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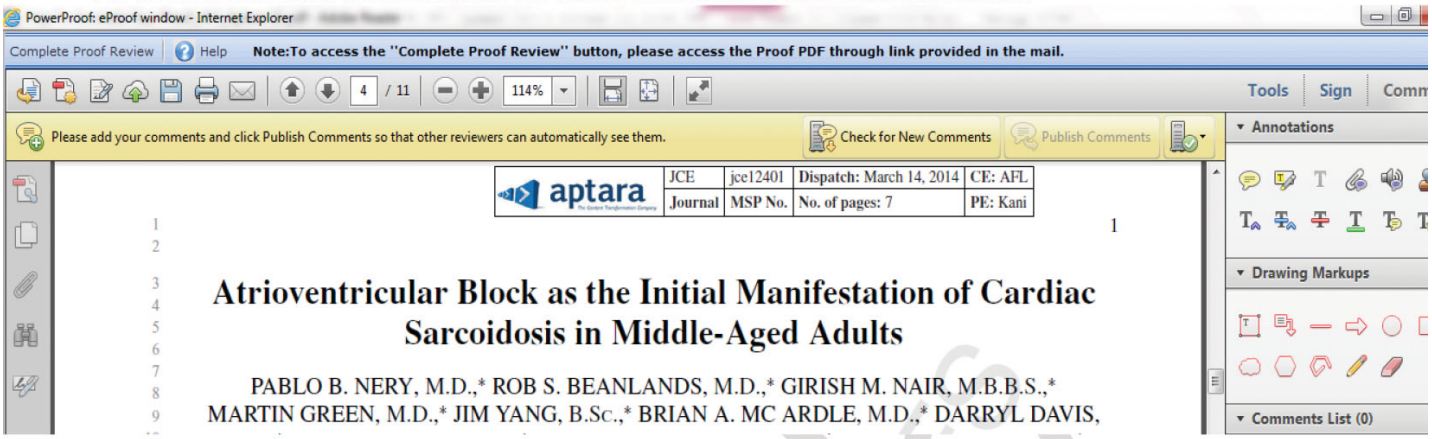

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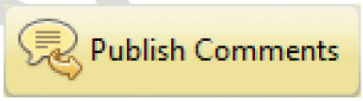

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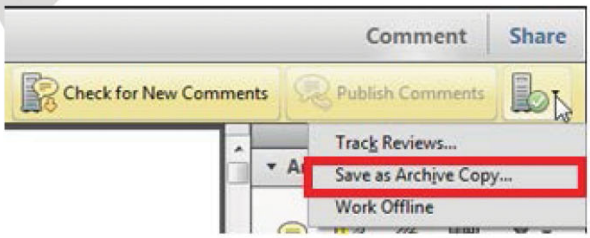

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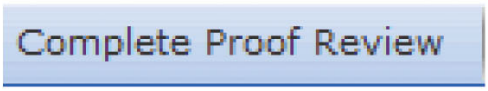

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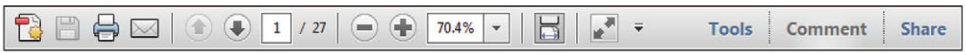

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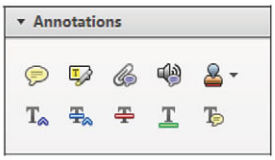

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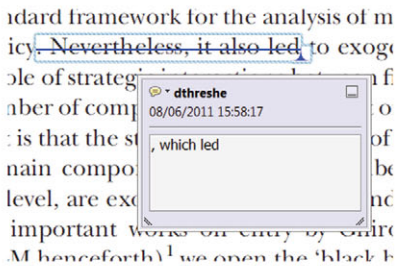

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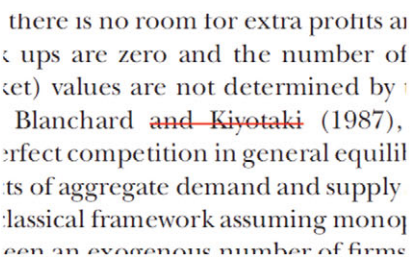

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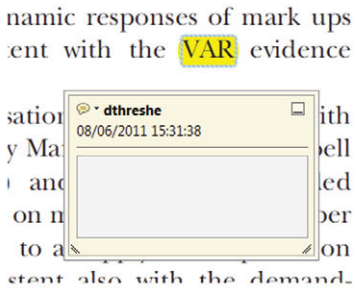

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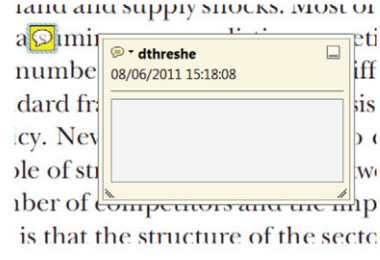

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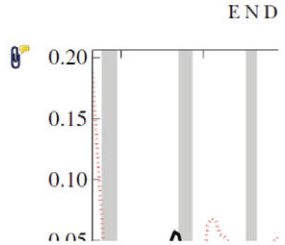

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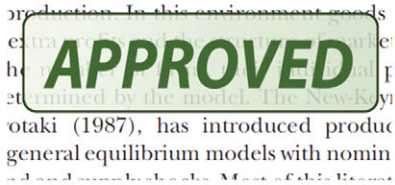

