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COPD-derived fibroblasts secrete higher levels of senescence-associated secretory phenotype proteins

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ABSTRACT

COPD-derived fibroblasts have increased cellular senescence. Senescent cell accumulation can induce tissue dysfunction by their senescence-associated secretory phenotype (SASP). We aimed to determine the SASP of senescent fibroblasts and COPD-derived lung fibroblasts, including severe, early-onset (SEO)-COPD. SASP protein secretion was measured after paraquat-induced senescence in lung fibroblasts using Olink Proteomics and compared between (SEO-)COPD-derived and control-derived fibroblasts. We identified 124 SASP proteins of senescent lung fibroblasts, of which 42 were secreted at higher levels by COPD-derived fibroblasts and 35 by SEO-COPD-derived fibroblasts compared with controls. Interestingly, the (SEO-)COPD-associated SASP included proteins involved in chronic inflammation, which may contribute to (SEO-)COPD pathogenesis.

INTRODUCTION

Accelerated lung ageing has been postulated to contribute to the pathogenesis of COPD.[1] Several mechanisms of accelerated ageing have been identified in COPD,[1 2] of which cellular senescence is most extensively described to be increased in lung tissue and structural cells from patients with COPD.[3] Cellular senescence is an irreversible cell cycle arrest that prevents cell death.[4] Senescent cells secrete (pro-inflammatory) proteins, called the senescence-associated secretory phenotype (SASP), to recruit immune cells for their clearance. However, on accumulation of senescent cells, high levels of SASP proteins can have detrimental effects on the surrounding tissue, by inducing chronic inflammation and tissue dysfunction.[5] The SASP is cell type specific and its potential (negative) impact on surrounding cells largely depends on the composition and level of secretion of these SASP proteins. Examples of previously described SASP proteins include interleukins, chemokines, growth factors and proteases.[6 7]

Recently, we demonstrated higher levels of cellular senescence in lung fibroblasts and lung tissue from patients with older, mild-moderate COPD and patients with severe, early-onset (SEO)-COPD compared with their matched controls.[8] Patients with SEO-COPD develop very severe COPD at a relatively early age with relatively low numbers of pack-years. Thus, accelerated lung ageing, including cellular senescence, may contribute to SEO-COPD. The SASP of senescent primary lung fibroblasts and COPD-derived fibroblasts is not defined yet and thus the potential impact of senescent fibroblasts on the surrounding lung tissue is unclear. Therefore, we aimed to first identify SASP proteins of senescent primary human lung fibroblasts and second to determine which of these SASP proteins are secreted at higher levels by COPD-derived fibroblasts, including SEO-COPD, compared with their matched non-COPD control-derived fibroblasts.

METHODS

Cell culture supernatants from lung fibroblasts from 10 patients with SEO-COPD and 11 patients with older, mild-moderate COPD and, respectively, 9 and 10 matched non-COPD controls were used (table 1), which were collected as previously described[8] (a detailed description of the methods can be found in the online supplemental). Briefly, cellular senescence was induced in fibroblasts from all subject groups by paraquat (PQ) treatment (250 µM for 24 hours), which by occupational exposure is a risk factor for COPD, and can induce senescence specifically via mitochondrial reactive oxygen species production.[9 10] Senescence induction was confirmed by a 40% increase in SA-β-gal positive cells and a sevenfold increase in p21 expression.[8] Cell culture supernatants were collected 4 days after senescence induction. The highly sensitive Olink Proteomics (Olink Proteomics, Uppsala, Sweden) panels *Inflammation* and *Cardiovascular III* were used to measure the secretion of 184 proteins, whereof 165 proteins passed quality control. Since cell numbers at the end of culture were significantly different between COPD and control and between PQ and untreated (online supplemental figure S1), levels of secreted proteins were corrected for these cell numbers. Significant differences between PQ treated and untreated cells were tested using Wilcoxon signed-rank test adjusted for multiple testing using Benjamini-Hochberg. Proteins were defined as SASP protein when a significant (FDR<0.05) ≥threefold increase in secretion was observed after PQ treatment. Next, statistical differences in SASP protein secretion between untreated COPD-derived and control-derived fibroblasts were tested using Mann-Whitney U test. FDR $p<0.05$ was considered statistically significant. Finally, pathway analysis of COPD-associated SASP proteins was performed using the STRING database (V.11.0) to provide more insight into the function of the SASP proteins and their potential role in COPD, while it should be noted that the selected panels may have caused a bias in the analysis.

