only one untreated sample appears to contain the transcript, suggests that GeneCalling (TM) is sufficiently sensitive to pick up changes which are only present in a subset of the pooled samples.

There have been a variety of other recent gene profiling studies of fibroblasts. Most relevant to this work are the studies of Verecchia *et al.* (14), and Chambers *et al.* (32). Our data overlap incompletely with the findings of Verecchia *et al.*, who used a nylon-based array. Among the genes in agreement are collagens I, III and XVI, versican, perlecan, sparc, thrombospondin, fibronectin and PAI-1, but several genes upregulated in their study, including MMP1 and proto-cadherin γ3 were downregulated in ours. Differences in general methodology and the fibroblast source may account for this. Our find-

ings of upregulation in ID1 and ID3 concur with the most striking observation of Chambers *et al*. (32).

Conclusions

Our studies demonstrate a change in the transcriptional repertoire of fibroblasts following TGFβ treatment. The changes seen are coherent and consistent with the previously reported effects of TGFβ on fibroblast metabolism. In general, the changes seen reflected the promotion of matrix protein synthesis, changes in cytoskeletal protein mRNAs compatible with myofibroblast transformation, and the upregulation of anti-apoptotic proteins. The latter findings are consistent with our previous studies which show that TGFβ induces resistance to apoptosis in dermal fibroblasts (2).

It is interesting that fibroblasts treated with TGFβ demonstrate a similar mRNAprofile to that of fibroblasts isolated from tissues of patients with fibrotic disease. Thus, fibroblasts from patients with scleroderma demonstrate increased mRNA for collagen and fibronectin, increased myofibroblast transformation and resistance to apoptosis. A more extensive comparison of the profile of TGFβ-treated fibroblasts with fibroblasts from pathologic fibrosis will elucidate whether TGFβ alone is responsible for phenotypic transformation in these disorders.

TGFβ induces fibroblast proliferation; nonetheless, at least at the 24-hour time point, most transcriptional changes appear to favor cytostasis over proliferation. Therefore the well established proliferative response of fibroblasts to TGFβ must be accounted for by later and possibly indirect events. Like many other transcription profiling studies, this one has revealed the presence and regulation of a variety of transcripts hitherto associated with other tissue types, particularly “neural” and “epithelial” genes such as semaphorin and claudin. It seems likely that many signals known to regulate neurons are also used by fibroblasts.

In summary, the effects of TGFβ on fibroblasts appear to be prosynthetic, while being protective against many apoptotic signals and pathways usually associated with inflammation and the acute phase response. A comparison of these results with the effects of TGFβ on epithelial cells, endothelial cells and smooth muscle cells will likely be very instructive and provide insight into mechanisms of both wound healing and pathologic fibrosis.

