Diplomarbeit

Senescence and autophagy in idiopathic pulmonary fibrosis and other fibrosing lung diseases

eingereicht von

Florian Gallob

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Diagnostic and Research Institute of Pathology

unter der Anleitung von Univ.-Prof. Dr. Helmut Popper und

Priv.-Doz. DDr. Luka Brcic

Graz, 07.11.2020

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 07.11.2020 *Florian Gallob eh*

Vorwort

Im Herbst 2018 entstand durch Univ.-Prof. Dr. Helmut Popper die Idee, einen Artikel zu den noch zum Teil ungeklärten Pathomechanismen von idiopathischer Lungenfibrose (IPF) zu verfassen.

Auf Anstoß meiner Diplomarbeitsbetreuer Univ.-Prof. Dr. Helmut Popper und Priv.-Doz. DDr. Luka Brcic nahmen dann die vorliegende Diplomarbeit und das mittlerweile publizierte Paper Form an.

Darauf folgte im Dezember 2018 eine erste Literaturrecherche. Dabei wurden Seneszenz und Autophagie als mögliche Pathomechanismen bei IPF identifiziert, die aber noch nicht endgültig verstanden oder geklärt sind, beziehungsweise wozu es bei Autophagie widersprüchliche Daten in der Literatur gibt.

Ausgewählte Gewebeproben wurden vom Krankenhaus München-Bogenhausen und dem Lungen Archiv vom Diagnostic and Research Institute of Pathology der Medizinischen Universität Graz bezogen und mit den nötigen klinischen Daten versehen.

In Folge konnten gemeinsam immunhistochemische Marker identifiziert werden, die es ermöglichten, Seneszenz und Autophagie im Gewebe nachzuweisen.

Im Verlauf wurden auch noch weitere Punkte von wissenschaftlichem Interesse identifiziert: Einerseits ist bei Annahme einer hochregulierten Autophagie- zu diesem Zeitpunkt konnten wir bereits erkennen, dass sich diese Autophagie-Aktivierung bei uns auch bestätigt- nicht klar, zu welchem Zwecke diese hochreguliert ist. Dabei könnte es sich entweder um einen protektiven Mechanismus gegen die chronische Entzündung, der dann ein Gegenspieler der Seneszenz wäre, handeln oder aber um eine Reaktion auf lokale Minderversorgung, ein Mechanismus, der aus dem Bereich der Tumorforschung bereits bekannt und beschrieben ist. Andererseits wurde bei der Literaturrecherche auch die Unklarheit bezüglich des zellulären Ursprungs des fibrotischen Gewebes erkannt. Um in Bezug auf diese beiden Fragenbereiche weitere Rückschlüsse zu ziehen, wurden auch hier entsprechende immunhistochemische Marker gefunden und benutzt, um dem zellulären Metabolismus und dem Ursprung von epithelialen Zellen in umgebauten Arealen nachzuforschen.

Hier möchte ich Frau Sylvia Eidenhammer danken, ohne deren Hilfe die immunhistochemische Aufbereitung und Analyse nicht möglich gewesen wäre.

Auch bei der Auswertung und der anschließenden Bewertung der Ergebnisse standen mir meine Diplombetreuer zur Seite. Letztendlich wurde das Manuskript von Virchows Archiv zur Veröffentlichung nach einer Revision angenommen.
Bereits 2019 konnte ein Poster mit dem Titel „Autophagy and senescence are activated mechanisms in idiopathic and autoimmunity caused usual interstitial pneumonia" mit einer ersten Auswertung der Ergebnisse erstellt werden, welches beim ERS Kongress 2019 in Madrid bei einer Poster Discussion vorgestellt wurde (das Poster ist im Anhang zu finden).
Recht zeitgleich mit der Publikation fand der virtuelle ERS Kongress 2020 (European Respiratory Society) statt, an dem ein gemeinsames E-Poster mit einer Tonaufnahme eingereicht und am virtuellen Kongress vorgestellt wurde. Dieses E-Poster mit dem Titel „What cells are replacing the epithelium in cystic remodeling in UIP and COPD" ist die anschließende Forschungsfrage, an die in der Publikation gewonnene Erkenntnis, dass die Zellen in umgebauten Arealen in UIP einen peripheren Ursprung nehmen (das E-Poster ist im Anhang zu finden). Wir konnten im Zuge dieses E-Posters auch Seneszenz in Lungengeweben von COPD Patienten und auch von Asthma Patienten nachweisen. Zusätzlich wurde hier aber auch- ausgehend von bisherigen Erkenntnissen- die Zytokeratin-Expression der jeweiligen Gewebearten untersucht.
Dieses E-Poster führte dann bereits zur Idee einer neuen Publikation, da hier Unterschiede im Zyotkeratin-Expressionsmuster zwischen UIP und COPD Lungengeweben identifiziert wurden. So läuft momentan noch die weitere Auswertung der verschiedenen Zytokeratinmuster in COPD und Asthma Lungengewebeproben. Zusätzlich wird auch versucht, neue Erkenntnisse über die Seneszenz in der Pathogenese der COPD zu gewinnen, nachdem das Thema zwar wissenschaftlich bereits behandelt, aber weiterhin nicht abschließend geklärt ist.
Abschließend möchte ich mich noch bei allen bedanken, die mir meinen ersten Kontakt zu wissenschaftlichem Arbeiten und der medizinischen Grundlagenforschung ermöglicht haben, allen voran natürlich meinen beiden Diplomarbeitsbetreuern.
Herzlichen Dank euch beiden!
Der Artikel ist bei Springer Nature unter dem Link https://doi.org/10.1007/s00428-020-02917-2 verfügbar.

Inhaltsverzeichnis

Vorwort 2

Abkürzungen 5

Abbildungsverzeichnis 6

Tabellenverzeichnis 7

Zusammenfassung 8

Abstract 9

Introduction 10

Material and methods 11

Study population and clinical data 11
Immunohistochemistry 11

Results 13

Study population 13
Senescence 13
Autophagy 14
Cellular metabolism with respect to hypoxia 14
Origin of epithelial cells 15

Discussion 15

Conclusion 17

References 17

Anhang 20

Abkürzungen

AuD	Autoimmune disease
CK 5/6	Cytokeratin 5/6
EMT	Epithelial-mesenchymal transition
GLUT1	Glucose transporter 1
HP	Hypersensitivity pneumonia
ILD	Interstitial lung disease
IPF	Idiopathic pulmonary fibrosis
LC3	(Microtubule-associated protein 1A/1B) Light chain 3
LDH	Lactate dehydrogenase
MAP1S	Microtubule-associated protein 1S
NSIP	Nonspecific interstitial pneumonia
pAMK	Phosphorylated adenosin-5' Monophosphate activated kinase
SIRT1	Sirtuin 1
TTF1	Thyroid transcription factor 1
TGF- ß	Transforming growth factor beta
UIP	Usual interstitial pneumonia
VATS	Video-assisted thoracoscopic surgery

Abbildungsverzeichnis

Figure 1: Senescence in IPF (a) p16 staining; (b) p21 staining. 14

Figure 2: Autophagy in IPF (a) LC3; (b) pAMK 15

Figure 3: Autophagy in IPF (a) and (b) SIRT1; (c) MAP1S...15

Figure 4: Cellular metabolism in IPF (a) LDH; (b) GLUT1. 16

Figure 5: Orgin of epithelial cells in IPF (a) TTF1; (b) CK 5/6 16

Tabellenverzeichnis

Table 1 Clinical and radiological data of patients 12
Table 2 Immunohistochemistry of the antibodies and methodology. 13
Table 3 Detailed results of the markers for senescence and autophagy in UIP/IPF and UIP/AuD. 14