Table 1 Subject characteristics of fibroblasts of combined groups and subgroups

Variable	Control	COPD	P value	Variable	Control (SEO-COPD-matched)	SEO-COPD	P value	Control (older COPD-matched)	Older, mild-moderate COPD	P value
Number	19	21		Number	9	10		10	11	
Age, mean years (range)	61 (42–81)	62 (44–81)	0.844	Age, mean years (range)	52 (42–59)	50 (44–55)	0.349	70 (65–81)	73 (66–81)	0.176
Male/female, n	9/10	12/9	0.548	Male/female, n	1/8	2/8	0.556	8/2	10/1	0.500
Pack-years	34 (28–40)	30 (15–50)	0.627	Pack-years	32 (28–35)	26 (14–30)	0.673	43 (28–51)	49 (19–53)	0.823
Stop-months,	120 (30–240)	78 (36–96)	0.337	Stop-months	84 (18–168)	78 (63–93)	0.677	186 (81–252)	66 (27–96)	0.421
Non-COPD, n	19	–		Non-COPD, n	9	–		10	–	
COPD, n	–	21		COPD, n	–	10		–	11	
GOLD 1	–	–		GOLD 1	–	–		–	–	
GOLD 2	–	7		GOLD 2	–	–		–	7	
GOLD 3	–	4		GOLD 3	–	–		–	4	
GOLD 4	–	10		GOLD 4	–	10		–	–	
FEV_1 %pred	88.1 (82.5–98.0)	38.8 (17.1–66.7)	**0.000**	FEV_1 %pred	87.0 (83.5–92.0)	16.5 (14.3–22.7)	**0.000**	90.7 (82.2–104.0)	66.7 (43.4–70.5)	**0.000**
FVC %pred	90.3 (83.0–107.5)	77.9 (44.2–83.5)	**0.005**	FVC %pred	92.8 (84.6–101.0)	42.6 (37.9–68.1)	**0.000**	89.5 (76.7–107.5)	83.5 (79.7–98.8)	0.647
FEV_1/FVC	73.6 (71.8–77.7)	41.8 (28.4–50.0)	**0.000**	FEV_1/FVC	75.9 (73.3–79.0)	27.6 (26.0–38.5)	**0.000**	72.1 (70.3–75.1)	50.0 (41.7–59.0)	**0.000**

Data are presented as medians with interquartile ranges unless otherwise stated.
Significant differences between groups were tested using Mann–Whitney U tests or unpaired t-tests. P values are stated.
Gold stage based on FEV_1 %pred.
%pred, % predicted; SEO, severe, early-onset.

RESULTS

First, the secretion of 124 proteins was significantly increased ≥threefold after senescence induction by PQ and these proteins were thus defined as SASP proteins of senescent primary lung fibroblasts (top-50 is shown in figure 1A, see online supplemental table S1 for all SASP proteins). We compared our SASP composition with the recently published SASP Atlas[7] and other literature and included the overlap in online supplemental table S1. From the 124 found SASP proteins 70 were previously described, including GDF-15 and CCL-3 (figure 1B). In addition, our approach revealed 54 potentially novel SASP proteins, including GDNF and TGF-α (figure 1C). We validated the Olink

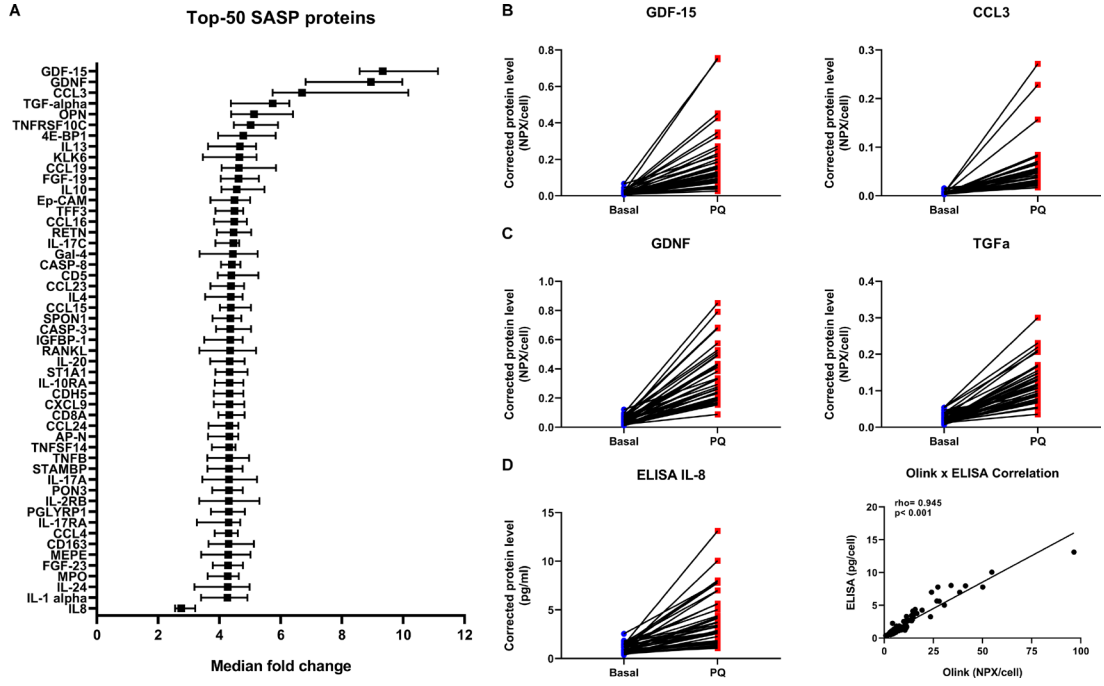

Figure 1 SASP of senescent primary lung fibroblasts. Graph showing top-50 of 124 significant SASP proteins with highest median fold change and IL-8, sorted on fold change (A). Significant differences were tested using Wilcoxon signed-rank tests (n=40). Benjamini-Hochberg adjusted FDR<0.05 was considered statistically significant. Medians with 95% CI are plotted. Examples of two previously described SASP proteins, that is, GDF-15 and CCL3 (B) and two not previously described SASP proteins, that is, GDNF and TGF-α (C) with the highest median fold change are plotted in dot plots (for more details see online supplemental table S1). Blue=basal and red=paraquat (PQ) treatment (both n=40). Protein levels are depicted as Olink NPX values corrected for total cell numbers. IL-8 protein levels were validated using Human DuoSet ELISA (R&D Systems, Abingdon, UK) (D) and correlated with Olink IL-8 levels (D, right panel). Spearman Rho and p value are plotted in the graph. FDR, false discovery rate; IL, interleukin; SASP, senescence-associated secretory phenotype.