References

1. DE FOUGEROLLES AR, CHI-ROSSO G, BAJARDI A, GOTWALS P, GREEN CD, KOTELIANSKY VE: Global expression analysis of extracellular matrix-integrin interactions in monocytes. *Immunity* 2000; 13: 749-58.
2. JELASKA A, KORN JH: Role of apoptosis and transforming growth factor beta1 in fibroblast selection and activation in systemic sclerosis. *Arthritis Rheum* 2000; 43: 2230-9.
3. SHIMKETS RA, LOWE DG, TAI JT *et al.*: Gene expression analysis by transcript profiling coupled to a gene database query. *Nat Biotechnol* 1999; 17: 798-803.
4. KEE BL,RIVERA RR,MURRE C: Id3 inhibits B lymphocyte progenitor growth and survival in response to TGF-beta. *Nat Immunol* 2001; 2: 242-7.
5. LING MTWX, TSAO SW, WONG YC: Down-regulation of Id-1 expression is associated with TGF beta 1-induced growth arrest in prostate epithelial cells. *Biochem Biophys Acta* 2002; 1570: 145-52.
6. BELLETTI B, DRAKAS R, MORRIONE A *et al.*: Regulation of Id1 protein expression in mouse embryo fibroblasts by the type 1 insulin-like growth factor receptor. *Exp Cell Res* 2002; 277: 107-18.
7. KANAMARU C, YASUDAH, FUJITA T: Involvement of Smad proteins in TGF-beta and activin A-induced apoptosis and growth inhibition of liver cells. *Hepatol Res* 2002; 23: 211-9.
8. HARPER JR, SPIRO RC, GAARDE WA *et al.*: Role of transforming growth factor beta and decorin in controlling fibrosis. *Methods Enzymol* 1994; 245: 241-54.
9. DODGE GR, HAWKINS D, BOESLER E, SAKAI L, JIMENEZ SA: Production of cartilage oligomeric matrix protein (COMP) by cultured human dermal and synovial fibroblasts. *Osteoarthritis Cartilage* 1998; 6: 435-40.
10. HECHT JT, NELSON LD, CROWDER E *et al.*: Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia. *Nat Genet* 1995; 10: 325-9.
11. YUAN W, VARGA J: Transforming growth factor-beta repression of matrix metalloproteinase- 1 in dermal fibroblasts involves Smad3. *J Biol Chem* 2001; 276: 38502-10.
12. HATTORI N, DEGEN JL, SISSON TH *et al.*: Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. *J Clin Invest* 2000; 106: 1341-50.
13. CHANG HY, CHI JT, DUDOIT S *et al.*: Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci USA* 2002; 99: 12877-82.
14. VERRECCHIA F, PESSAH M, ATFI A, MAUVIEL A: Tumor necrosis factor-alpha inhibits transforming growth factor-beta/Smad signaling in human dermal fibroblasts via AP-1 activation. *J Biol Chem* 2000; 275: 30226-31.
15. CHANG NS: Hyaluronidase enhancement of TNF-mediated cell death is reversed by TGF-beta 1. *Am J Physiol* 1997; 273(6 Pt. 1): C1987-94.
16. SOMA Y, MIZOGUCHI M, YAMANE K *et al.*: Specific inhibition of human skin fibroblast chemotaxis to platelet-derived growth factor A-chain homodimer by transforming growth factor-beta1. *Arch Dermatol Res* 2002; 293: 609-13.
17. VELLING T, KUSCHE-GULLBERG M, SEJERSEN T, GULLBERG D: cDNA cloning and chromosomal localization of human alpha-(11) integrin. A collagen-binding, I domain-containing, beta(1)-associated integrin alpha-chain present in muscle tissues. *J Biol Chem* 1999; 274: 25735-42.
18. KAMATA T, TAKADA Y: Direct binding of collagen to the I domain of integrin alpha 2 beta 1 (VLA-2, CD49b/CD29) in a divalent cation-independent manner. *J Biol Chem* 1994; 269: 26006-10.
19. HEINO J, IGNOTZ RA, HEMLER ME, CROUSE C, MASSAGUE J: Regulation of cell adhesion receptors by transforming growth factor-beta. Concomitant regulation of integrins that share a common beta 1 subunit. *J Biol Chem* 1989; 264: 380-8.
20. CHIN BY, MOHSENIN A, LI SX, CHOI AM, CHOI ME: Stimulation of pro-alpha(1)(I) collagen by TGF-beta(1) in mesangial cells: role of the p38 MAPK pathway. *Am J Physiol Renal Physiol* 2001; 280: F495-504.
21. LIU C, GACA MD, SWENSON ES, VELLUCCI VF, REISS M, WELLS RG: Smads 2 and 3 are differentially activated by TGF-beta in quiescent and activated hepatic stellate cells: Constitutive nuclear localization of Smads in activated cells is TGF-beta independent. *J Biol Chem* 2003; 22: 22.
22. ROBERTS AB, PIEK E, BOTTINGER EP, ASHCROFTG,MITCHELLJB,FLANDERSKC: Is Smad3 a major player in signal transduction pathways leading to fibrogenesis ? *Chest* 2001; 120 (Suppl. 1): 43S-47S.
23. CHEN W, WOODRUFF TK, MAYO KE: Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins. *Endocrinology* 2000; 141: 1263-72.
24. TSANG KJ, TSANG D, BROWN TN, CROWE DL: A novel dominant negative Smad2 mutation in a TGF-beta resistant human carcinoma cell line. *Anticancer Res* 2002; 22 (1A): 13-9.
25. DENISSOVA NG, POUPONNOT C, LONG J, HE D, LIU F: Transforming growth factor beta-inducible independent binding of SMAD to the Smad7 promoter. *Proc Natl Acad Sci USA* 2000; 97: 6397-402.
26. LEEKSMA OC, VAN ACHTERBERG TA, TSUMURA Y *et al.*: Human sprouty 4, a new ras antagonist on 5q31, interacts with the dual specificity kinase TESK1. *Eur J Biochem* 2002; 269: 2546-56.
27. ECHOLS N, HARRISON P, BALASUBRAMANIAN S *et al.*: Comprehensive analysis of amino acid and nucleotide composition in

eukaryotic genomes, comparing genes and pseudogenes. *Nucleic Acids Res* 2002; 30: 2515-23.

28. LEITCH V, AGRE P, KING LS: Altered ubiquitination and stability of aquaporin-1 in hypertonic stress. *Proc Natl Acad Sci USA* 2001; 98: 2894-8.
29. ARGRAVES KM, BATTEY FD, MACCALMAN CD *et al.*: The very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase-plasminogen activator inhibitor type I complexes. *J Biol Chem* 1995; 270: 26550-7.
30. SCHALLER MD: Paxillin: a focal adhesion-associated adaptor protein. *Oncogene* 2001; 20: 6459-72.
31. VAN DER VELDEN LA, MANNI JJ, RAMAEKERS FC, KUIJPERS W: Expression of intermediate filament proteins in benign lesions of the oral mucosa. *Eur Arch Otorhinolaryngol* 1999; 256: 514-9.
32. CHAMBERS RC, LEONI P, KAMINSKI N, LAURENT GJ, HELLER RA: Global expression profiling of fibroblast responses to transforming growth factor-beta(1) reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. *Am J Pathol* 2003; 162: 533-46.
33. CZUWARA-LADYKOWSKA J, SHIRASAKI F, JACKERS P, WATSON DK, TROJANOWSKA M: Fli-1 inhibits collagen type I production in dermal fibroblasts via an Sp1-dependent pathway. *J Biol Chem* 2001; 276: 20839-48.
34. MITCHELL TC, HILDEMAN D, KEDL RM *et al.*: Immunological adjuvants promote activated T cell survival via induction of Bcl-3. *Nat Immunol* 2001; 2: 397-402.