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ScienceDirect
Matrix Biology 26 (2007) 625–632
MATRIX BIOLOGY
www.elsevier.com/locate/matbio

Endogenous endothelin-1 signaling contributes to type I collagen and CCN2 overexpression in fibrotic fibroblasts

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Received 16 January 2007; received in revised form 6 June 2007; accepted 7 June 2007

Abstract

Fibrosis is excessive scarring caused by the accumulation of extracellular matrix proteins and is a common end pathway in many chronic diseases. Endothelin-1 is a possible contributor to the persistent fibrotic phenotype of fibroblasts isolated from fibrotic lesions. In this report we used a specific dual endothelin A/B receptor antagonist, bosentan, to determine the role of endogenous endothelin signaling in maintaining the profibrotic phenotype of lung fibroblasts from scleroderma patients. Bosentan treatment of lung fibroblasts cultured from normal individuals and individuals with scleroderma was assessed using Affymetrix genome-wide expression profiling, real-time polymerase chain reaction and Western blot analysis and revealed that approximately one-third of the transcripts elevated greater than two-fold in fibrotic fibroblasts were reduced by Bosentan treatment. Genes whose overexpression in fibrotic fibroblasts that were dependent on endogenous endothelin signaling included the matrix or matrix-associated genes type I collagen, fibronectin and CCN2. The elevated adhesive property of fibrotic fibroblasts was also reduced by endothelin receptor antagonism. Basal expression of collagen, fibronectin and CCN2 and adhesion to matrix was not affected. Thus endogenous endothelin signaling contributes to the fibrotic phenotype of fibrotic fibroblasts, suggesting that antagonizing endothelin receptors may be of benefit in combating fibrotic disease.

Keywords: Fibrosis; Connective tissue; Fibroblast; Endothelin; CCN2; Type I collagen; Adhesion

1. Introduction

The normal tissue repair program requires the *de novo* production of connective tissue, which is comprised of mesenchymal cells and extracellular matrix (ECM). Should the repair program be appropriately terminated, normal tissue function is essentially restored. However, if the tissue repair program continues unabated, excessive production and contraction of can ECM occurs, resulting in pathological scarring. Scars are characterized by the presence of a specialized form of fibroblast, termed the myofibroblast (Desmouliere, 1995; Harrison et al., 1991), which is responsible both for normal tissue repair and also the excessive production and contraction of extracellular matrix (ECM) within fibrotic lesions (Desmouliere, 1995; Harrison et al., 1991). Excessive scarring can, in turn, result in pathological fibrotic diseases, which can culminate in organ failure and death.

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0945-053X/$ - see front matter © 2007 Published by Elsevier B.V./International Society of Matrix Biology.
doi:10.1016/j.matbio.2007.06.003

Table 1
mRNAs increased >2-fold in FASSc lung fibroblasts also inhibited >2-fold by bosentan. Average expression values compared to expression in normal fibroblasts is shown