Zusammenfassung

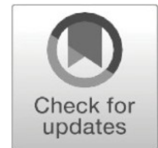
Wegen der sehr schlechten Prognose und Großteils fehlender Therapiemöglichkeiten ist bei idiopathischer Lungenfibrose weiterführende Forschungsarbeit besonders relevant. Unlängst konnte mehr und mehr der Pathophysiologie von IPF verstanden werden, dabei waren vor allem auch Seneszenz und die Rolle der Autophagie von besonderer Bedeutung. Daher wurden 23 Fälle von mikroskopisch gewöhnlicher interstitieller Pneumonie (UIP) ausgewählt, der ätiologisch einerseits IPF und andererseits chronische Autoimmunkrankheiten zugrunde lagen. Von diesen 23 Fällen wurden Proben entnommen, diese wurden mit Formalin fixiert und auf Paraffin eingebettet und anschließend mit immunhistochemischen Antikörpern eingefärbt. Als Marker für Seneszenz wurden p16, p21 und ß-Galactosidase, als Marker für Autophagie LC3, SIRT1, MAP1S und pAMKa, als Marker für den zellulären Metabolismus LDH und GLUT1 und als Marker für den zellulären Ursprung TTF1 und CK5 verwendet. In diesen Färbungen zeigte sich, dass epitheliale Zellen, nicht aber Myofibroblasten in veränderten Lungenarealen positiv auf P21 und P16 gefärbt wurden, beides Marker für Seneszenz. Dabei zeigte sich P16 als spezifischerer Marker. Autophagie konnte mittels der vier Marker LC3, SIRT1, MAP1S und pAMKa sowohl in epithelialen Zellen, als auch in Myofibroblasten, im Bronchialepithel und auch in Pneumozyten in gesunden Lungenarealen nachgewiesen werden. Ob die Autophagie als anti-inflammatorischer Prozess auftritt, also damit gegen IPF arbeiten würde, oder ob der hochregulierten Autophagie eine zelluläre Hypoxie zu Grunde liegt, konnte mit Hilfe von LDH und GLUT1 beantwortet werden. Beide Marker waren positiv in Epithelien und in Myofibroblasten in umgebauten Arealen, womit die zelluläre Hypoxie als Ursache für die Aktivierung von Autophagie anzunehmen ist. Unsere Hypothese, dass die Zellen in ihren Zellverbänden mit eingeschränkter Versorgung die Autophagie als Energiequelle nutzen, wurde entsprechend diskutiert. Der Ursprung der epithelialen Zellen in UIP wird ebenfalls divers gesehen (Annahme, daß die Zellen aus dem zentralen Bronchialsystem stammen), daher wurde versucht mit der Färbung auf TTF1 und CK5 zwischen einem lokalen Ursprung oder einer Theorie von eingewanderten Zellen zu differenzieren. Nachdem die Zellen in umgebauten Arealen sowohl TTF1, als auch CK5 exprimierten, ist von einem Ursprung dieser Zellen aus den Basalzellen der Bronchiolen auszugehen.

Abstract

Idiopathic pulmonary fibrosis (IPF) is a disease with a dismal prognosis. Currently, the causing agent(s) are poorly understood. Recent data suggest that senescence and autophagy might play a role in its development, as well as changes in metabolism due to hypoxic conditions. In this study, the expression of senescence markers in 23 cases of usual interstitial pneumonia (UIP)/IPF and UIP/chronic autoimmune diseases (UIP/AuD) was investigated. The status of autophagy was evaluated with respect to either antiinflammatory or antihypoxia function. Formalin-fixed paraffin-embedded tissues of UIP were selected for immunohisto- chemistry with antibodies for p21, p16, and ß-galactosidase (senescence); for LC3, SIRT1, MAP1S, and pAMKa (autophagy); and for LDH and GLUT1 (metabolism). Epithelial cells in cystic remodeled areas of UIP stained for p16 and p21, p16 being more specific compared with p21. Myofibroblasts were negative in all cases. An upregulation of all four autophagy markers was seen not only in epithelia within remodeled areas and proliferating myofibroblasts, but also in bronchial epithelia and pneumocytes. Upregulated autophagy points to a compensatory mechanism for hypoxia; therefore, LDH and GLUT1 were investigated. Their expression was present in epithelia within cystic remodeling and in myofibroblasts. The cells within the remodeled areas stained for cytokeratin 5, but coexpressed TTF1, confirming their origin from basal cells of bronchioles. Within this population, senescent cells arise. Our results indicated that autophagy in UIP very likely helps cells to survive in hypoxic condition. By phagocytosis of cellular debris, they supplement their need for nutrition, and by upregulating LDH and GLUT1, they compensate for local hypoxia.

Virchows Archiv
https://doi.org/10.1007/s00428-020-02917-2

ORIGINAL ARTICLE



Senescence and autophagy in usual interstitial pneumonia of different etiology

Florian Gallob[1] & Luka Brcic [1] & Sylvia Eidenhammer[1] & Florian Rumpp [2] & Andreas Nerlich[3] & Helmut Popper [1]

Received: 5 April 2020 / Revised: 15 July 2020 / Accepted: 19 August 2020
The Author(s) 2020

Abstract
Idiopathic pulmonary fibrosis (IPF) is a disease with a dismal prognosis. Currently, the causing agent(s) are poorly understood. Recent data suggest that senescence and autophagy might play a role in its development, as well as changes in metabolism due to hypoxic conditions. In this study, the expression of senescence markers in 23 cases of usual interstitial pneumonia (UIP)/IPF and UIP/chronic autoimmune diseases (UIP/AuD) was investigated. The status of autophagy was evaluated with respect to either antiinflammatory or antihypoxia function. Formalin-fixed paraffin-embedded tissues of UIP were selected for immunohistochemistry with antibodies for p21, p16, and ß-galactosidase (senescence); for LC3, SIRT1, MAP1S, and pAMKα (autophagy); and for LDH and GLUT1 (metabolism). Epithelial cells in cysticremodeled areas of UIP stained for p16 and p21, p16 being more specific compared with p21. Myofibroblasts were negative in all cases. An upregulation of all four autophagy markers was seen not only in epithelia within remodeled areas and proliferating myofibroblasts, but also in bronchial epithelia and pneumocytes. Upregulated autophagy points to a compensatory mechanism for hypoxia; therefore, LDH and GLUT1 were investigated. Their expression was present in epithelia within cystic remodeling and in myofibroblasts. The cells within the remodeled areas stained for cytokeratin 5, but coexpressed TTF1, confirming their origin from basal cells of bronchioles. Within this population, senescent cells arise. Our results indicated that autophagy in UIP very likely helps cells to survive in hypoxic condition. By phagocytosisofcellulardebris, they supplement their need for nutrition, and by upregulating LDH and GLUT1, they compensate for local hypoxia.

Keywords IPF · UIP · Senescence · p16 · Autophagy · LC3 · SIRT · MAP1S · pAMKα · Regeneration in cysts · Cytokeratin 5 · TTF1 · Myofibroblast · Hypoxia · LDH · GLUT1

Introduction

Interstitial lung diseases (ILDs) are a heterogenous group of diseaseswith differentetiology, clinical, radiological, and histologic presentation. They can be classified based on etiology (known and unknown) or based on histology, with usual interstitial pneumonia (UIP) as the most common pattern [24, 44, 49, 60]. Idiopathic pulmonary fibrosis (IPF) is the most severe disease characterized by an UIP pattern. Many aspects of the pathogenesis have been reported, such as premature aging of pneumocytes due to defects in the telomere or the surfactant gene system [15, 30, 31, 36, 42, 46, 47, 53, 55]. Initially, the occurrence of UIP pattern in diseases other than IPF was not accepted; however, this changed. Furthermore, it was recognized that the disease course in chronic autoimmune diseases with UIP pattern (UIP/AuD) has a similar dismal course as IPF (UIP/IPF) [1]. The incidence of IPF in Europe ranges between 5 and 8.5 per 100,000 individuals per year with short mean survival of 2.5 to 5 years after diagnosis [21, 40]. Current therapy includes deceleration of the disease progression with antifibrotic drugs Nintedanib and Pirfenidon, symptom release with oxygen, and, as ultima ratio, lung transplantation [13, 32]. Two processes might play a key role in development of UIP/IPF: senescence and autophagy [37, 45].