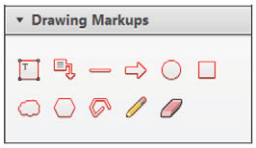

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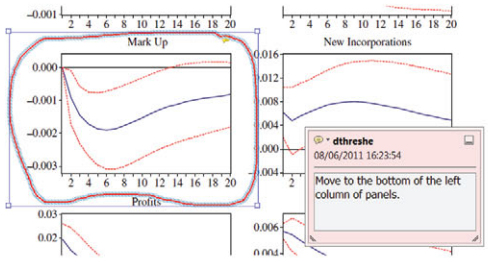

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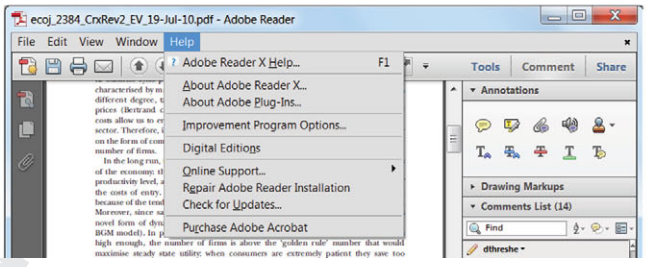

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RESEARCH ARTICLE

Cigarette smoke alters the secretome of lung epithelial cells

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Cigarette smoke is the most relevant risk factor for the development of lung cancer and chronic obstructive pulmonary disease. Many of its more than 4500 chemicals are highly reactive, thereby altering protein structure and function. Here, we used subcellular fractionation coupled to label-free quantitative MS to globally assess alterations in the proteome of different compartments of lung epithelial cells upon exposure to cigarette smoke extract. Proteomic profiling of the human alveolar derived cell line A549 revealed the most pronounced changes within the cellular secretome with preferential downregulation of proteins involved in wound healing and extracellular matrix organization. In particular, secretion of secreted protein acidic and rich in cysteine, a matricellular protein that functions in tissue response to injury, was consistently diminished by cigarette smoke extract in various pulmonary epithelial cell lines and primary cells of human and mouse origin as well as in mouse ex vivo lung tissue cultures. Our study reveals a previously unrecognized acute response of lung epithelial cells to cigarette smoke that includes altered secretion of proteins involved in extracellular matrix organization and wound healing. This may contribute to sustained alterations in tissue remodeling as observed in lung cancer and chronic obstructive pulmonary disease.

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Cigarette smoke / Lung / Proteomic profiling / Secretome

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

According to the World Health Organization (2015), around 6 million deaths are attributable annually to tobacco-related diseases [1]. Tobacco smoking is the most relevant risk factor for a variety of lung diseases, including lung cancer and chronic obstructive pulmonary disease (COPD). Cigarette smoke contains more than 4500 chemicals, many of which

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Q4 **Abbreviations**: **COPD**, chronic obstructive pulmonary disease; **CSE**, cigarette smoke extract; **ECM**, extracellular matrix; **HBEC**, human bronchial epithelial cell; **MMP2**, matrix metalloproteinase-2; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **PGRN**, progranulin; **SPARC**, secreted protein acidic and rich in cysteine; **TGF**, transforming growth factor; **TIMP**, tissue inhibitor of metalloproteinases

*These authors contributed equally to this work.

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Significance of the study

According to the World Health Organization, around 6 million deaths/year are attributable to tobacco-related diseases among them lung cancer and chronic obstructive pulmonary disease. More than 4500 reactive compounds are found in cigarette smoke, which potentially alter protein structure, abundance, and function. Here, we analyzed the proteomic changes induced by cigarette smoke in different compartments of lung epithelial cells. Subcellular fractionation coupled to label-free quantitative MS revealed that the most pronounced changes in lung alveolar cells are observed within the cellular secretome. In particular, we identified differential regulation of several proteins involved in wound-healing responses and extracellular matrix organization, among them secreted protein acidic and rich in cysteine, a matricellular protein that functions in tissue response to injury. A similar but distinct response was observed in bronchial epithelial cells. This acute response of lung epithelial cells to cigarette smoke involves altered secretion of proteins involved in extracellular matrix organization and wound-healing responses may contribute to sustained alterations in tissue remodeling as observed in lung cancer and chronic obstructive pulmonary disease.

are free radicals that act strongly oxidizing, pro-inflammatory, and carcinogenic [2, 3]. These effects are most pronounced in the pulmonary epithelium, the first barrier of the lung. Cigarette smoke easily interacts with DNA, lipids, and proteins and modifies them. In particular, cigarette smoke affects expression and/or posttranslational modifications of proteins, thereby altering their structure, abundance, and function [3, 4].