Affymetrix ID	Gene name		Fold up In FASSc	Bosentan inhibition in normal	Bosentan inhibition in FASSc
Cell/cell cell/matrix adhesion					
215646_s_at	Versican	CSPG2	3.9	1.1	0.5
209101_at	Connective tissue growth factor (CCN2)	CTGF	7.8	1.0	0.5
214702_at	Fibronectin 1	FN1	2.6	1.2	0.5
203440_at	*N*-cadherin	CDH2	4.0	1.2	0.4
Signalling molecules					
204790_at	SMAD 7	SMAD7	3.2	1.2	0.5
206796_at	WNT1 inducible signaling pathway protein 1	WISP1	2.1	0.8	0.4
205020_s_at	ADP-ribosylation factor-like 4A	ARL4A	2.2	1.0	0.5
212706_at	RAS p21 protein activator 4	RASA4	2.0	1.4	0.5
222077_s_at	Rac GTPase activating protein 1	RACGAP1	2.7	1.1	0.3
221246_x_at	Tensin	TNS	2.1	1.0	0.5
212977_at	Chemokine orphan receptor 1	CMKOR1	2.9	0.9	0.5
219179_at	Dapper homolog 1	DACT1	6.5	1.1	0.4
217979_at	Transmembrane 4 superfamily member 13	TM4SF13	6.5	1.3	0.2
Matrix production, metabolism					
202619_s_at	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	PLOD2	2.5	0.9	0.5
202620_s_at	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	PLOD2	3.1	1.0	0.5
202311_s_at	Collagen, type I, alpha 1	COL1A1	2.1	0.9	0.3
209765_at	A disintegrin and metalloproteinase domain 19	ADAM19	3.3	1.4	0.5
201262_s_at	Biglycan	BGN	2.7	1.1	0.5
202627_s_at	Serine (or cysteine) proteinase inhibitor, member 1	SERPINE1	5.9	0.9	0.3
202628_s_at	Serine (or cysteine) proteinase inhibitor, member 1	SERPINE1	7.0	0.9	0.4
202952_s_at	A disintegrin and metalloproteinase domain 12	ADAM12	2.2	0.9	0.4
Cytoskeletal genes					
206116_s_at	Tropomyosin 1 (alpha)	TPM1	5.3	1.0	0.5
206117_at	Tropomyosin 1 (alpha)	TPM1	3.8	1.0	0.5
210986_s_at	Tropomyosin 1 (alpha)	TPM1	3.9	1.1	0.4
210987_x_at	Tropomyosin 1 (alpha)	TPM1	4.4	0.9	0.5
200974_at	Actin, alpha 2, smooth muscle, aorta	ACTA2	3.7	0.7	0.4
203243_s_at	LIM protein	LIM	3.2	1.3	0.4
Development and differentiation					
207030_s_at	Cysteine and glycine-rich protein 2	CSRP2	2.4	1.6	0.3
211126_s_at	Cysteine and glycine-rich protein 2	CSRP2	2.3	1.5	0.4
Metabolism					
203159_at	Glutaminase	GLS	2.9	1.3	0.5
221510_s_at	Glutaminase	GLS	2.2	1.1	0.4
208447_s_at	Phosphoribosyl pyrophosphate synthetase 1	PRPS1	3.1	1.0	0.4
209440_at	Phosphoribosyl pyrophosphate synthetase 1	PRPS1	3.0	0.9	0.3
201037_at	Phosphofructokinase, platelet	PFKP	2.8	0.9	0.5
201830_s_at	Neuroepithelial cell transforming gene 1	NET1	3.0	1.0	0.5
219773_at	NADPH oxidase 4	NOX4	4.5	1.0	0.4
210041_s_at	Phosphoglucomutase 3	PGM3	2.2	1.2	0.5
203810_at	DnaJ (Hsp40) homolog, subfamily B, member 4	DNAJB4	2.3	1.1	0.4
201242_s_at	ATPase, Na+/K+ transporting, beta 1 polypeptide	ATP1B1	2.7	1.0	0.3
Cytokines					
206924_at	Interleukin 11	IL11	7.8	0.8	0.4
Transcription factors					
208937_s_at	Inhibitor of DNA binding 1	ID1	24.1	1.2	0.2
201565_s_at	Inhibitor of DNA binding 2	ID2	2.8	1.1	0.4
207826_s_at	Inhibitor of DNA binding 3	ID3	18.6	1.7	0.1
202393_s_at	TGFB inducible early growth response	TIEG	2.7	1.0	0.3
201170_s_at	Basic helix-loop—helix domain containing, class B, 2	BHLHB2	3.6	1.7	0.4
209291_at	Inhibitor of DNA binding 4	ID4	2.9	1.0	0.3
201862_s_at	Leucine rich repeat (in FLII) interacting protein 1	LRRFIP1	2.6	1.2	0.4