Senescence is the state of cell cycle arrest, which is initiated by cyclin-dependent kinase inhibitors such as p16, p21, and

Master thesis work of Florian Gallob

* Helmut Popper
helmut.popper@medunigraz.at

[1] Diagnostic and Research Institute of Pathology, Medical University of Graz, Neue Stiftingtalstrasse 6, 8010 Graz, Austria

[2] Pulmonology Department, Clinics Munich-Bogenhausen, Munich, Germany

[3] Pathology Department, Clinics Munich-Bogenhausen, Munich, Germany

Published online: 27 August 2020

p53. It is caused by DNA damage and is usually age related [54]. Senescent cells secrete various mediators (e.g., IL-1, IL-6, IL-10, TGF-ß), which promote fibrosis and may play a role in the epithelial mesenchymal transition in UIP, which may be brought on by dysfunctional autophagy [6, 8, 38, 45, 52, 53]. Senescent cells can be identified by the surrogate markers ß-galactosidase, and p21 and p16, inducing senescence through cell cycle inhibition [11, 16, 20, 27, 34]. These markers may be used for immunohistochemical detection [41]. Autophagy is a cellular process for degradation and recycling of cellular debris, importantforhomeostasis. Itcan downregulate inflammation, thus inhibiting the action of senescent cells [7, 23, 58]. Under specific conditions, such us hypoxia, starvation, or the absence of growth factors, autophagy is considerably increased [26]. It was observed that markers for autophagy (LC3-II and the number of autophagosomes) are reduced in IPF lung cell lysates, indicating that dysfunctional autophagy might lead to senescence and myofibroblast transdifferentiation into epithelial cells [5, 43]. However, more recent studies demonstrated that autophagy was necessary for TGF-ß-induced fibrosis in UIP/IPF and that markers for autophagy are seen in both epithelial and mesenchymal cells, whereas samples from UIP/AuD donors showed less autophagic activity [17]. Autophagic activity can be demonstrated using antibodiesfor adenosin-5' monophosphate-activated kinase (AMPK), known as an activator of autophagy, or for microtubule-associated protein 1A/1B-light chain 3 (LC3) and microtubule-associated protein 1S (MAP1S), both involved in the development and degradation of autophagosomes [18, 43, 51, 63]. Nuclear protein SIRT1, a member of the silent information regulator (SIR) gene family, takes part in DNA damage prevention and repair and induces autophagy, and might also be used as a marker of autophagy [25]. Yet, there are controversial studies about the protective effect of SIRT1 against senescence, some stating that SIRT1 may even reverse senescence; other observed that continuous SIRT1 stimulation leads to irreversible senescence [33, 56].

Epithelial-mesenchymal transition (EMT) has also a role in the development of UIP/IPF. However, the exact origin of the cells replacing damaged pneumocytes in UIP is not known. One theory is that basal cells from the upper airways, which have stem cell properties, migrate to remodeled areas in UIP [39, 61]. The staining pattern of cytokeratin 5 (CK5) may be one way to investigate the origin of cells replacing the damaged pneumocytes in UIP. CK5, physiologically expressed by basal cells in upper airways, was observed in the epithelia of remodeled areas in UIP samples, and was also pronounced in the distal airways and alveoli when compared with healthy samples [48, 64]. Staining for the thyroid transcription factor 1 (TTF1) will additionally point towards the origin of these epithelial cells from peripheral lung epithelia [57]. The origin of myofibroblasts in UIP is not known. Some theories prefer EMT; other suggest involvement of circulating mesenchymal precursor cells from the bone marrow [3]. Another important factor for the induction of myofibroblast differentiation, and therefore for the pathogenesis of lung fibrosis, is lactic acid. It was shown that lactate-dehydrogenase-A (LDH-A), the enzyme which produces lactate, is overexpressed in UIP, and a decrease in extracellular pH induces a rise in TGF-ß, a known mediator of pulmonary fibrosis [22, 28]. Therefore, the dysregulation of cellular metabolism might be a key factor in the genesis of lung fibrosis. However, how GLUT1 is connected to this dysregulation and the associated hypoxia is not clear. In normal lungs, GLUT1 is expressed only in erythrocytes. In UIP, some state that in areas of fibrosis, fibroblasts induce increased levels of GLUT1-dependent glycolysis to compensate for an elevated energy demand [9]. On the contrary, others state that overexpression of GLUT1 in UIP/IPF is not due to expression in fibroblasts, but in erythrocytes and inflammatory cells (macrophages), probably caused by increased neovascularization and inflammation [12].

The aim of our study was to determine the presence of senescence cells in UIP and to evaluate if autophagy is upregulated ordownregulated. Downregulationof autophagy might counteract senescence by removal of cellular debris, whereas upregulation might point to a mechanism of protecting senescence cells from hypoxic stress. This might lead to a connection with metabolism under hypoxic condition in UIP. The final question was where the cells in remodeled areas (including senescent cells) originate.

Material and methods

Study population and clinical data

During the period from 2012 to 2019, 12 cases from the Hospital München-Bogenhausen and the Lung Archive of the Diagnostic and Research Institute of Pathology, Medical University of Graz, were selected based on pathologic reports and if a definite clinical diagnosis was available. There were 10 cases of IPF/UIP and 13 of UIP due to chronic autoimmune diseases (UIP/AuD) (Table 1). All slides were re-evaluated by two of the authors (HP, LB). The criteria for UIP were focal fibrosis, myofibroblastic foci, temporal heterogeneity (normal lunglobules, myofibroblastic foci, fibrosis), geographical heterogeneity (peripheral accentuation—only in cases with VATS biopsies), and cystic remodeling with bronchiolar metaplasia.

Immunohistochemistry

The tissues were either videothoracoscopic biopsies (17 from all autoimmune disease and some IPF patients and 6 cryobiopies from the remaining IPF patients). Serial sections were taken from selected tissue blocks. One slide was stained

Table 1 Clinical and radiological data of patients; all patients had definite UIP by histology. *ILD* interstitial lung disease, *HP* hypersensitivity pneumonia

Age	Gender	Smoking history	CT scan	Clinical diagnosis (working hypothesis)	Final diagnosis including histology
51	F	No	ILD, NSIP?	Undifferentiated collagen vascular disease	Systemic sclerosis
82	M	Yes	UIP	Collagen vascular disease	Rheumatoid arthritis
78	F	No	UIP	Systemic disease	Rheumatoid arthritis
72	M	No	ILD	Systemic disease	Rheumatoid arthritis
74	M	No	IPF	Did not fit to IPF	Systemic sclerosis
51	M	No	UIP/IPF	Collagen vascular disease	Rheumatoid arthritis
69	M	Yes	ILD	Mixed collagen vascular disease	Mixed collagen vascular disease
57	F	No	ILD	Interstitial lung disease of unknown cause	Systemic sclerosis
50	M	No	ILD	Interstitial lung disease of unknown cause	Mixed collagen vascular disease
75	M	Yes	HP	Do not fit into HP	Chronic autoimmune disease
73	M	Yes	HP	Do not fit into HP	Chronic autoimmune disease
73	M	No	ILD	Interstitial lung disease of unknown cause	Dermatomyositis
76	M	Yes	ILD	Interstitial lung disease of unknown cause	Chronic autoimmune disease
72	M	Yes	UIP	Probable IPF	IPF
60	M	Yes	Probable UIP	Probable IPF	IPF
60	M	Yes	Probable UIP	Probable IPF	IPF
75	M	Yes	UIP	Probable IPF	IPF
68	M	Yes	Probable UIP	Probable IPF	IPF
69	M	Yes	UIP	IPF	IPF
54	M	Yes	ILD	ILD of unknown cause	IPF, very early case
82	M	Yes	UIP	IPF	IPF
69	M	Yes	Probable UIP	Probable IPF	IPF
74	M	Yes	UIP	IPF	IPF

with hematoxylin-eosin, and further sections were incubated with antibodies for p16, p21, ß-galactosidase, MAP1S, pAMPKa, LC3, SIRT1, TTF1, CK5/6, GLUT1, and LDH (detailed immunohistochemistry protocol is provided in Table 2).

ß-Galactosidase, p21, and p16 were used as markers for senescence. Autophagy was investigated by immunohistochemical staining for phosphorylated adenosin-5' monophosphate activated kinase (pAMPKa), LC3, MAP1S, and SIRT1. In order to investigate the possible migration of circulating precursor cells of bronchiolar metaplasia cells, TTF1 was used as a surrogate marker for small airway epithelium and CK5/6 as marker for basal cells of the bronchiolar epithelium. Immunostaining for GLUT1 and LDH was done in order to quantify potential changes in cellular metabolism due to increased energy demand.

The presence (positive reaction) and absence of staining (negative reaction) with abovementioned antibodies was evaluated semiquantitatively for the following compartments: bronchial epithelium, airway-associated smooth muscle cells, vascular endothelium and smooth muscle cells, myofibroblasts, macrophages, bronchiolar metaplasia, and pneumocytes in unaffected lung areas and in areas of microcystic degeneration of lung parenchyma. Positivity was expressed as the percentage of analyzed cells for the markers of senescence (ß-galactosidase, p21, and p16) and autophagy (pAMPKa, LC3, MAP1S, and SIRT1). The evaluation was done independently by two authors (HP, LB). In rare discrepant cases, slides were discussed on a multiheaded microscope and consensus was reached.

