Genomics 94 (2009) 101–109

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Differences in gene expression profiles from asbestos-treated SPARC-null and wild-type mouse lungs

Mark A. Pershouse, Aubrey M. Smartt, Corbin Schwanke, Elizabeth A. Putnam *

Center for Environmental Health Sciences, Department of Biomedical and Pharmaceutical Sciences, The University of Montana, 32 Campus Dr., Missoula MT 59812-1552, USA

ARTICLE INFO

Article history:
Received 7 November 2008
Accepted 30 April 2009
Available online 13 May 2009

Keywords:
Knockout mice
Osteonectin
SPARC
Hevin
Microarray analysis
Asbestos
Amphibole
Fibrosis

ABSTRACT

The role of SPARC in the in vivo lung response to crocidolite asbestos was addressed by instillation of crocidolite asbestos in a series of wild-type or SPARC-null mice. Animals were sacrificed at one week, one month, and three months post-instillation to assess the impact of SPARC on multiple stages in the development of fibrosis. RNA was harvested from 10 animals/time point, pooled, and used to probe a mouse array containing ~10,000 probes. Gene expression data were analyzed for fold change, and for broader functional group alterations. As expected, the one-week time point displayed alterations in genes involved in immune recognition, energy utilization, and growth factor production. Later time points showed expression alterations for genes involved in protein degradation, Wnt receptor signaling, membrane protein activity, and transport. Molecules in the Wnt pathway have been implicated in bone growth, mediation of fibroblast activity, and have been directly linked to SPARC regulation.

Introduction

As a member of the matricellular class of secreted proteins, SPARC (a *s*ecreted *p*rotein *a*cidic and *r*ich in *c*ysteine) is involved in the regulation of extracellular matrix (ECM) – cellular interactions. SPARC has been localized to fibroblasts found in idiopathic pulmonary fibrosis [1]. After embryogenesis, SPARC is most often expressed in tissues undergoing remodeling [1,2]. SPARC is a calcium-binding glycoprotein that interacts with many ECM components including the fibrillar collagens (types I, II, III, and V) as well as type IV, thrombospondin1 (THBS1), vitronectin (VTN), and fibrinogen fragments D and E [3,4]. SPARC has been shown to be a modulator of growth factors involved in fibrosis and has also been implicated in the development of cancers [5–7]. These characteristics may be linked to the activities of SPARC that include stimulating the TGF-β signaling system, serving as a target for and/or inducer of matrix metalloproteinases, and modulating integrin-linked kinase activity [5,8,9]. It has also been shown that SPARC interacts with the TGF-β-receptor complex to modulate phosphorylation of Smad-2 (SMAD2), to affect the activity of JNK (MAPK8), and to increase the expression of c-*jun* (Jun) [10]. More recently, SPARC has been shown to interact with Stabilin-1 (STAB1), a scavenger receptor expressed on alternatively-activated tissue macrophages and sinusoidal endothelial cells [11]. This interaction regulates the extracellular concentration of SPARC and thus potentially modulates ECM remodeling.

The 32 kDa SPARC protein is a member of a gene family with structural similarities in the arrangements of the protein modules. Other matricellular proteins in this family include SC1/Hevin (SPARCL1), QR1 (NQO1), and Testican (SPOCK1) [12,13]. The protein domains include a N-terminal acidic domain that contains a low-affinity calcium-binding domain and a transglutaminase cross-linking site. This domain has been shown to inhibit cell spreading. The central domain of the protein (aa 50–130) is similar to follistatin (FST), and characteristically acts by inhibiting proliferation and abrogating focal adhesions; however, release of an internal peptide ([K]GHK) stimulates proliferation and angiogenesis. The C-terminal domain (E–C) contains a high affinity calcium binding E–F hand domain that inhibits cell spreading and proliferation, and is responsible for binding to cells and matrix [3,12].