Several proteomic studies have been applied to human lung tissues and cells in order to identify and quantify protein alterations caused by exposure to cigarette smoke [3]. Most of these studies involved comparative 2D gel electrophoresis coupled with MS. Some studies analyzed sputum, bronchoalveolar lavage or epithelial lining fluids of nonsmokers, healthy smokers, and COPD subjects identifying alterations in mucin proteins and peptidase regulators [5], differential regulation of proteins involved in tumor growth and invasions [6], or in oxidative and inflammatory responses [5–9]. Proteomic analysis of lung tissue from nonsmokers, current smokers, and ex-smokers revealed cigarette smoke mediated induction of an unfolded protein response [10]. Although several proteomic studies have been conducted on pulmonary human fibroblasts exposed to cigarette smoke extract (CSE) [11] and on bronchial airway epithelium from current and never smokers [12], little is known on the effect of cigarette smoke on alveolar epithelial cells.

In this study, we investigated the effects of cigarette smoke on the proteome of A549 human airway epithelial cells by performing cellular fractionation coupled with label-free quantitative MS (i.e. LC-MS/MS). Subcellular fractionation enabled detection of proteins of low abundance, thus allowing for improved sensitivity and identification of proteins that would otherwise be difficult to identify and quantify in a complex cellular extract. Moreover, cellular fractionation also provides unique data on compartment-specific alterations of protein expression. This information can be particularly valuable as it allows identifying proteins that may shuttle between one subcellular compartment and the other upon exposure of cells to cigarette smoke.

2 Materials and methods

2.1 Cell culture

A549 (human adenocarcinoma cell line) and MLE12 (SV40-immortalized mouse alveolar cell line) cell lines were obtained from ATCC (Manassas, USA). A549 were maintained in DMEM (Life Technologies, Carlsbad, USA), MLE12 in RPMI (Life Technologies), and 16HBE14o$^-$ human bronchial epithelial cells (HBECs) were cultivated in MEM. Media was supplemented with 10% FBS (PAA Laboratories, Cölbe, Germany) and 100 U/mL penicillin/streptomycin (Life Technologies). Primary HBECs and isolated mouse airways were obtained and cultivated as described previously [13, 14]. For cell fractionation and MS analysis, phenol red free media without FBS was used. Primary mouse alveolar type II cells were isolated from C57BL6/N mice (Charles River Laboratories, Sulzfeld, Germany) as previously described [15]. All cells were grown at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were repeated several times to obtain independent biological replicates.

2.2 Preparation of CSE

Stocks of CSE were prepared by bubbling smoke from six 3R4F research-grade cigarettes (Tobacco and Health Research Institute, University of Kentucky, Lexington, KY, USA) through 100 mL of DMEM phenol-red free cell culture media as described (see Supporting Information, [16]).

2.3 Preparation of three-dimensional ex vivo lung tissue cultures and exposure to CSE

Three-dimensional ex vivo lung tissue cultures were prepared from mouse lung tissue as published [17] and detailed in the Supporting Information.

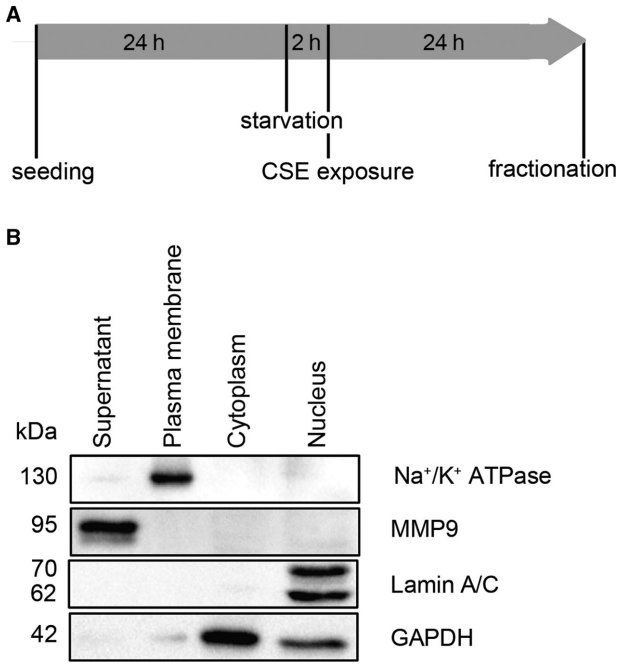

Figure 1. Subcellular fractionation of A549 lung epithelial cells exposed to cigarette smoke extract. (A) Experimental setup. (B) Western blot analysis of cell compartment specific proteins.

2.4 Cytotoxicity and proliferation assays

Cytotoxicity of CSE was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [16] and proliferation using the BrdU assay (both Roche Diagnostics, Mannheim, Germany) [18].

2.5 Cell fractionation

Triplicates for each sample of A549, 16HBE14o$^-$, and MLE12 cells were exposed to 50, 25, or 10% CSE, respectively, for 24 h or to normal media. For each fraction, we used 2–4 million cells 24 h after seeding, which were prestarved for 2 h. Cells were exposed for additional 24 h to serum-free CSE-containing (treated) or normal media (control) (Fig. 1A). Fractionation was carried out as previously described [19]. Briefly, cell supernatants were collected, filtered, and concentrated with Vivaspin 6 concentrators (Sartorius, Göttingen, Germany). Cells were washed and surface proteins were labeled with biotin. After lysis, cells were scraped and cytosolic and nuclear proteins were separated through differential centrifugation while surface proteins were pulled down with streptavidin beads.