Of note, the term myofibroblast was preferred over fibroblasts, as these proliferating cells not only synthesize different

Table 2 Immunohistochemistry of the antibodies and methodology. *MAP1S* microtubule-associated protein 1S, *pAMKa* phosphorylated adenosin-5' monophosphate activated kinase a, *LC3* microtubule-associated protein 1A/1B-light chain 3, *SIRT1* silent information regulator T1, *TTF1* thyroid transcription factor 1, *CK* 5/6 cytokeratin 5/6, *GLUT1* glucose transporter type 1, *LDH* lactate-dehydrogenase

Antibod	P16	P2	ß-Galactosidase	MAP1s	PAMPKa	LC3	SIRT	TTF	CK	GLUT	LD
Producer	ENZ	DAK	Abcam	Novu	Cell Signaling	Nano-tools	LSBio	DAK	DAK	Neo-markers	Abcam
Clone	ENZ-ABS-377-0100		Ab4761	NBP2-47383		0231-100/LC3-5F10	LS-B1895	M357	B4rtu (DAKO GA780)	RB-9052-P	EP1566Y ab52488
Dilution	1:100	1:30	1:150	1:100	1:100	1:200	1:500	1:100	Ready to use	1:200	1:100
Pretreatment	CC1 Ventana	MW9,0 (DAKO S2367)	CC1 Ventana Ultra	CC1 Ventana Ultra	CC1 Ventana Ultra	MW9,0 (DAKO S2367)	MW9,0 (DAKO S2367)	MW9,0 (DAKO S2367)	Flex highpH	MW Natrium-citrat 6,0	MW9,0 (DAKO S2367)
Detection kit	Ventana optiview amplification	Envision DAKO K5007	Ventana optiview amplification	Ventana Ultra-View	Ventana Ultra-View	Envision DAKO K5007	Envision DAKO K5007	Envision DAKO K5007	HRP (GV800)	Envision DAKO K5007	Envision DAKO K5007

collagens, but also contain myofilaments enabling them to migrate (they are stained less intense with smooth muscle actin compared with smooth muscle cells). Furthermore, the term cystic remodeling was preferred over honeycombing: the latter is a radiological term designating cystic structures in secondary lobules, while histopathologically remodeling can already be seen in primary lung lobules.

The study protocol was approved by the Ethics Committee of the Medical University of Graz (EK Number 24-135 ex 11/12).

Results

Study population

A total of 23 patients all with the histological pattern of UIP were investigated. Two groups were formed, one ($n = 10$) with UIP pattern and clinical diagnosis of IPF (UIP/IPF) and a second group ($n = 13$), for which clinical data pointed to an underlying immune disease (UIP/AuD). The age distribution was in the range of 52 to 82; the majority were man. All UIP/IPF patients were cigarette smokers, in contrast to 5/13 UIP/AuD patients. Based on clinical and laboratory data (not shown) as well as the histological findings, a final diagnosis could be established in all cases, although in some cases, a definite type of autoimmune disease was not possible (Table 1).

Senescence

The staining pattern was evaluated separately for different compartments as stated previously. Epithelial cells (transformed pneumocytes and bronchiolar metaplasia) in remodeled areas were p16 and p21 positive in 22% and 57% of analyzed cases, respectively (Fig. 1a). Staining for p21 was positive in approximately 60% of normal pneumocytes as well (Fig. 1b), whereas p16 showed no positivity in normal pneumocytes. Neither p21 nor p16 was positive in myofibroblasts. A low percentage of normal bronchial/bronchiolar epithelial cells were positive for p21 in 19% of all cases, exclusively in UIP/AuD, whereas negative in UIP/IPF samples. p16 was negative in normal bronchial and bronchiolar epithelia in all cases. Endothelia, smooth muscle cells, and macrophages were negative for both p21 and p16. In other compartments, there was no difference concerning the expression of senescence markers in the two groups. Staining for ß-galactosidase was not successful in formalin-fixed paraffin-embedded tissue despite several attempts with different protocols (Table 3).

Table 3 Detailed results of the markers for senescence and autophagy in UIP/IPF and UIP/AuD

		Degenerative/remodeled epithelium	Myofibroblasts	Normal pneumocytes	Bronchial epithelium	Endothelium	Smooth muscle cells	Macrophages
Senescence								
p21	UIP/IPF	9/9	1/9	8/9	0/9	0/9	0/9	0/9
	UIP/AuD	13/13	0/13	13/13	6/13	0/13	0/13	0/13
p16	UIP/IPF	9/9	0/9	1/9	0/9	0/9	0/9	0/9
	UIP/AuD	13/13	1/13	0/13	0/13	0/13	0/13	1/13
Autophagy								
SIRT1	UIP/IPF	10/10	7/10	7/10	6/10	0/10	0/10	10/10
	UIP/AuD	13/13	12/13	11/13	9/13	3/13	0/13	12/13
MAP1S	UIP/IPF	10/10	4/10	8/10	7/10	4/10	0/10	10/10
	UIP/AuD	13/13	6/13	7/13	7/13	2/13	0/13	12/13
LC3	UIP/IPF	10/10	10/10	10/10	10/10	9/10	0/10	10/10
	UIP/AuD	13/13	12/13	13/13	13/13	8/13	3/13	13/13
pAMPKa	UIP/IPF	10/10	9/10	9/10	10/10	2/10	2/10	10/10
	UIP/AuD	13/13	13/13	13/13	12/13	2/13	1/13	13/13

• In one case, the relevant lesions were not anymore present in some of the serial sections; therefore, this case was excluded from the evaluation

Autophagy

Epithelial cells in remodeled areas were positive in more than 90% of all cases for SIRT1, MAP1S, LC3, and pAMKa (Table 3). Positive staining for LC3 (Fig. 2a) and pAMKa (Fig. 2b) was observed in normal pneumocytes in more than 90% of all cases, while SIRT1 (Fig. 3a, b) and MAP1S (Fig. 3c) were only occasionally seen in some normal pneumocytes. Myofibroblasts were LC3 and pAMKa positive in more than 90% of cases, whereas positive staining for SIRT1 and MAP1S was observed only in some cases.

More than 80% of cases demonstrated expression of LC3 and pAMKa in bronchial epithelia. SIRT1 and MAP1S, on the contrary, were only observed in some cases inbronchial epithelial cells. LC3 showed positivity in endothelia in most cases, whereas MAP1S, SIRT1, and pAMKa were observed in endothelial cells only in few cases. Positive staining for LC3 and pAMKa in smooth muscle cells was seen in few cases. SIRT1 and MAP1S were not observed in smooth muscle cells. Positive staining for SIRT1, MAP1S, LC3, and PAMKa was observed in alveolar macrophages in more than 60% of cases.

When comparing the staining pattern in UIP/IPF and UIP/AuD, there were no differences in the expression of autophagy markers (Table 3).

Cellular metabolism with respect to hypoxia

Epithelial cells in remodeled areas stained positive for not only LDH, but also bronchial epithelia and macrophages (Fig. 4a). In few cases, LDH was expressed in endotheliaas well, especially in larger vessels within remodeled areas. LDH was not expressed in pneumocytes in unaffected lung parenchyma, nor in myofibroblasts or smooth muscle cells. Positive staining for GLUT1 was observed in erythrocytes, epithelia in remodeled areas (Fig. 4b), and in bronchial epithelia of areas

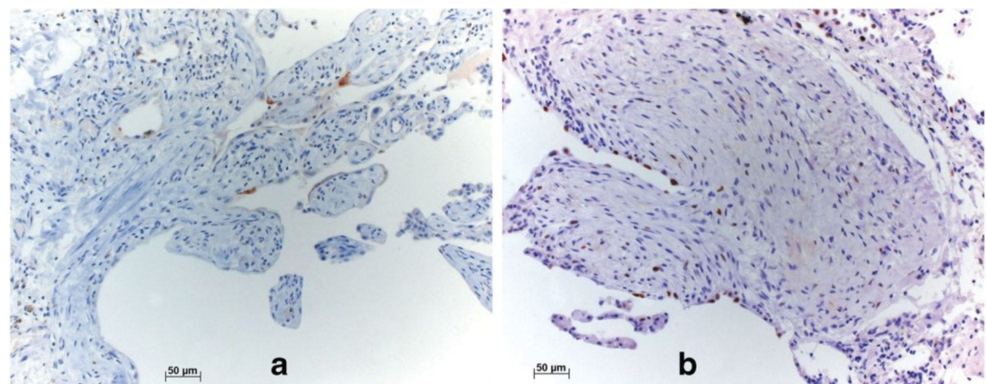


Fig. 1 a Positive p16 reaction in senescent cells. b Presentation of staining with p21. Since the number of positive cells is high, this shows that p21 is expressed not only by senescent cells. Bars 50 µm