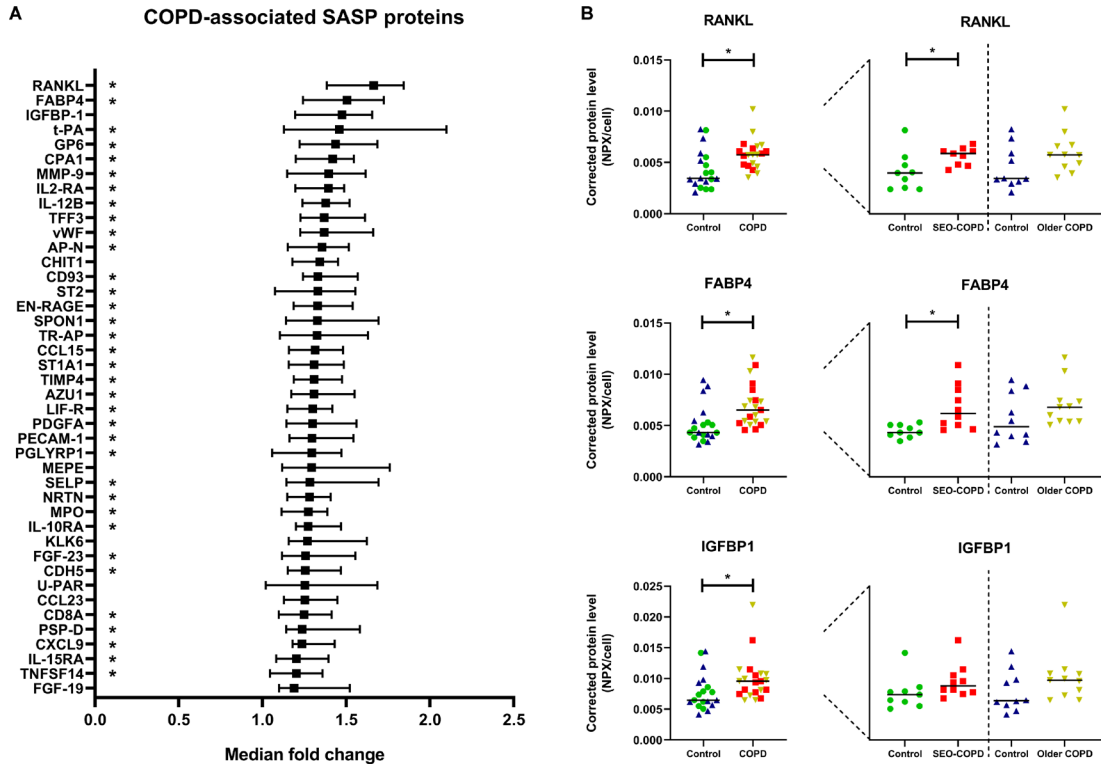

Figure 2 Higher levels of SASP protein secretion by COPD-derived fibroblasts. Graph showing all 42 significant SASP proteins with higher protein secretion in COPD-derived fibroblasts (n=21) compared with non-COPD controls (n=19), sorted on fold change (A) (for more details see online supplemental table S2). Significant differences were tested using Mann-Whitney U tests. Benjamini-Hochberg adjusted FDR<0.05 was considered statistically significant. Medians with 95% CI are plotted. The SEO-COPD-associated SASP proteins are indicated with a star in the graph behind the protein names. No older, mild-moderate COPD-associated SASP proteins were found. The three COPD-associated SASP proteins with the highest fold change in medians are plotted in dot plots (B). Green=SEO-COPD-matched controls (n=9), red=SEO COPD (n=10), blue=older, mild-moderate COPD-matched controls (n=10), yellow=older, mild-moderate COPD (n=11). Protein levels are depicted as Olink NPX values corrected for cell numbers. Lines represent medians. SASP, senescence-associated secretory phenotype; SEO, severe, early-onset. FDR, false discovery rate.

Proteomics platform by measuring IL-8 using ELISA. A similar increase in IL-8 secretion was detected by ELISA after PQ-induced senescence with a significant positive correlation with IL-8 levels measured by Olink Proteomics (figure 1D).

Next, the secreted levels of these 124 defined SASP proteins were evaluated in untreated cell culture supernatants from patients with COPD compared with their matched control-derived fibroblasts. We observed higher levels of 42 SASP proteins in supernatants from COPD-derived fibroblasts (figure 2A, see online supplemental table S2 for a detailed overview). The three proteins with the highest median fold change were RANKL, FABP4 and IGFBP-1 (figure 2B). Several of the COPD-associated SASP proteins were previously found to be higher expressed at the transcription level in COPD-derived lung tissue compared with controls, including vWF, CHIT1, SPON1, TR-AP, TIMP4, PECAM1, CDH5, PSP-D, IL-15RA.[11] Furthermore, several COPD-associated SASP proteins were associated with ageing in lung tissue at the transcription level, including t-PA, CHIT1, SPON1, IL-10RA and CXCL9.[12] On subgroup analyses, 35 of the 42 COPD-associated proteins were secreted at higher levels by fibroblasts from patients with SEO-COPD compared with their matched controls (online supplemental table S2), whereas this was not the case for the patients with older, mild-moderate COPD compared with their matched controls.

Finally, STRING pathway analysis revealed that responses to stimuli, immune responses and cytokine-related pathways are associated with the COPD-associated SASP proteins (data not shown). COPD-associated SASP proteins include cytokines (IL12B, TNFSF14 and RANKL) and chemokines (CCL15, CCL23 and CXCL9) that are known to be involved in inflammatory processes. These findings suggest that the SASP proteins that are secreted at higher levels by COPD-derived fibroblasts might be involved in the chronic inflammatory response in COPD.