2.6 Mass spectrometry

Protein concentration of the different fraction was measured with Pierce BCA protein assay (Thermo Fisher Scientific, Schwerte, Germany). Secreted, nuclear, and cytoplasmic proteins were digested with Lys-C and trypsin with a filter-aided sample preparation procedure as previously described [19, 20] and subjected to proteomic analysis. Biotinylated and affinity-enriched surface proteins were digested with trypsin and PNGaseF directly on the streptavidin beads. LC-MS/MS analysis was performed on an Ultimate 3000 nano-RSLC coupled to a LTQ OrbitrapXL mass spectrometer (Thermo Fisher Scientific) as described [21, 22]. The acquired spectra were loaded to Progenesis LC-MS software (version 2.5; Nonlinear Dynamics) for label-free quantification and analyzed as published [21, 22]. MS/MS spectra were used for identification with Mascot (version 2.3; Matrixscience) as previously described [19] using organism-specific proteins databases from Ensembl (Ensembl mouse, release 75, 51 765 sequences; Ensembl human, release 69, 96 556 sequences). Search parameters used were: 10 ppm peptide mass tolerance and 0.6 Da fragment mass tolerance, one missed cleavage allowed, carbamidomethylation was set as fixed modification, methionine oxidation, and asparagine or glutamine deamidation were allowed as variable modifications. A Mascot-integrated decoy database search calculated an average false discovery of $<1\%$ when searches were performed with a mascot percolator score cut-off of 15 and an appropriate significance threshold p. After inverse hyperbolic transformation (arcsinh function), normalized abundances of proteins were used for statistical analysis by Student's t-test in order to identify proteins, which were significantly altered after CSE treatment ($p < 0.05$).

2.7 GO cellular component enrichment analysis

The list of proteins identified in each fraction was subjected to GO enrichment analysis using STRAP software [23].

2.8 Network analysis

Fifty-five significantly different and at least twofold altered proteins after CSE treatment were fed into the Genomatix GePS software (http://www.genomatix.de/index.html). The interaction filter was set to "validated regulatory level" and the generated network was extended with five proteins with most frequent co-citation (gray).

2.9 Antibodies, western blot, and quantitative real-time RT-PCR

Details are provided in the Supporting Information.

2.10 Statistical analysis

Details on the statistical analyses used are given in the respective figure legends. Statistical analysis was performed using the GraphPad Prism software (version 5.00; GraphPad Software, LaJolla, CA, USA).

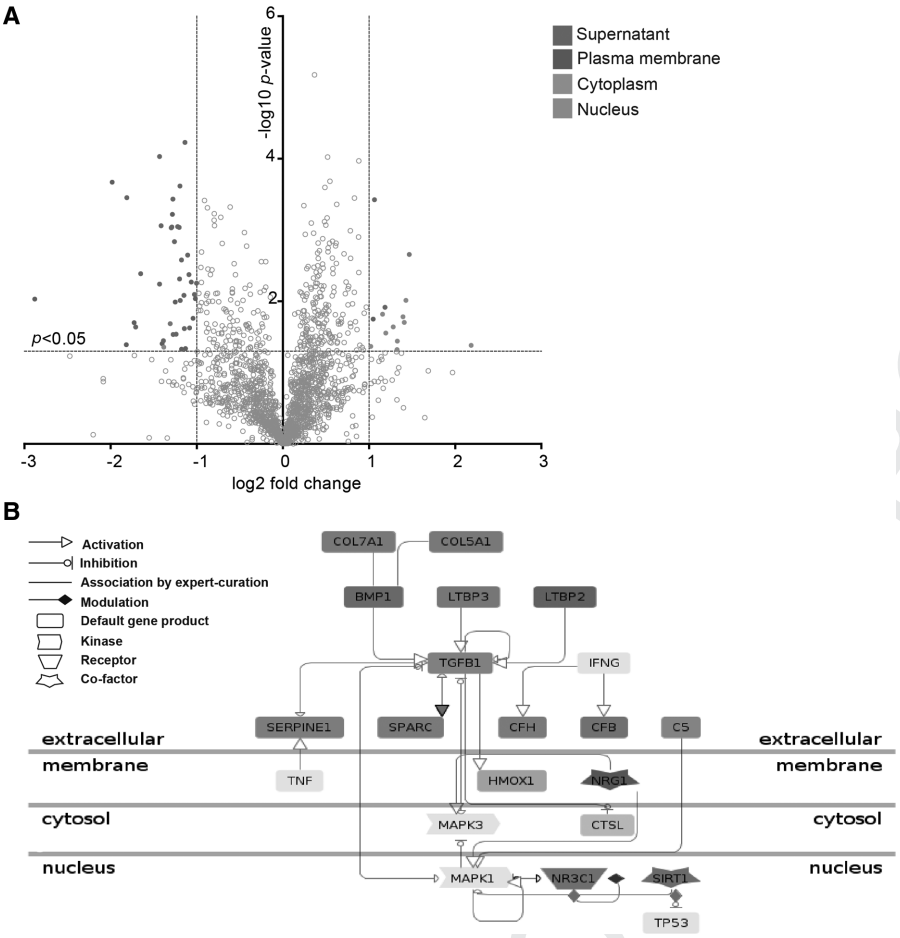

Figure 2. Cigarette smoke extract alters the cellular secretome. (A) Volcano plot showing the A549 proteome exposed to 50% CSE for 24 h. Colored dots indicate proteins that were significantly ($p < 0.05$) altered by at least twofold. Gray dots represent proteins whose expression was not significantly altered and/or was less than twofold regulated compared to control, $n = 3$. (B) Genomatix GePS network analysis of CSE-regulated proteins: proteins downregulated (blue), proteins upregulated (red), proteins used to extend the network according to frequent co-citation (gray). Unconnected proteins were discarded.