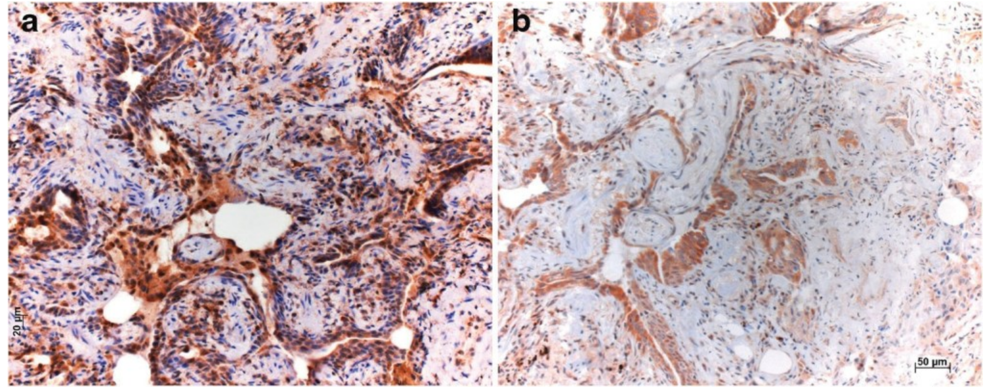


Fig. 2 Autophagy markers LC3 (a) and phosphorylated AMPK (b) stain epithelial cells, including those in remodeled areas, but also myofibroblasts, Bars 50 µm

unaffected by fibrosis. The staining in cells of epithelia in remodeled areas was exclusively cytoplasmic and was not observed in normal pneumocytes. Staining for GLUT1 was absent in myofibroblasts, endothelia, smooth muscle cells, and macrophages. There was no difference with respect to UIP/IPF and UIP/AuD samples.

Origin of epithelial cells

Epithelial cells in remodeled areas were positive for both TTF1 and CK5/6 (Fig. 5a, b). Positive staining of CK5/6 was observed in basal cells in normal epithelium as well. Columnar and cuboidal cells of normal epithelium were negative. In addition, positivity was seen in squamous metaplasia within the remodeled areas. TTF1 showed positivity in normal epithelium as well as in pneumocytes and regenerating cells in remodeled areas. Myofibroblasts, smooth muscle cells, endothelium, and macrophages were all negative. Here as well, there was no difference between UIP/IPF and UIP/AuD samples.

Discussion

Senescence has been identified as a strong factor contributing to fibrosis in the lung, kidney, and liver [2, 35, 45, 65]. In lung diseases, it is activated in UIP. Senescent cells release different inflammatory mediators, most prominently interleukin-1a, interleukin-1b, interleukin-6, interleukin-10, and TGF-ß. This release causes a prolonged repair process, finally resulting in

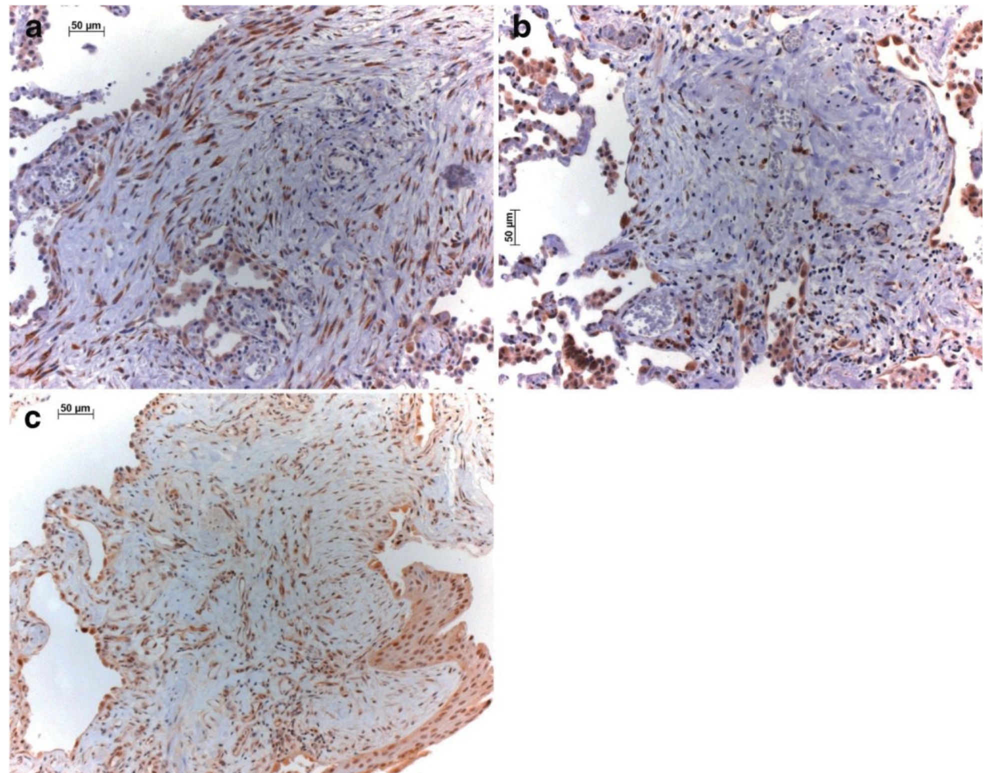


Fig. 3 Autophagy markers SIRT in (a) young myofibroblasts, with a gradual loss of staining in older myofibroblasts and fibrocytes (b); MAP1S, another autophagy marker, is expressed in myofibroblasts and epithelial cells within remodeled areas (c). Bars 50 and 20 µm

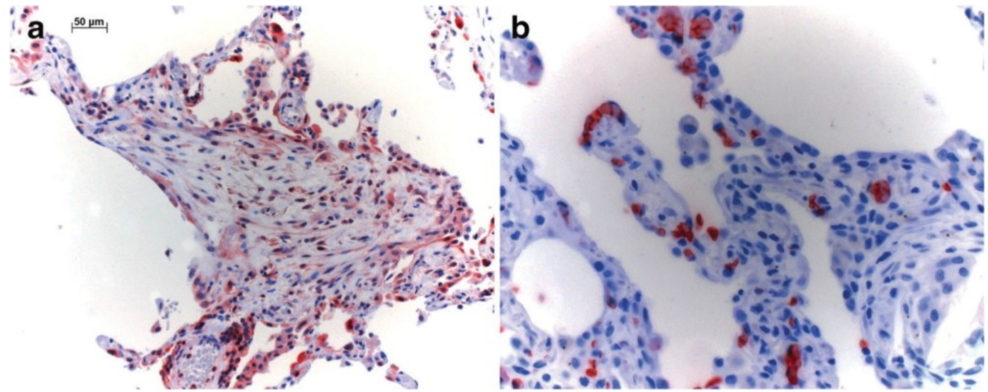


Fig. 4 a Epithelial cells in remodeled areas, but also normal bronchial epithelia are positive for LDH; myofibroblasts also have upregulated this enzyme, but less intense. b Expression of GLUT1 is seen in red blood cells, but also in few epithelial cells within the remodeling area. Bar 50 μm and magnification x 400

remodeling of the peripheral lung tissue and fibrosis [29]. Several markers of senescent cells have been identified, such as p16, p21, and ß-galactosidase [19, 45]. In our study, p16 and p21 expression was present in remodeled areas of UIP, where p16 is much more specific for senescent cells, whereas p21 is expressed in additional cells, such as pneumocytes and normal bronchiolar cells. We did not succeed in staining for ß-galactosidase in formalin-fixed paraffin-embedded tissues. Senescence plays a role in UIP regardless of the underlying etiology, as the expression of p16 and p21 was similar in UIP/IPF and UIP/AuD cases. Interestingly, myofibroblasts did not express p16 or p21; therefore, they do not undergo senescence. This implies that proliferation of myofibroblasts might be stimulated by growth factors released by the senescent epithelial cells.