CONCLUSION

By using a proteomic-based approach, we provide insight into the SASP of primary human lung fibroblasts. Interestingly, 42 of the 124 identified SASP proteins were secreted at higher levels by fibroblasts from patients with COPD compared with matched controls. The COPD-associated SASP proteins include proteins that have been implicated in chronic inflammation, and thus may contribute to disease pathology in COPD. Remarkably, 35 of these 42 COPD-associated SASP proteins are secreted at higher levels by patients with SEO-COPD compared with their matched controls, whereas none were significantly different between patients with older, mild-moderate COPD compared with their matched controls. This lack of significance is likely due to higher biological variation in these older subgroups as the fold changes are comparable (online supplemental table S2) and the interquartile ranges are higher in these groups (online supplemental figure S2). These results suggest a role for these SASP proteins in COPD. The fact that both cellular senescence and SASP protein secretion were higher in COPD-derived lung

fibroblasts compared with their matched controls suggests that senescence accumulation is involved in the pathogenesis of COPD. It should be noted that until now it is unknown whether the higher senescence observed in COPD is driven by acute exposures or chronic exposures, which may result in a different SASP profile. In addition, different senescence-inducing stimuli may result in a different SASP profile as well. The identified (COPD-associated) SASP proteins of primary lung fibroblasts can be used for further studies to understand the role of senescent cell accumulation and its potential detrimental impact in SEO-COPD pathogenesis.

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REFERENCES

1 Ito K, Barnes PJ. Copd as a disease of accelerated lung aging. *Chest* 2009;135:173–80.
2 Meiners S, Eickelberg O, Königshoff M. Hallmarks of the ageing lung. *Eur Respir J* 2015;45:807–27.
3 Brandsma C-A, de Vries M, Costa R, *et al*. Lung ageing and COPD: is there a role for ageing in abnormal tissue repair? *Eur Respir Rev* 2017;26. doi:10.1183/16000617.0073-2017. [Epub ahead of print: 31 Dec 2017].
4 Kuilman T, Michaloglou C, Mooi WJ, *et al*. The essence of senescence. *Genes Dev* 2010;24:2463–79.
5 Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol* 2014;15:482–96.
6 Coppé J-P, Patil CK, Rodier F, *et al*. Senescence-Associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic Ras and the p53 tumor suppressor. *PLoS Biol* 2008;6:e301–2868.
7 Basisty N, Kale A, Jeon OH, *et al*. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol* 2020;18:e3000599.
8 Woldhuis RR, de Vries M, Timens W, *et al*. Link between increased cellular senescence and extracellular matrix changes in COPD. *Am J Physiol Lung Cell Mol Physiol* 2020;319:L48–60.
9 Castello PR, Drechsel DA, Patel M. Mitochondria are a major source of paraquat-induced reactive oxygen species production in the brain. *J Biol Chem* 2007;282:14186–93.
10 Chinta SJ, Woods G, Demaria M, *et al*. Cellular senescence is induced by the environmental neurotoxin paraquat and contributes to neuropathology linked to Parkinson's disease. *Cell Rep* 2018;22:930–40.
11 Brandsma C-A, van den Berge M, Postma DS, *et al*. A large lung gene expression study identifying fibulin-5 as a novel player in tissue repair in COPD. *Thorax* 2015;70:21–32.
12 de Vries M, Faiz A, Woldhuis RR, *et al*. Lung tissue gene-expression signature for the ageing lung in COPD. *Thorax* 2018;73:609–17.

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Online Supplement

Complete methods

Subjects

Primary lung fibroblasts from subjects undergoing lung transplantation or tumour resection surgery were used. Resected lung tissue was isolated distal from the tumour and was macroscopically and histologically normal. Primary parenchymal lung fibroblasts were isolated and cultured as described before [1]. Briefly, parenchymal lung tissue was cut into small cubes and cultured in 12-wells plates in Ham's F12 medium supplemented with 10% foetal calf serum (FCS), 2mM L-Glutamine, 100µg/ml Streptomycin and 100U/ml penicillin at 37°C and 5% CO_2. Medium was refreshed every week and after four weeks fibroblasts were trypsinized and placed into 25 cm^2 flasks. When cultures reached confluency, fibroblasts were frozen and stored in liquid nitrogen. The following inclusion criteria were used:

1) SEO-COPD patients; FEV1/FVC <70% and FEV1 <30%pred measured at an age <53 (according to [2]) and with age <56 at time of lung transplant surgery
2) non-COPD control subjects (SEO-COPD-matched); FEV1/FVC >70%, age <60 at time of surgery
3) Older, mild-moderate, COPD patients; FEV1/FVC <70% and FEV1 30-80%pred, age >65 at time of surgery
4) non-COPD control subjects (Older COPD-matched); FEV1/FVC >70%, age >65 at time of surgery

None of the COPD patients was alpha-1 antitrypsin deficient. To get sufficient SEO-COPD-matched non-COPD control subjects, subjects at an age <60 at the time of surgery were included, taken into account the age-matching with the SEO-COPD group.

The study protocol was consistent with the Research Code of the University Medical Centre Groningen and national ethical and professional guidelines ("Code of conduct; Dutch federation of biomedical scientific societies", htttp://www.federa.org). Lung fibroblasts and lung tissues used in this study are derived from left-over lung material after lung surgery and transplant procedures. This material was not subject to the act on medical research involving human subjects in the Netherlands and therefore an ethics waiver was provided by the Medical Ethical Committee of the University Medical Centre Groningen (METc UMCG). All samples and clinical information were de-identified before experiments were performed.