3 Results

3.1 Fractionation of the A549 proteome allowed identification of more than 2500 proteins

To investigate the effect of CSE on protein expression, A549 cells were exposed for 24 h to serum-free media containing 50% CSE (Fig. 1A). While metabolic activity and proliferation were reduced, morphology of the cells was not grossly altered indicating that this dose was well tolerated by A549 cells (Supporting Information Fig. E1). To obtain large coverage of the A549 proteome, we performed subcellular fractionation prior to MS analysis into four main compartments: cellular supernatant, plasma membrane, cytoplasm, and nucleus. Efficient enrichment of compartment-specific proteins was confirmed by western blot analysis of cell compartment specific markers such as the integral membrane protein Na^+/K^+ ATPase, the secretory matrix metalloproteinase-9, nuclear lamin A/C, and cytoplasmic glyceraldehyde 3-phosphate dehydrogenase (Fig. 1B). For each fraction, we performed LC-MS/MS analysis and subsequent label-free quantification resulting in the identification of 2715 proteins (Supporting Information Table E1). GO cellular component enrichment analysis revealed that each fraction was clearly enriched for proteins of the respective cellular compartment.

3.2 CSE predominantly alters protein expression in the secretome of A549 cells

We next identified proteins that were differentially regulated by CSE. For that, we considered only proteins that were unambiguously identified by at least two unique peptides (1839 proteins, Supporting Information Table E1). Fifty-five of them were significantly ($p < 0.05$) regulated by CSE with a minimum of twofold change compared to controls (Fig. 2A and Supporting Information Table E2), 41 of these were downregulated. Remarkably, the majority of CSE-regulated proteins (29 of 55) belonged to the cellular secretome (Fig. 2A, red dots). Most of these proteins are involved in the organization of the ECM such as fibrillin and collagens, proteins of the transforming growth factor β (TGF-β) superfamily (BMP1, LTBP2, LTBP3, and TGFB1), the ECM glycoproteins EGF containing fibulin-like extracellular matrix protein 1, and members of the secreted protein acidic and rich in cysteine (SPARC) family (SPARC and SPOCK1). Subsequent network

analysis using the Genomatix Pathway System Software identified concerted downregulation of proteins involved in TGF-β signaling, wound-healing responses, as well as interferon-γ and tumor necrosis factor responsive proteins (Fig. 2B). We did not observe any significant changes in the subcellular distribution of these proteins in response to CSE exposure. These data strongly indicate that nontoxic smoke exposure of lung epithelial cells to CSE has an acute inhibitory effect on the cellular secretome with reduced collagen expression and impaired wound-healing responses.

3.3 CSE downregulates proteins involved in wound healing and ECM organization in alveolar epithelial cells

As A549 cells are human alveolar adenocarcinoma cells, we next confirmed CSE-mediated regulation of the secretome using a noncarcinoma lung alveolar epithelial cell line. For that we analyzed the secretome of mouse alveolar epithelial MLE12 cells by LC-MS/MS after exposure to CSE for 24 h. CSE doses were adjusted according to the increased sensitivity of these cells to CSE as determined by MTT assay (Supporting Information Fig. E2) [15]. We identified and quantified the abundance of more than 100 proteins that were differentially regulated upon CSE exposure (Supporting Information Table E4). Importantly, we again observed predominant downregulation of proteins involved in ECM organization and wound-healing responses such as bone morphogenetic proteins, latent TGF-β binding proteins, and SPARC; thus, confirming our data obtained with A549 cells.

Several of the ECM organizing proteins that were downregulated in both A549 and MLE12 cells were found to be also regulated on the mRNA level in MLE12 alveolar cells: mRNA expression of BMP1, progranulin (PGRN), and SPARC were dose-dependently reduced with 10 and 25% CSE, while LTBP3 was not downregulated on the mRNA level (Fig. 3A). In addition, we observed concerted downregulation of several other well-known ECM molecules and organizing cytokines, i.e. fibronectin and TGF-β2 and 3, while collagen Iα1 and collagen IVα1 as well as TGF-β1 were not altered (Fig. 3A). Downregulation of fibronectin was confirmed on the protein level by 1.6-fold in our proteomics data of MLE12 cells.