The role of autophagy in UIP is controversially discussed. Some studies reported downregulation, othersupregulation; however, some were experimental studies, and others conducted an examination of human UIP/IPF samples with immunohistochemical staining [5, 17, 37, 43]. Autophagy has two roles: one is to remove debris and downregulate inflammation and the other, frequently seen in cancer, is to phagocytose cellular debris, degrade it, and use it for metabolic purposes of the cells [50]. The second mechanism might be especially important in areas of hypoxia—a common phenomenon in cancer. In UIP, regardless of the underlying etiology, we found high expression of allfour markers of autophagy (LC3, SIRT1, MAP1S, and pAMPKa) in epithelial cells within the remodeled areas and in myofibroblasts. Interestingly, expression was predominantly seen in myofibroblasts ("young lesions"), whereas fibrotic areas ("old lesions") whwereereuse were instead of where matured and no longer proliferating fibrocytes were present, were less metabolic active. Upregulation of autophagy is here very likely the answer of epithelial cells and myofibroblasts to local hypoxia. This corresponds well with morphology: the thickness of alveolar septa is increased in these areas, and there is no neoangiogenesis (in contrast to organizing pneumonia); therefore, the cells have difficulties to support their metabolic needs. The expression of autophagy markers was also present in macrophages. This fits well into the picture, as they are within the alveolar lumina, have no access to nutrients from capillaries, and act very likely in the same way for their metabolic needs. Interestingly, hypoxia may also be responsible for the proliferation of metaplastic cells in the remodeled areas [59]. Very likely, hypoxia also induces the proliferation or differentiation of myofibroblasts [4].

We tried to tackle the question of hypoxia in remodeled areas using the expression of LDH and GLUT1. LDH is a marker for anaerobic glycolysis or the metabolic pathway of the Warburg type [43]. A positive staining was observed in cells within the remodeled areas, which can be interpreted as a sign of hypoxia. Whether anaerobic glycolysis takes place, because oxygen is scarce due to the increased demand and the impairment of vascularization through fibrosis, or because glycolysis takes place in exchange for oxidative

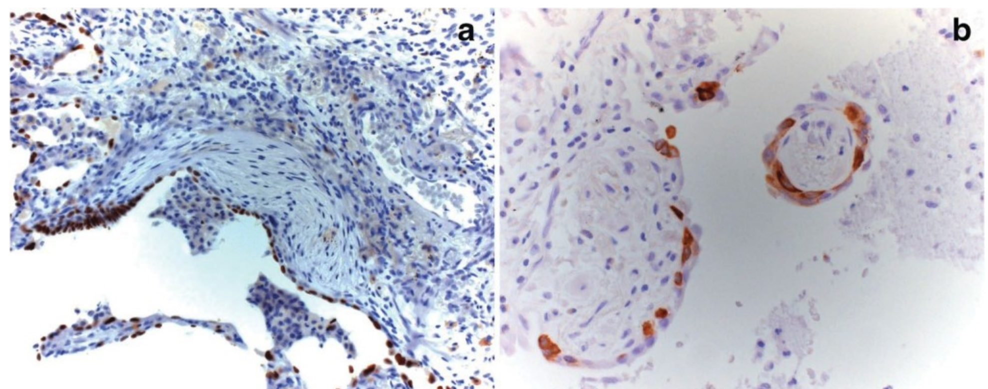


Fig. 5 Epithelial cells in bronchioles and in regenerating cystic areas express TTF1 (a), a marker of the terminal bronchoalveolar unit as well as cytokeratin 5/6 (b), also found in basal cells of bronchioles and small bronchi. Magnification x 200 and x 400

phosphorylation (Warburg effect) as seen in neoplastic processes, needs further investigation [14]. Positive staining for GLUT1 in remodeled areas is another indicator for a high cellular energy demand, since GLUT1 expression reflects an increased cellular glucose uptake and glycolysis [10, 62]. Regarding the GLUT1 staining pattern, it must be noted that due to high-intensity staining of erythrocytes, the evaluation of the cells within remodeled areas and also unaffected lung tissue was difficult to determine.

There have been some speculations about the origin of cells within remodeled areas of the peripheral lung. As these cells sometimes differentiate into squamous cell metaplasia, an origin from large airways was discussed [48]. To contribute to this discussion, we performed immunohistochemistry using markers for peripheral cells. TTF1 and CK5/6 were expressed in epithelial cells in remodeled areas. CK5/6 was also seen in basal cells of the bronchioles. This could imply that cells from the bronchiolar epithelium move into the denuded alveolar region and repopulate these areas. The expression of TTF1 in these cells was a strong argument for their peripheral origin, as cells from bronchi do not express this protein.

Conclusion

UIP regardless of the underlying etiology is driven by p16/p21-positive senescent cells within the remodeled area, which sustain the proliferation of myofibroblasts by inflammatory cytokines. Upregulation of autophagy in the setting of UIP seemsto protect epithelia andmyofibroblastsagainst hypoxia. In addition, the metabolism is changed to a glycolysis pathway demonstrated by the expression of LDH and GLUT1 within the remodeled foci. Because pneumocytes undergo apoptosis in areas of active remodeling (myofibroblastic foci), the epithelial surface layer is regenerated by basal cells from terminal bronchioles expressing TTF1 and CK5/6.

Author contributions HP designed the study; LB, AN, and HP evaluated the histological diagnosis and quality of the tissues; AR provided the clinical data; FG, LB, and HP evaluated the immunohistochemical staining and defined the compartments for evaluation; SE tested the antibodies, evaluated the staining intensities, and performed all staining procedures. FG, LB, AR, AN, and HP worked on the manuscript, and all authors approved the final version.

Funding Information Open access funding provided by Medical University of Graz.

Data availability All data are available within the manuscript. No additional data are deposited.

Compliance with ethicalstandards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Alhamad EH, Cal JG, Alboukai AA, Shaik SA, Omair MA (2016) Autoimmune symptoms in idiopathic pulmonary fibrosis: clinical significance. Clin Respir J 10:350–358. https://doi.org/10.1111/crj.12224
2. Alvarez D, Cardenes N, Sellares J, Bueno M, Corey C, Hanumanthu VS, Peng Y, D'Cunha H, Sembrat J, Nouraie M, Shanker S, Caufield C, Shiva S, Armanios M, Mora AL, Rojas M (2017) IPF lung fibroblasts have a senescent phenotype. Am J Physiol Lung Cell Mol Physiol 313:L1164–l1173. https://doi.org/10.1152/ajplung.00220.2017
3. Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, Westergren-Thorsson G, Selman M (2008) Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonaryfibrosis. Int J Biochem Cell Biol 40:2129–2140. https://doi.org/10.1016/j.biocel.2008.02.012
4. Aquino-Galvez A, Gonzalez-Avila G, Jimenez-Sanchez LL, Maldonado-Martinez HA, Cisneros J, Toscano-Marquez F, Castillejos-L6pez M, Torres-Espindola LM, Velazquez-Cruz R, Rodriguez VHO, Flores-Soto E, Solis-Chagoyan H, Cabello C, Zt'.fiiga J, Romero Y (2019) Dysregulated expression of hypoxia-inducible factors augments myofibroblasts differentiation in idiopathic pulmonary fibrosis. Respir Res 20:130. https://doi.org/10.1186/s12931-019-1100-4
5. Araya J, Kojima J, Takasaka N, Ito S, Fujii S, Hara H, Yanagisawa H, Kobayashi K, Tsurushige C, Kawaishi M, Kamiya N, Hirano J, Odaka M, Morikawa T, Nishimura SL, Kawabata Y, Hano H, Nakayama K, Kuwano K (2013) Insufficient autophagy in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 304:L56–L69. https://doi.org/10.1152/ajplung.00213.2012
6. Bernales S, McDonald KL, Walter P (2006) Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS Biol 4:2311–2324. https://doi.org/10.1371/journal.pbio.0040423
7. Bunney PE, Zink AN, Holm AA, Billington CJ, Kotz CM (2017) Orexin activation counteracts decreases in nonexercise activity thermogenesis (NEAT) caused by high-fat diet. Physiol Behav 176:139–148. https://doi.org/10.1016/j.physbeh.2017.03.040
8. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L, Piccoli P, Cangi G, Semenzato G, Doglioni C (2002) Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. Lab Investig 82:1335–1345
9. Cho SJ, Moon JS, Lee CM, Choi AM, Stout-Delgado HW (2017) Glucose transporter 1-dependent glycolysis is increased during aging-related lung fibrosis, and phloretin inhibits lung fibrosis. Am J Respir Cell Mol Biol 56:521–531. https://doi.org/10.1165/rcmb.2016-0225OC