SPARC-null mice were developed independently by two groups [14,15]. These mice are born phenotypically normal but develop early cataracts, kinked tails, osteopenia and increased adipose tissue. SPARC-null mice also demonstrate enhanced growth and metastasis of implanted tumors, accelerated closure of cutaneous wounds, collagen fibrils with smaller and more regular diameters and a diminished ability to encapsulate subcutaneously implanted foreign bodies (see Framson and Sage [6] for review). It is unknown how the lung will respond to asbestos exposures in the absence of SPARC. Our investigations are designed to discover

* Corresponding author. Fax: +1 406 243 5228.
E-mail address: elizabeth.putnam@umontana.edu (E.A. Putnam).

0888-7543/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2009.04.009

Table 1
Number of gene transcripts altered >2 fold for each condition.

Mouse type	Exposure length	1 wk	1 month	3 month
WT	Up	59	31	44
	Down	3	3	21
KO	Up	24	2	7
	Down	88	57	35

the role SPARC plays in the development of lung fibrosis in response to asbestos exposure by examining gene expression in lungs derived from asbestos-exposed SPARC-null and wild-type mice. The specific analyses in this report target those genes or classes of genes in mouse lung tissue that change their expression during exposure to asbestos, with or without the presence of SPARC. This approach would logically describe a set of genes that can only be activated by the combination of SPARC activity and asbestos exposure.

Results and discussion

The central questions in this study center concern 1) the types and magnitude of gene expression changes in SPARC-null mouse lungs versus wild-type mouse lungs and 2) the types and magnitude of changes seen in SPARC-null mouse lung following the stress induced by asbestos exposure. SPARC-null and wild-type mice were exposed to saline or crocidolite asbestos for periods of one week, one month, or three months. Animals were euthanized and lungs were removed and submitted for RNA expression analysis. Microarray analysis was performed in a standard test versus reference RNA co-hybridization on MWG 10K Group A mouse oligo-based arrays. The use of reference RNA allowed the comparison of values from multiple time points and exposure conditions. Ten mice were pooled from each treatment group for each data point. Pooling tends to obscure subtle gene expression changes as the variance of ten biological specimens (mice) generates a high baseline of transcriptional noise. Typically only those expression changes that are of a large magnitude are seen in pooled data. In order to focus primarily on genes that reproducibly were altered, we performed two sequential threshold or filter steps. Three replicate arrays were analyzed for each experiment and mean and standard deviation was established for each gene. Genes achieving a significant ($p<0.01$) difference in expression compared to the saline control experiments were further analyzed. An arbitrary cutoff of two-fold up or down regulation (ratio of <0.50 or >2) was used to further filter the data to delineate genes that were highly activated or suppressed by asbestos exposure. A general description of the numbers of genes meeting these criteria is delineated in Table 1. The largest sets of gene expression alterations occurred early (one week) in both wild-type and SPARC-null mice, reflecting an acute response, but significantly more changes occurred in SPARC-null mice than the wild-type mice at this time point (112 genes up or down in SPARC-null vs. 62 in wild-type mice). Smaller sets of gene expression changes take place at the one month and three month time points (Table 1). The resulting sets of gene transcripts for each time point and condition are listed in Tables 2–4. The datasets are from one-week exposures of crocidolite vs. saline in both wild-type and SPARC-null mice (Table 2), one-month exposures of crocidolite vs. saline in both wild-type and SPARC-null mice (Table 3), and three-month exposures of crocidolite vs. saline in both wild-type and SPARC-null mice (Table 4). Table 5 presents transcripts that were altered in expression level in SPARC-null mice versus wild-type mice without the addition of asbestos. This represents the steady state gene expression differences within the lung and a potential list of genes related to constitutive SPARC function. Genes with