Primary parenchymal lung fibroblast culture

The primary parenchymal lung fibroblasts were defrosted and cultured in batches of four, including fibroblasts from each subgroup in equal numbers, as described before [1]. At passage 5, 25000 fibroblasts were seeded in Ham's F12 medium + 5% FCS in 12-well plates and after two days treated with or without 250 µM Paraquat dichloride hydrate (PQ) (Sigma-Aldrich, Zwijndrecht, the Netherlands) for 24 hours to induce cellular senescence [3]. After 24 hours, PQ was removed and cells were kept in culture for four days in Ham's F12 medium + 5% FCS. These time-points were carefully chosen based on pilot study results.

Olink Proteomics

The highly sensitive Olink Proteomics (Olink Proteomics, Uppsala, Sweden) panels *Inflammation* and *Cardiovascular III*, were used to measure the secretion of 184 proteins, whereof 165 proteins passed QC. The Olink Proteomics analysis uses an antibody-based method called Proximity Extension Assay technology. Briefly, oligonucleotide-labelled antibody pairs bind the target protein and when oligonucleotides are in close proximity, these hybridize and get extended by a DNA polymerase. This created DNA barcode is amplified and quantified by qPCR. A full explanation about this analysis can be found on their website: https://www.olink.com/data-you-can-trust/technology/. Levels of secreted proteins were corrected for total cell numbers four days after senescence induction.

Secreted protein analyses

Cell-free supernatants were harvested four days after PQ removal and stored in -80°C prior to analyses. Secreted IL-8 levels were measured using Human DuoSet ELISA (R&D Systems, Abingdon, United Kingdom). As the numbers of cells were different at the end of culture between COPD and control-derived fibroblasts, and between untreated and PQ-treated, we corrected the secreted protein levels for cell numbers counted at the end of culture.

Statistical analyses

SPSS software was used for the statistical analyses. Significant differences between PQ treated and untreated cells were tested using Wilcoxon signed-rank test adjusted for multiple testing using Benjamini-Hochberg. Proteins were defined as SASP protein when a significant (FDR <0.05) ≥3-fold increase in secretion was observed upon PQ treatment. Next, statistical differences in SASP protein secretion between untreated COPD- and control-derived fibroblasts were tested using Mann-Whitney U. FDR P<0.05 was considered statistically significant.

Table S1: Overview of all 124 defined SASP proteins

PROTEIN	FOLD CHANGE	P-VALUE	FDR	DESCRIBED OR NOVEL?
GDF-15	9.331	5.255E-08	7.226E-08	SASP Atlas
GDNF	8.946	5.255E-08	7.226E-08	Potentially novel
CCL3	6.695	5.683E-08	7.442E-08	SASP Atlas
TGF-ALPHA	5.737	5.255E-08	7.226E-08	Potentially novel
OPN	5.129	5.255E-08	7.226E-08	Potentially novel
TNFRSF10C	5.017	5.253E-08	7.226E-08	Previously described
4E-BP1	4.776	5.255E-08	7.226E-08	SASP Atlas
IL13	4.668	5.255E-08	7.226E-08	Previously described
KLK6	4.651	5.253E-08	7.226E-08	Potentially novel
CCL19	4.636	5.253E-08	7.226E-08	SASP protein family
FGF-19	4.621	5.253E-08	7.226E-08	SASP protein family
IL10	4.564	5.255E-08	7.226E-08	Previously described
EP-CAM	4.490	9.008E-07	1.047E-06	Potentially novel
TFF3	4.486	5.255E-08	7.226E-08	Potentially novel
CCL16	4.484	5.255E-08	7.226E-08	Previously described
RETN	4.459	5.255E-08	7.226E-08	Potentially novel
IL-17C	4.455	5.255E-08	7.226E-08	Previously described
GAL-4	4.435	5.255E-08	7.226E-08	Potentially novel
CASP-8	4.409	5.255E-08	7.226E-08	Potentially novel
CD5	4.387	5.255E-08	7.226E-08	Potentially novel
CCL23	4.379	5.253E-08	7.226E-08	SASP protein family
IL4	4.373	5.255E-08	7.226E-08	Previously described
CCL15	4.370	5.253E-08	7.226E-08	SASP protein family
SPON1	4.359	5.255E-08	7.226E-08	Potentially novel
CASP-3	4.351	5.253E-08	7.226E-08	Previously described
IGFBP-1	4.350	5.255E-08	7.226E-08	SASP protein family
RANKL	4.346	5.255E-08	7.226E-08	Potentially novel
IL-20	4.335	5.255E-08	7.226E-08	SASP protein family
ST1A1	4.332	5.255E-08	7.226E-08	Potentially novel
IL-10RA	4.331	5.255E-08	7.226E-08	SASP protein family
CDH5	4.330	5.255E-08	7.226E-08	Potentially novel
CXCL9	4.328	5.255E-08	7.226E-08	SASP protein family
CD8A	4.322	5.253E-08	7.226E-08	Potentially novel
CCL24	4.321	5.255E-08	7.226E-08	SASP protein family
AP-N	4.320	5.255E-08	7.226E-08	SASP Atlas
TNFSF14	4.316	5.255E-08	7.226E-08	SASP protein family
TNFB	4.316	5.255E-08	7.226E-08	SASP protein family
STAMBP	4.311	5.253E-08	7.226E-08	Potentially novel
IL-17A	4.309	5.253E-08	7.226E-08	Previously described
PON3	4.309	5.255E-08	7.226E-08	Potentially novel
IL-2RB	4.308	5.255E-08	7.226E-08	SASP protein family
PGLYRP1	4.305	5.255E-08	7.226E-08	Potentially novel
IL-17RA	4.302	5.255E-08	7.226E-08	SASP protein family