10. Cho SJ, Moon J-S, Nikahira K, Yun HS, Harris R, Hong KS, Huang H, Choi AMK, Stout-Delgado H (2019) GLUT1-dependent glycolysis regulates exacerbation of fibrosis via AIM2 inflammasome activation. Thorax 1:1–10. https://doi.org/10.1136/thoraxjnl-2019-213571
11. Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O (2009) Protocols to detect senescence-associated beta-galactosidase (SA-ßgal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 4:1798–1806. https://doi.org/10.1038/nprot.2009.191
12. El-Chemaly S, Malide D, Yao J, Nathan SD, Rosas IO, Gahl WA, Moss J, Gochuico BR (2013) Glucose transporter-1 distribution in fibrotic lung disease: association with [(1)(8)F]-2-fluoro-2-deoxyglucose-PET scan uptake, inflammation, and neovascularization. Chest 143:1685–1691. https://doi.org/10.1378/chest.12-1359
13. Fujimoto H, Kobayashi T, Azuma A (2015) Idiopathic pulmonary fibrosis treatment—OFEV® (nintedanib). Capsules 9:179–185. https://doi.org/10.4137/CCRPM.S23321.TYPE
14. Gallo M, Sapio L, Spina A, Naviglio D, Calogero A, Naviglio S (2015) Lacticdehydrogenaseandcancer: anoverview. Front Biosci (Landmark Ed) 20:1234–1249. https://doi.org/10.2741/4368
15. Garcia CK (2011) Idiopathic pulmonary fibrosis: update on genetic discoveries. Proc Am Thorac Soc 8:158–162. https://doi.org/10.1513/pats.201008-056MS
16. Gessner C, Wirtz H, Sack U, Winkler J, Stiehl P, Schauer J, Wolff G (2002) BALF N-acetylglucosaminidase and ß-galactosidase activities in idiopathic pulmonary fibrosis. Respir Med 96:751–756. https://doi.org/10.1053/rmed.2002.1344
17. Ghavami S, Yeganeh B, Zeki AA, Shojaei S, Kenyon NJ, Ott S, Samali A, Patterson J, Alizadeh J, Moghadam AR, Dixon IMC, Unruh H, Knight DA, Post M, Klonisch T, Halayko AJ (2017) Autophagy and the unfolded protein response promote profibrotic effects of TGF-ß 1 in human lung fibroblasts. Am J Phys Lung Cell Mol Phys 314:L493–L504. https://doi.org/10.1152/ajplung.00372.2017
18. Han X, Tai H, Wang X, Wang Z, Zhou J, Wei X, Ding Y, Gong H, Mo C, Zhang J, Qin J, Ma Y, Huang N, Xiang R, Xiao H (2016) AMPK activation protects cells from oxidative stress-induced senescence via autophagic flux restoration and intracellular NAD elevation. Aging Cell 15:416–427. https://doi.org/10.1111/acel.12446
19. Hernandez-segura A, Nehme J, Demaria M (2018) Hallmarks of cellular senescence. Trends Cell Biol 28:436–453. https://doi.org/10.1016/j.tcb.2018.02.001
20. Hildebrand DG, Lehle S, Borst A, Haferkamp S, Essmann F, Schulze-Osthoff K (2013) a-Fucosidase as a novel convenient biomarker for cellular senescence. Cell Cycle 12:1922–1927. https://doi.org/10.4161/cc.24944
21. Hutchinson J, Fogarty A, Hubbard R, McKeever T (2015) Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur Respir J 46:795–806. https://doi.org/10.1183/09031936.00185114
22. Judge JL, Nagel DJ, Owens KM, Rackow A, Phipps P, Sime PJ, Kottmann RM (2018) Prevention and treatment of bleomycin-induced pulmonary fibrosis with the lactate dehydrogenase inhibitor gossypol. PLoS One 13(5):e0197936. https://doi.org/10.1371/journal.pone.0197936
23. Kang C, Xu Q, Martin TD et al (2015) The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. Science 349. https://doi.org/10.1016/j.physbeh.2017.03.040
24. Kim HC, Ji W, Kim MY, Colby TV, Jang SJ, Lee CK, Han SB, Kim DS (2015) Interstitial pneumonia related to undifferentiated connective tissue disease: pathologic pattern and prognosis. Chest 147:165–172. https://doi.org/10.1378/chest.14-0272
25. Kitada M, Ogura Y, Koya D (2016) Role of Sirt1 as a regulator of autophagy autophagy: cancer, other pathologies, inflammation, immunity, infection, and aging 89–100. https://doi.org/10.1016/B978-0-12-802937-4.00003-X
26. Klionsky RJDDJ(2008) Autophagy: mechanismandphysiological relevance 'brewed' from yeast studies. Bone 23:1–7. https://doi.org/10.1038/jid.2014.371
27. Knaś M, Zalewska A, Kretowski R, Niczyporuk M, Waszkiewicz N, Cechowska-Pasko M, Waszkiel D, Zwierz K (2012) The profile of lysosomal exoglycosidases in replicative and stress-induced senescence in early passage human fibroblasts. Folia Histochem Cytobiol 50:220–227. https://doi.org/10.5603/FHC.2012.0031
28. Kottmann RM, Kulkarni AA, Smolnycki KA, Lyda E, Dahanayake T, Salibi R, Honnons S, Jones C, Isern NG, Hu JZ, Nathan SD, Grant G, Phipps RP, Sime PJ (2012) Lactic acid is elevated in idiopathic pulmonary fibrosis and induces myofibroblast differentiation via pH-dependent activation of transforming growth factor-ß. Am J Respir Crit Care Med 186:740–751. https://doi.org/10.1164/rccm.201201-0084OC
29. Kuwano K, Araya J, Hara H, Minagawa S, Takasaka N, Ito S, Kobayashi K, Nakayama K (2016) Cellular senescence and autophagy in the pathogenesis of chronic obstructive pulmonarydisease (COPD) and idiopathic pulmonary fibrosis (IPF). Respir Investig 54:397–406. https://doi.org/10.1016/j.resinv.2016.03.010
30. Lawson WE, Grant SW, Ambrosini V, Womble KE, Dawson EP, Lane KB, Markin C, Renzoni E, Lympany P, Thomas AQ, Roldan J, Scott TA, Blackwell TS, Phillips JA 3rd, Loyd JE, du Bois RM (2004) Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax 59:977–980. https://doi.org/10.1136/thx.2004.026336
31. Lawson WE, Loyd JE, Degryse AL (2011) Genetics in pulmonary fibrosis—familial cases provide clues to the pathogenesis of idiopathic pulmonary fibrosis. Am J Med Sci 341:439–443. https://doi.org/10.1097/MAJ.0b013e31821a9d7a
32. Lehmann M, Buhl L, Alsafadi HN, Klee S, Hermann S, Mutze K, Ota C, Lindner M, Behr J, Hilgendorff A, Wagner DE, Königshoff M(2018) Differential effects of Nintedanib and Pirfenidone on lung alveolar epithelial cell function in ex vivo murine and human lung tissue cultures of pulmonary fibrosis. Respir Res 19:175. https://doi.org/10.1186/s12931-018-0876-y
33. Liu T, Ma X, Ouyang T, Chen H, Lin J, Liu J, Xiao Y, Yu J, Huang Y (2018) SIRT1 reverses senescence via enhancing autophagy and attenuates oxidative stress-induced apoptosis through promoting p53 degradation. Int J Biol Macromol 117:225–234. https://doi.org/10.1016/J.IJBIOMAC.2018.05.174
34. Lozano-Torres B, Estepa-Fernandez A, Rovira M, Orzaez M, Serrano M, Martinez-Mafiez R, Sancen6n F (2019) The chemistry of senescence. Nat Rev Chem 3. https://doi.org/10.1038/s41570-019-0108-0
35. Luo C, Zhou S, Zhou Z, Liu Y, Yang L, Liu J, Zhang Y, Li H, Liu Y, Hou FF, Zhou L (2018) Wnt9a promotes renal fibrosis by accelerating cellular senescence in tubular epithelial cells. J Am Soc Nephrol 29:1238–1256.https://doi.org/10.1681/ASN.2017050574
36. Maher TM, Wells AU, Laurent GJ (2007) Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? Eur Respir J 30:835–839
37. Margaritopoulos GA, Tsitoura E, Tzanakis N, Spandidos DA, Siafakas NM, Sourvinos G, Antoniou KM (2013, 2013) Self-eating: friend or foe? The emerging role of autophagy in idiopathic pulmonary fibrosis. Biomed Res Int. https://doi.org/10.1155/2013/420497
38. Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, Kawaishi M, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K (2011) Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-ß-induced senescence