For further validation of our proteomic data, we chose two newly identified CSE-responsive target proteins: PGRN, which functions as a wound-healing mediator in tissue regeneration [24, 25], and SPARC, a major ECM organizing protein [26, 27]. In our proteomic profiling, CSE exposure reduced PGRN expression by fourfold in the secretome of MLE12 cells (Supporting Information Table E4). Using a specific ELISA for PGRN, we confirmed dose-dependent downregulation of secreted PGRN in cellular supernatants of MLE12 cells by CSE (Fig. 3B). Furthermore, the matricellular protein SPARC was downregulated both in A549 and MLE12 proteomic profiling by about twofold (Supporting Information Tables E2 and E4). Western blot analysis confirmed reduction of SPARC protein levels in supernatants of MLE12 cells by about 65% with 25% CSE (Fig. 3C). Of note, downregulation of SPARC and also fibronectin was even more pronounced when fresh medium was added to the MLE12 cells for recovery (Fig. 4A and B), indicating that the observed transcriptional regulation of wound-healing mediators effectively relays the acute effects of CSE to sustained ECM matrix regulation.

We further confirmed reduced secretion of wound-healing mediators by CSE in supernatants of primary mouse alveolar type II cells: PGRN ELISA and western blot analysis for SPARC and fibronectin revealed significant downregulation of these mediators also in primary alveolar epithelial cells (Fig. 4C and Supporting Information Fig. E2 for MTT assay). These data clearly indicate that acute exposure to cigarette

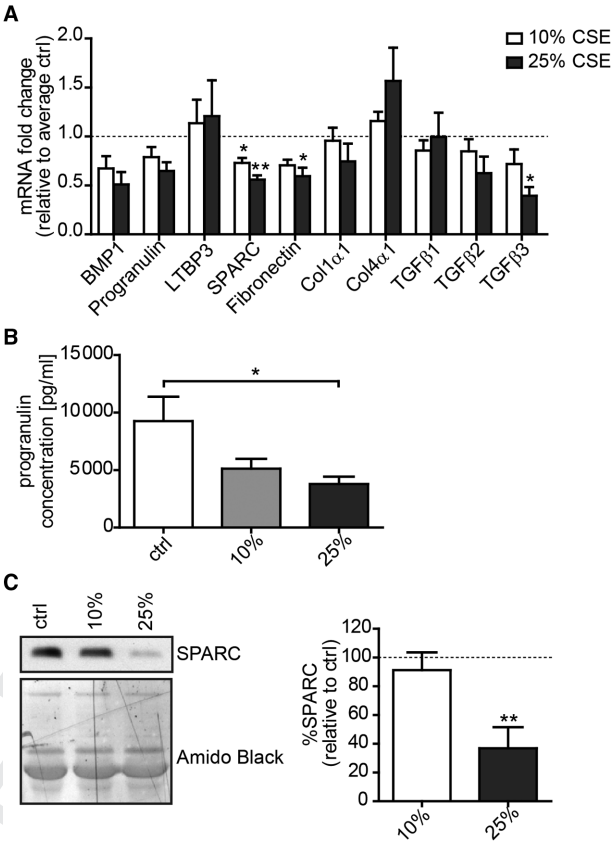

Figure 3. Validation of the dose-dependent effects of CSE on selected ECM organizers and wound-healing mediators. (A) Dose-dependent effects of CSE (10 or 25%) on mRNA expression in MLE12 cells after 24 h, normalized to housekeeper and controls (ctrl), $n = 3$, mean ± SEM. (B) ELISA-based detection of secreted progranulin in supernatants of MLE12 cells exposed to 10 or 25% CSE for 24 h, $n = 4$, mean ± SEM. (C) Western blot analysis and quantification of SPARC in MLE12 supernatants after exposure to 10 or 25% CSE for 24 h ($n = 4$); mean ± SEM. Amidoblack staining confirmed equal protein loading of cell culture supernatants. One-way ANOVA with Dunnett's multiple comparison test was used.

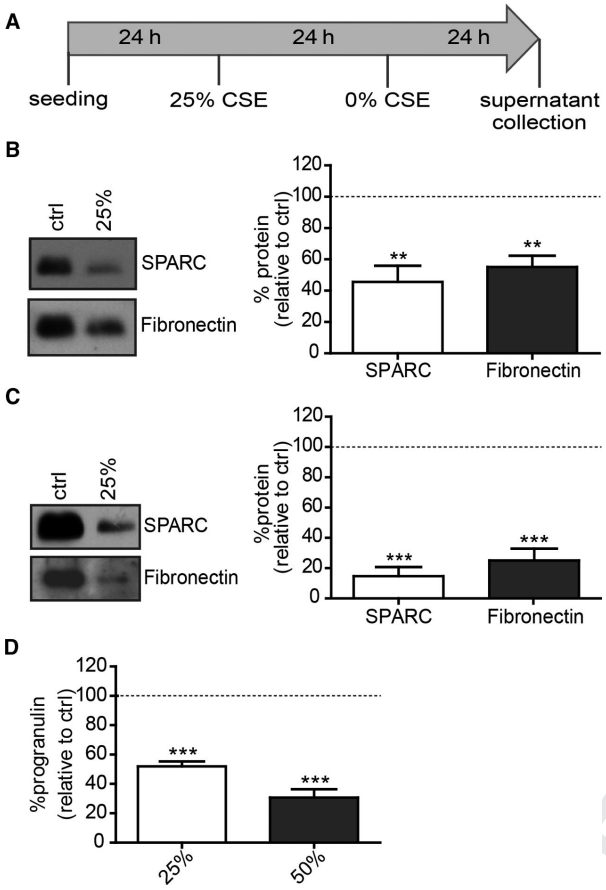

Figure 4. CSE-mediated downregulation of SPARC, fibronectin, and progranulin in primary mouse ATII cells. (A) Scheme of MLE12 recovery experiment. (B) Western blot analysis and quantification of SPARC and fibronectin after recovery ($n = 3$); mean ± SEM. (C) Representative western blots and relative to control (ctrl) quantification of SPARC and fibronectin protein expression in primary mouse alveolar type II cells (pmATII) supernatants after 24-h exposure to 25% CSE; $n = 3$, mean ± SEM. (D) ELISA-based detection of progranulin in supernatants of pmATII exposed to 25% CSE for 24 h, $n = 3$, mean ± SEM. One-way ANOVA with Dunnett's multiple comparison test was used.