CCL4	4.301	5.255E-08	7.226E-08	SASP protein family
CD163	4.301	5.255E-08	7.226E-08	Potentially novel
MEPE	4.287	5.255E-08	7.226E-08	Potentially novel
FGF-23	4.278	5.251E-08	7.226E-08	SASP protein family
MPO	4.271	5.255E-08	7.226E-08	Previously described
IL-24	4.269	5.255E-08	7.226E-08	SASP protein family
IL-1 ALPHA	4.262	3.782E-07	4.588E-07	Previously described
PSP-D	4.249	5.255E-08	7.226E-08	Potentially novel
CCL28	4.247	5.255E-08	7.226E-08	SASP protein family
SELP	4.239	5.255E-08	7.226E-08	Potentially novel
LIF-R	4.225	5.253E-08	7.226E-08	Potentially novel
TNFRSF14	4.224	5.255E-08	7.226E-08	SASP protein family
VWF	4.217	5.255E-08	7.226E-08	Potentially novel
SIRT2	4.214	5.253E-08	7.226E-08	Potentially novel
AZU1	4.212	5.253E-08	7.226E-08	Potentially novel
FGF-21	4.211	5.255E-08	7.226E-08	SASP protein family
CD6	4.190	5.255E-08	7.226E-08	Potentially novel
MMP-9	4.183	5.255E-08	7.226E-08	SASP Atlas
CCL25	4.182	5.255E-08	7.226E-08	Previously described
SCGB3A2	4.179	5.253E-08	7.226E-08	Potentially novel
TR	4.175	5.253E-08	7.226E-08	SASP Atlas
CPA1	4.172	5.253E-08	7.226E-08	Potentially novel
CD244	4.168	5.255E-08	7.226E-08	Potentially novel
PECAM-1	4.166	5.255E-08	7.226E-08	Potentially novel
TNF	4.166	5.251E-08	7.226E-08	Previously described
NOTCH 3	4.159	5.253E-08	7.226E-08	Potentially novel
IL-22 RA1	4.153	5.255E-08	7.226E-08	SASP protein family
OSM	4.151	5.251E-08	7.226E-08	Potentially novel
TR-AP	4.141	5.255E-08	7.226E-08	Potentially novel
IL-20RA	4.129	5.255E-08	7.226E-08	SASP protein family
IL-1RT2	4.125	5.255E-08	7.226E-08	SASP protein family
EN-RAGE	4.121	4.070E-07	4.831E-07	Potentially novel
NRTN	4.114	5.255E-08	7.226E-08	Potentially novel
IL2	4.105	5.253E-08	7.226E-08	Previously described
ADA	4.097	5.253E-08	7.226E-08	Potentially novel
IFN-GAMMA	4.095	5.255E-08	7.226E-08	Previously described
U-PAR	4.093	5.255E-08	7.226E-08	SASP Atlas
ICAM-2	4.090	5.255E-08	7.226E-08	Potentially novel
AXIN1	4.089	5.255E-08	7.226E-08	Potentially novel
TIMP4	4.081	5.253E-08	7.226E-08	SASP protein family
CHIT1	4.078	5.255E-08	7.226E-08	Potentially novel
CPB1	4.068	5.255E-08	7.226E-08	Potentially novel
GP6	4.050	5.255E-08	7.226E-08	Potentially novel
ARTN	4.048	5.255E-08	7.226E-08	Potentially novel
VEGFA	4.047	5.255E-08	7.226E-08	Previously described
IL18	4.025	9.669E-07	1.101E-06	SASP Atlas

DNER	4.018	5.255E-08	7.226E-08	Potentially novel
TSLP	3.994	5.255E-08	7.226E-08	Potentially novel
IL33	3.989	5.255E-08	7.226E-08	SASP protein family
IL5	3.985	5.255E-08	7.226E-08	SASP protein family
PDGFA	3.950	5.255E-08	7.226E-08	Previously described
SHPS-1	3.948	5.255E-08	7.226E-08	Potentially novel
CD93	3.944	5.253E-08	7.226E-08	Potentially novel
ST2	3.938	5.255E-08	7.226E-08	SASP protein family
IL2-RA	3.912	5.253E-08	7.226E-08	SASP protein family
LTBR	3.896	5.255E-08	7.226E-08	Potentially novel
PCSK9	3.847	5.255E-08	7.226E-08	Potentially novel
SELE	3.833	5.251E-08	7.226E-08	Potentially novel
IL-18BP	3.785	5.255E-08	7.226E-08	SASP protein family
IL-15RA	3.781	5.255E-08	7.226E-08	SASP protein family
EPHB4	3.756	5.253E-08	7.226E-08	Potentially novel
TNFRSF9	3.736	5.255E-08	7.226E-08	SASP protein family
TLT-2	3.680	5.255E-08	7.226E-08	Potentially novel
FABP4	3.667	5.255E-08	7.226E-08	Previously described
NT-PROBNP	3.666	5.255E-08	7.226E-08	Potentially novel
GAL-3	3.548	5.253E-08	7.226E-08	SASP Atlas
CX3CL1	3.547	5.683E-08	7.442E-08	Previously described
BETA-NGF	3.487	5.255E-08	7.226E-08	Previously described
IL-10RB	3.474	5.255E-08	7.226E-08	SASP protein family
SCF	3.449	5.255E-08	7.226E-08	Previously described
CCL20	3.442	1.196E-06	1.351E-06	Previously described
IL-18R1	3.440	5.255E-08	7.226E-08	SASP protein family
T-PA	3.424	5.683E-08	7.442E-08	SASP Atlas
CXCL11	3.302	5.255E-08	7.226E-08	Previously described
TNF-R2	3.263	5.253E-08	7.226E-08	Previously described
IL-12B	3.259	5.253E-08	7.226E-08	SASP protein family
PD-L1	3.166	5.255E-08	7.226E-08	Potentially novel
CTSZ	3.100	5.255E-08	7.226E-08	SASP Atlas
FGF-5	3.042	5.255E-08	7.226E-08	SASP protein family
CXCL16	3.029	5.255E-08	7.226E-08	SASP protein family
CD40	3.011	4.070E-07	4.831E-07	Previously described