Table 2

A. Genes altered in expression after 1 wk exposure to crocidolite vs saline in WT mice

Name	Symbol	ID	Entrez GeneID	Cro/sal ratio
IQ motif-containing E	Iqce	AK006472	74239	5.26
RIKEN cDNA 2610110G12 gene	2610110G12Rik	AK002569	73242	4.57
Nurim (nuclear envelope membrane protein)	Nrm	AK017439	106582	4.31
CDK5 regulatory subunit associated protein 1	Cdk5rap1	NM_025876	66971	4.14
Keratin 85	Krt85	AY028606	114566	3.90
RIKEN cDNA 1200014M14 gene	1200014M14Rik	NM_026173	67463	3.66
La ribonucleoprotein domain family, member 6	Larp6	NM_026235	67557	3.55
Zinc finger protein 143	Zfp143	NM_009281	20841	3.54
Tripartite motif protein 21	Trim21	NM_009277	20821	3.53
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit F	Atp5j	NM_016755	11957	3.52
Phosphatidylinositol transfer protein, beta	Pitpnb	NM_019640	56305	3.40
Lactalbumin, alpha	Lalba	NM_010679	16770	3.33
5-hydroxytryptamine (serotonin) receptor 5A	Htr5a	NM_008314	15563	3.32
Epidermal growth factor receptor pathway substrate 8	Eps8	NM_007945	13860	3.32
Regulator of G-protein signaling 5	Rgs5	NM_009063	19737	3.29
Mitogen activated protein kinase 8	Mapk8	NM_016700	26419	3.26
Eph receptor A4	Epha4	NM_007936	13838	3.23
Zinc finger protein 706	Zfp706	AB041652	68036	3.21
RIKEN cDNA 1700010I14 gene	1700010I14Rik	NM_025851	66931	3.08
Wild-type p53-induced gene 1	Zmat3	NM_009517	22401	2.98
V-set and transmembrane domain containing 2B	Vstm2b	NM_021387	58188	2.95
5′-nucleotidase domain containing 2	Nt5dc2	AK010966	70021	2.95
Neugrin, neurite outgrowth associated	Ngrn	NM_031375	83485	2.88
Phosphoenolpyruvate carboxykinase 2 (mitochondrial)	Pck2	BC023437	74551	2.80
ELK4, member of ETS oncogene family	Elk4	NM_007923	13714	2.78
Ribonuclease H2, subunit C	Rnaseh2c	NM_026616	68209	2.70
Transmembrane protein 101	Tmem101	BC011109	76547	2.67
TLC domain containing 1	Tlcd1	BC005702	68385	2.65
RIKEN cDNA 5730559C18 gene	5730559C18Rik	NM_028872	67313	2.61
Ubiquitin carboxy-terminal hydrolase L1	Uchl1	NM_011670	22223	2.52
RIKEN cDNA 2700049A03 gene	2700049A03Rik	AK012399	76967	2.47
RIKEN cDNA 4930404N11 gene	4930404N11Rik	AK015088	432479	2.43
Succinate-Coenzyme A ligase, GDP-forming, beta subunit	Suclg2	AF058956	20917	2.39
BarH-like 1 (*Drosophila*)	Barhl1	NM_019446	54422	2.38
Ectodermal-neural cortex 1	Enc1	NM_007930	13803	2.31
Interleukin 1 receptor accessory protein	Il1rap	NM_008364	16180	2.30
Calcium homeostasis endoplasmic reticulum protein	Cherp	AK017525	27697	2.28
CAMP-regulated phosphoprotein 19	Arpp19	NM_021548	59046	2.24
Purinergic receptor P2X, ligand-gated ion channel, 1	P2rx1	NM_008771	18436	2.24
Zinc finger protein of the cerebellum 4	Zic4	NM_009576	22774	2.24
CD68 antigen	Cd68	NM_009853	12514	2.24
Poly A binding protein, cytoplasmic 1	Pabpc1	AK017655	66978	2.22
Tumor-associated calcium signal transducer 2	Tacstd2	NM_020047	56753	2.18
Tripartite motif-containing 54	Trim54	NM_021447	58522	2.17
Protein arginine N-methyltransferase 3	Prmt3	AK008118	71974	2.15
RIKEN cDNA 1600016N20 gene	1600016N20Rik	AK005476	72000	2.15
Proprotein convertase s ubtilisin/kexin type 1 inhibitor	Pcsk1n	NM_013892	30052	2.15
Dynamin 2	Dnm2	NM_010561	16201	2.13
GA repeat binding protein, beta 2	Gabpb2	AK020406	213054	2.12