smoke causes an altered alveolar epithelial response that involves ECM remodeling and tissue injury mechanisms.

3.4 Acute CSE exposure alters expression of distinct ECM organizers in bronchial epithelial cells

We also performed CSE exposure of a HBEC line, i.e. the 16HBE14o$^-$ cells, and subsequent LC-MS/MS analysis of supernatants to investigate whether CSE induces a similar response in bronchial epithelial cells. Twenty-four hours exposure of 16HBE14o$^-$ cells to nontoxic doses of 10% CSE (Supporting Information Fig. E2) induced only minor changes in the supernatant with a total of 22 proteins more than twofold differentially regulated (Supporting Information Table E3). Among them were only two proteins with distinct functions in ECM remodeling, i.e. matrix metalloproteinase-2 (MMP2) and the tissue inhibitor of metalloproteinases (TIMP) metallopeptidase inhibitor 1. Notably, there was only a minor overlap of CSE-regulated proteins in 16HBE14o$^-$ cells with those of A549 and MLE12 cells including vascular endothelial growth factor A, some cadherin, and TIMP members. While TIMP1 and MMP2 were increased about twofold by CSE in 16HBE14o$^-$ cells, TIMP2 and MMP2 were markedly downregulated in A549 and MLE12 cells. As these data suggested a differential responsiveness of bronchial epithelial cells to CSE compared to alveolar cells, we exposed primary HBECs to nontoxic doses of CSE for 24 h to validate this assumption [13, 14]. On the RNA level, expression of several ECM organizing molecules such as SPARC, fibronectin, collagen Iα1 and IVα1, as well as TGF-β2 and PGRN was downregulated by CSE (Supporting Information Fig. E3A). Total protein levels of fibronectin were also downregulated, although not significantly, while SPARC levels were maintained as determined by western blotting (Supporting Information Fig. E3B). These data indicate that bronchial epithelial cells also downregulate several ECM organizers in response to CSE in a similar but distinct way compared to alveolar epithelial cells.

3.5 Acute CSE exposure downregulates ECM organizers and wound-healing mediators in ex vivo lung tissues

To study the effect of cigarette smoke in a more physiologically relevant setting, we used isolated airways from mice and exposed them to 10% CSE for 24 h. RNA expression of both SPARC and fibronectin was significantly downregulated by CSE exposure (Fig. 5A). Moreover, we exposed mouse three-dimensional ex vivo lung tissue cultures to 5% CSE. Very similar to our previous results, we observed predominant downregulation of several wound-healing mediators and ECM organizing molecules on the mRNA level (Fig. 5B). In particular, SPARC, fibronectin, collagen IVα1, and TGF-β1 were downregulated. Reduced expression of SPARC was also observed on the protein level (Fig. 5C). These results clearly confirm an acute effect of CSE on matrix organizing molecules and mediators of wound healing in the pulmonary epithelium. We did not, however, detect any significant alteration in SPARC and fibronectin levels in the lungs of mice that had been smoked for 10 days possibly due to the low basal SPARC expression in alveolar epithelial cells of the lung (Supporting Information Fig. E4).

4 Discussion

In the present study, we used subcellular fractionation coupled to label-free quantitative MS to identify compartment-specific changes in the composition of the proteome of lung