- *Fold change: Median of fold changes between PQ treated and untreated primary lung fibroblasts.*
- *P-value: tested using Wilcoxon signed-rank tests.*
- *FDR: P-value adjusted for multiple testing using Benjamini-Hochberg correction.*
- *Last column describes overlap with SASP Atlas [4], PubMed search for previous described, and protein families of described SASP proteins [5].*

Table S2: Overview of all 42 COPD associated SASP proteins

	COPD VS CONTROLS			SEO-COPD VS MATCHED CONTROLS			OLDER, MM COPD VS MATCHED CONTROLS		
PROTEIN	**FC**	**P-value**	**FDR**	**FC**	**P-value**	**FDR**	**FC**	**P-value**	**FDR**
RANKL	1.6630	0.0054	**0.0379**	1.4804	0.0193	**0.0379**	1.6704	0.0783	0.1820
FABP4	1.5049	0.0018	**0.0379**	1.4286	0.0071	**0.0375**	1.3885	0.1809	0.2111
IGFBP-1	1.4742	0.0113	**0.0450**	1.1928	0.0500	0.0568	1.5197	0.0910	0.1820
T-PA	1.4575	0.0132	**0.0450**	1.6420	0.0114	**0.0375**	1.1056	0.3981	0.4180
GP6	1.4362	0.0051	**0.0379**	1.4196	0.0179	**0.0379**	1.4577	0.0910	0.1820
CPA1	1.4189	0.0011	**0.0379**	1.3138	0.0033	**0.0375**	1.4426	0.0573	0.1820
MMP-9	1.3942	0.0122	**0.0450**	1.5136	0.0043	**0.0375**	1.1451	0.4813	0.4931
IL2-RA	1.3935	0.0030	**0.0379**	1.3822	0.0143	**0.0375**	1.3723	0.0573	0.1820
IL-12B	1.3780	0.0070	**0.0379**	1.5486	0.0243	**0.0379**	1.2342	0.1590	0.1964
TFF3	1.3685	0.0076	**0.0379**	1.2113	0.0338	**0.0443**	1.4524	0.0671	0.1820
VWF	1.3678	0.0076	**0.0379**	1.3410	0.0222	**0.0379**	1.2822	0.1590	0.1964
AP-N	1.3555	0.0047	**0.0379**	1.3294	0.0222	**0.0379**	1.4039	0.0573	0.1820
CHIT1	1.3418	0.0047	**0.0379**	1.2939	0.0500	0.0568	1.4140	0.0411	0.1820
CD93	1.3304	0.0008	**0.0379**	1.2934	0.0143	**0.0375**	1.3839	0.0242	0.1820
ST2	1.3296	0.0030	**0.0379**	1.4317	0.0114	**0.0375**	1.1581	0.1213	0.1960
EN-RAGE	1.3288	0.0169	**0.0500**	1.3847	0.0305	**0.0427**	1.2412	0.2050	0.2265
SPON1	1.3282	0.0076	**0.0379**	1.3119	0.0222	**0.0379**	1.3587	0.0910	0.1820
TR-AP	1.3258	0.0055	**0.0379**	1.3555	0.0338	**0.0443**	1.3781	0.0783	0.1820
CCL15	1.3137	0.0060	**0.0379**	1.2205	0.0275	**0.0412**	1.3429	0.0910	0.1820
ST1A1	1.3074	0.0024	**0.0379**	1.4220	0.0118	**0.0375**	1.3526	0.0671	0.1820
TIMP4	1.3072	0.0070	**0.0379**	1.2771	0.0143	**0.0375**	1.3158	0.1590	0.1964
AZU1	1.3067	0.0039	**0.0379**	1.3340	0.0143	**0.0375**	1.2909	0.1213	0.1960
LIF-R	1.3004	0.0145	**0.0450**	1.3056	0.0152	**0.0375**	1.3207	0.1213	0.1960
PDGFA	1.2988	0.0033	**0.0379**	1.3515	0.0412	**0.0495**	1.4048	0.0346	0.1820
PECAM-1	1.2950	0.0036	**0.0379**	1.2810	0.0179	**0.0379**	1.5383	0.0671	0.1820
PGLYRP1	1.2943	0.0097	**0.0445**	1.4064	0.0222	**0.0379**	1.2557	0.1590	0.1964
MEPE	1.2928	0.0132	**0.0450**	1.3013	0.0864	0.0864	1.2737	0.0783	0.1820
SELP	1.2824	0.0036	**0.0379**	1.3386	0.0114	**0.0375**	1.2940	0.0783	0.1820
NRTN	1.2803	0.0064	**0.0379**	1.1821	0.0305	**0.0427**	1.4130	0.0573	0.1820
MPO	1.2742	0.0097	**0.0445**	1.2814	0.0143	**0.0375**	1.3080	0.1392	0.1964
IL-10RA	1.2725	0.0059	**0.0379**	1.2351	0.0118	**0.0375**	1.3135	0.1053	0.1960
KLK6	1.2679	0.0122	**0.0450**	1.4624	0.0604	0.0634	1.1514	0.1590	0.1964
FGF-23	1.2570	0.0157	**0.0474**	1.2329	0.0118	**0.0375**	1.3974	0.2313	0.2491
CDH5	1.2547	0.0036	**0.0379**	1.2409	0.0090	**0.0375**	1.4702	0.0910	0.1820
U-PAR	1.2530	0.0142	**0.0450**	1.4439	0.0864	0.0864	1.2140	0.0486	0.1820
CCL23	1.2522	0.0076	**0.0379**	1.2071	0.0576	0.0621	1.2669	0.0486	0.1820
CD8A	1.2480	0.0124	**0.0450**	1.2829	0.0380	**0.0469**	1.2830	0.1590	0.1964
PSP-D	1.2365	0.0122	**0.0450**	1.6285	0.0025	**0.0375**	1.0308	0.5262	0.5262
CXCL9	1.2358	0.0145	**0.0450**	1.2279	0.0380	**0.0469**	1.3673	0.1590	0.1964
IL-15RA	1.2023	0.0124	**0.0450**	1.2376	0.0243	**0.0379**	1.1905	0.2050	0.2265