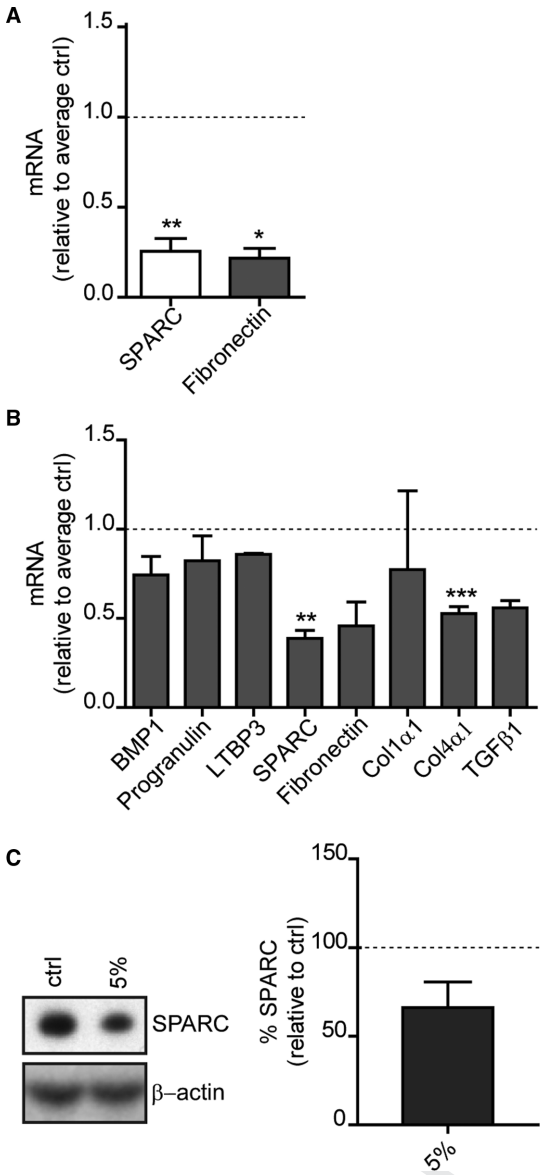

Figure 5. Regulation of ECM organizers and wound-healing mediators in ex vivo mouse lung tissue. (A) RT-qPCR analysis of isolated mouse airways treated with 10% CSE for 24 h ($n = 3$, unpaired *t*-test). (B) RT-qPCR analysis of 5% CSE-treated three-dimensional ex vivo lung tissue cultures (3D-LTC) after 24 h ($n = 3$, unpaired *t*-test). (C) Representative western blot and relative (to control and β-actin housekeeper) quantification of SPARC in 3D-LTC after exposure to 5% CSE for 72 h with $n = 7$; mean ± SEM, unpaired *t*-test.

epithelial cells in response to acute and nontoxic CSE exposure. This approach resulted in the identification of more than 2500 proteins. Compared to other shotgun proteomic studies of lung cells or tissues, our fractionation approach thus increased total proteome coverage by at least threefold [7, 9, 10, 12]. It also allowed us to identify the secretome as the cellular compartment with the most prominent changes in protein composition: while exposure of lung epithelial cells to CSE did not influence overall subcellular distribution of proteins, we observed distinct changes in the abundance levels of secreted proteins in the supernatant fraction. Other proteomic studies that analyzed epithelial lining fluids and bronchoalveolar lavages upon cigarette smoke exposure did not detect similar changes [7–9, 28]. This might be due to the generally low abundance of these proteins and the possibility of directional secretion as suggested recently by a comprehensive proteomic study of the secretome of human bronchial cells [29]. Gene enrichment analysis of differentially expressed proteins in the secretome identified the genes involved in ECM organization as mostly regulated. All proteins in this pathway were downregulated including several mediators of the TGF-β signaling pathway and ECM proteins and regulators thereof. Concerted downregulation of TGF-β signaling in response to cigarette smoke was confirmed for the immortalized mouse alveolar epithelial MLE12 cells on the mRNA and protein level. This finding, however, is in contrast to other studies, which observed increased secretion of TGF-β in alveolar and small airways epithelial cells from smokers and COPD patients [30, 31]. Our data obtained with alveolar and bronchial epithelial cells of different origin, however, agree with previous observations that cigarette smoke can inhibit the capacity of HBECs to release TGF-β [32]. Contrasting findings might be due to the different in vitro settings used for the exposure to cigarette smoke. Importantly, our results support the hypothesis that cigarette smoke inhibits wound repair in lung epithelial cells [32, 33]. In particular, we observed smoke-mediated downregulation of PGRN, a central wound-healing mediator in tissue regeneration [24, 25], in the secretome of both MLE12 and primary mouse ATII cells. As a wound-related growth factor, PGRN promotes the granulation phase of wound healing and supports vascularization and formation of a fibronectin scaffold necessary for subsequent collagen deposition [24]. Another protein that was downregulated in lung epithelial cells in response to cigarette smoke is SPARC. SPARC is a matricellular protein that binds several resident proteins of the ECM and alters the activity of extracellular proteases and growth factors. It thereby participates in the assembly and organization of ECM and is essential for proper wound-healing responses [26, 34]. Indeed, SPARC is highly expressed during development and at sites of injury and disease where tissues undergo constant repair and remodeling [34, 35]. SPARC also alters ECM organization by reducing levels of fibronectin via outside–in signaling [36]. In our study, both SPARC and fibronectin were significantly downregulated by acute cigarette smoke exposure in vitro in alveolar and bronchial epithelial cells as well as in ex vivo lung tissue. As we did not observe any pronounced changes in SPARC expression in mice that had been exposed to cigarette smoke for 10 days, it is well feasible that the observed changes in vitro might be initially counteracted in vivo by adaptive changes. Upon chronic exposure to cigarette smoke, however, altered secretion of ECM organizers may contribute

to pathogenic lung tissue remodeling as observed in COPD patients.

Taken together, our results show that lung epithelial cells acutely respond to nontoxic doses of cigarette smoke by impaired secretion of key factors for wound healing and tissue remodeling. This acute response of the pulmonary epithelium to cigarette smoke may contribute to the detrimental tissue damage observed in COPD patients. Indeed, it has been suggested that tissue destruction in COPD patients is a consequence of the inadequate capacity of damaged cells of the lung to successfully repair lung tissue and maintain lung structure [32, 33, 37].

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PROTEOMICS

Supporting Information for Proteomics

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Cigarette smoke alters the secretome of lung epithelial cells