TNFSF14	1.2016	0.0114	**0.0450**	1.1898	0.0243	**0.0379**	1.2558	0.1809	0.2111
FGF-19	1.1882	0.0145	**0.0450**	1.1634	0.0576	0.0621	1.3390	0.1213	0.1960

- *FC (Fold change): Fold change in medians of COPD compared to control-derived fibroblasts.*
- *P-value: tested using Mann-Whitney U tests.*
- *FDR: P-value adjusted for multiple testing using Benjamini-Hochberg correction. Boldfaced when significant.*

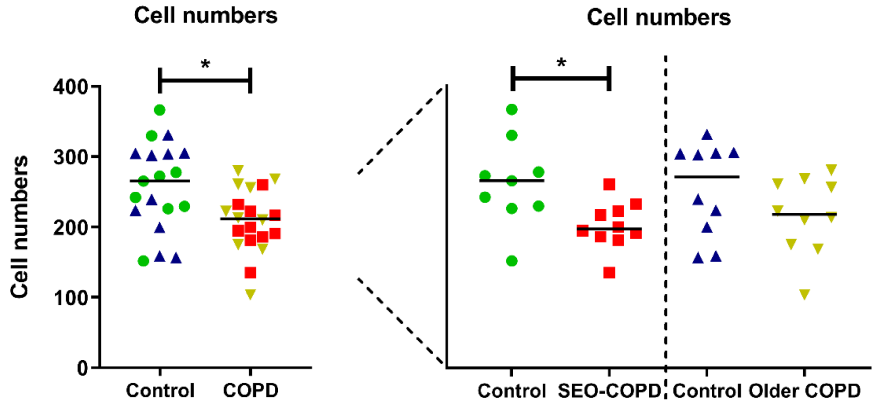

Figure S1: Cell number differences between fibroblasts from COPD patients and controls at baseline. *Dot plots show total cell number at the end of culture of all 4 patient groups. Green = SEO-COPD-matched control, red = SEO-COPD, blue = older COPD-matched control, yellow = older, mild-moderate, COPD. Lines represent medians. Significant differences tested with Mann-Whitney U tests. * P-value < 0.05.*

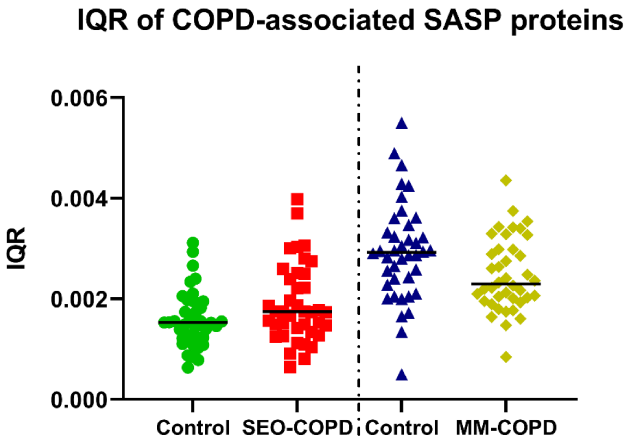

Figure S2: Interquartile ranges of COPD-associated proteins per subgroup*. Interquartile ranges (IQR) of the 42 COPD-associated SASP proteins per subgroup. Green = SEO-COPD-matched controls, red = SEO-COPD, blue = older, mild-moderate COPD-matched controls, yellow = older, mild-moderate COPD.*

References

1 Noordhoek JA, Postma DS, Chong LL*, et al*. Different modulation of decorin production by lung fibroblasts from patients with mild and severe emphysema. *COPD.* 2005;**2** (suppl 1):17-25.

2 Silverman EK, Chapman HA, Drazen JM*, et al*. Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease. Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med.* 1998;**157** (suppl 6 Pt 1):1770-1778.

3 Chinta SJ, Woods G, Demaria M*, et al*. Cellular Senescence Is Induced by the Environmental Neurotoxin Paraquat and Contributes to Neuropathology Linked to Parkinson's Disease. *Cell Rep.* 2018;**22** (suppl 4):930-940.

4 Basisty N, Kale A, Jeon OH*, et al*. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol.* 2020;**18** (suppl 1):e3000599.

5 Coppe JP, Desprez PY, Krtolica A*, et al*. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol.* 2010;**5**:99-118.