of human bronchial epithelial cells. Am J Phys Lung Cell Mol Phys 300:L391–L401. https://doi.org/10.1152/ajplung.00097.2010
39. Musah S, Chen J, Hoyle GW (2012) Repair of tracheal epithelium by basal cells after chlorine-induced injury. Respir Res 13:107. https://doi.org/10.1186/1465-9921-13-107
40. Navaratnam V, Fleming KM, West J, Smith CJP, Jenkins RG, Fogarty A, Hubbard RB (2011) The rising incidence of idiopathic pulmonary fibrosis in the UK. Thorax 66:462–467. https://doi.org/10.1136/thx.2010.148031
41. Okuda R, Aoshiba K, Matsushima H, Ogura T, Okudela K, Ohashi K (2019) Cellular senescence and senescence-associated secretory phenotype: comparison of idiopathic pulmonary fibrosis, connective tissue disease-associated interstitial lung disease, and chronic obstructive pulmonary disease. J Thorac Dis 11:857–864. https://doi.org/10.21037/jtd.2019.02.11
42. Pardo ASM (2002) Idiopathic pulmonary fibrosis: new insights. Int J Biochem Cell Biol 34:1534
43. Patel AS, Lin L, Geyer A, Haspel JA, An CH, Cao J, Rosas IO, Morse D(2012) Autophagy in idiopathic pulmonary fibrosis. PLoS One 7. https://doi.org/10.1371/journal.pone.0041394
44. Poletti V, Kitaichi M (2000) Facts and controversies in the classification of idiopathic interstitial pneumonias. Sarcoidosis Vasc Diffuse Lung Dis 17:229–238
45. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, Oberg AL, Birch J, Salmonowicz H, Zhu Y, Mazula DL, Brooks RW, Fuhrmann-Stroissnigg H, Pirtskhalava T, Prakash YS, Tchkonia T, Robbins PD, Aubry MC, Passos JF, Kirkland JL, Tschumperlin DJ, Kita H, LeBrasseur NK (2017) Cellular senescence mediates fibrotic pulmonary disease. Nat Commun 8:14532. https://doi.org/10.1038/ncomms14532
46. Selman M, Pardo A (2014) Revealing the pathogenic and aging-related mechanisms of the enigmatic idiopathic pulmonary fibrosis. an integral model. Am J Respir Crit Care Med 189:1161–1172. https://doi.org/10.1164/rccm.201312-2221PP
47. Selman M, Lin HM, Montafio M, Jenkins AL, Estrada A, Lin Z, Wang G, DiAngelo SL, Guo X, Umstead TM, Lang CM, Pardo A, Phelps DS, Floros J (2003) Surfactant protein A and B genetic variants predispose to idiopathic pulmonary fibrosis. Hum Genet 113:542–550. https://doi.org/10.1007/s00439-003-1015-4
48. Smirnova NF, Schamberger AC, Nayakanti S, Hatz R, Behr J, Eickelberg O (2016) Detection and quantification of epithelial progenitor cell populations in human healthy and IPF lungs. Respir Res 17:83. https://doi.org/10.1186/s12931-016-0404-x
49. Song JW, Do KH, Kim MY, Jang SJ, Colby TV, Kim DS (2009) Pathologic and radiologic differences between idiopathic and collagen vascular disease-related usual interstitial pneumonia. Chest 136:23–30. https://doi.org/10.1378/chest.08-2572
50. Su Z, Yang Z, Xu Y, Chen Y, Yu Q (2015) Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer 1–14. https://doi.org/10.1186/s12943-015-0321-5
51. Tanida I, Ueno T, Kominami E (2008) LC3 and autophagy—methods in molecular biology. Methods Mol Biol 445:77–88. https://doi.org/10.1007/978-1-59745-157-4-4
52. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, Blackwell TS(2011) Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. J Biol Chem 286:30972–30980. https://doi.org/10.1074/jbc.M110.181164
53. Tanjore H, Blackwell TS, Lawson WE (2012) Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 302: L721–L729. https://doi.org/10.1152/ajplung.00410.2011
54. Van Deursen JM (2014) The role of senescent cells in ageing. Nature 509:439–446. https://doi.org/10.1038/nature13193
55. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK (2009) Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. Am J Hum Genet 84:52–59. https://doi.org/10.1016/j.ajhg.2008.11.010
56. Wang Y, Liang Y, Vanhoutte PM (2011) SIRT1 and AMPK in regulating mammalian senescence: a critical review and a working model. Mol Med Rep 14(2):1309–15. https://doi.org/10.3892/mmr.2016.5346
57. Wang G, Lou HH, Salit J, Leopold PL, Driscoll S, Schymeinsky J, Quast K, Visvanathan S, Fine JS, Thomas MJ, Crystal RG (2019) Characterization of an immortalized human small airway basal stem/progenitor cell line with airway region-specific differentiation capacity. Respir Res 20:1–14. https://doi.org/10.1186/s12931-019-1140-9
58. Wang S, Wang X, Cheng Y, Ouyang W, Sang X, Liu J, Su Y, Liu Y, Li C, Yang L, Jin L, Wang Z (2019) Autophagy dysfunction, cellular senescence, and abnormal immune-inflammatory responses in AMD: from mechanisms to therapeutic potential. Oxidative Med Cell Longev. https://doi.org/10.1155/2019/3632169
59. Weng T, Poth JM, Karmouty-Quintana H, Garcia-Morales LJ, Melicoff E, Luo F, Chen NY, Evans CM, Bunge RR, Bruckner BA, Loebe M, Volcik KA, Eltzschig HK, Blackburn MR (2014) Hypoxia-induced deoxycytidine kinase contributes to epithelial proliferation in pulmonary fibrosis. Am J Respir Crit Care Med 190:1402–1412. https://doi.org/10.1164/rccm.201404-0744OC
60. Wuyts WA, Cavazza A, Rossi G, Bonella F, Sverzellati N, Spagnolo P (2014) Differential diagnosis of usual interstitial pneumonia: when is it truly idiopathic? Eur Respir Rev 23:308–319. https://doi.org/10.1183/09059180.00004914
61. Xian W, Mckeon F (2012) Adult stem cells underlying lung regeneration. Cell Cycle 11(5):887–94. https://doi.org/10.4161/cc.11.5.1932
62. Xiao H, Wang J, Yan W, Cui Y, Chen Z, Gao X, Wen X, Chen J (2018) GLUT1 regulates cell glycolysis and proliferation in prostate cancer. Prostate 78:86–94. https://doi.org/10.1002/pros.23448
63. Xie R, Nguyen S, McKeehan K, Wang F, McKeehan WL, Liu L (2011) Microtubule-associated protein 1S (MAP1S) bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. J Biol Chem 286: 10367–10377. https://doi.org/10.1074/jbc.M110.206532
64. Yee M, Domm W, Gelein R, De Mesy Bentley KL, Kottmann RM, Sime PJ, Lawrence BP, O'Reilly MA (2017) Alternative progenitor lineages regenerate the adult lung depleted of alveolar epithelial type 2 cells. Am J Respir Cell Mol Biol 56:453–464. https://doi.org/10.1165/rcmb.2016-0150OC
65. Zhang Z, Yao Z, Zhao S, Shao J, Chen A, Zhang F, Zheng S (2017) Interaction between autophagy and senescence is required for dihydroartemisinin to alleviate liver fibrosis. Nature Publishing Group. https://doi.org/10.1038/cddis.2017.255

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

PA5379

Medical University of Graz

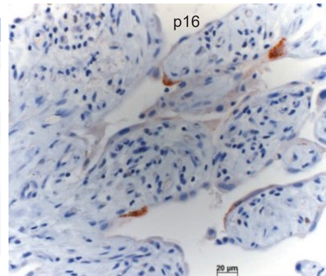
Autophagy and senescence are activated mechanisms in idiopathic and autoimmunity caused usual interstitial pneumonia

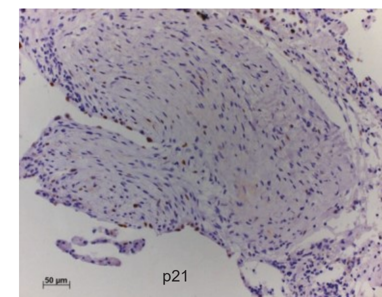
Helmut Popper, Luka Brcic, Sylvia Eidenhammer, Florian Gallob, Florian Rampp, Andreas Nerlich
Center for Diagnostics and Research in Molecular Biomedicine, Pathology Institute for Diagnostics and Research, Medical University Graz, Neue Stiftingtalstraße 6, Graz, 8036, Austria
Center for Lung Diseases Munich, Institute of Pathology, Clinics Bogenhausen, City Clinics Munich, Germany

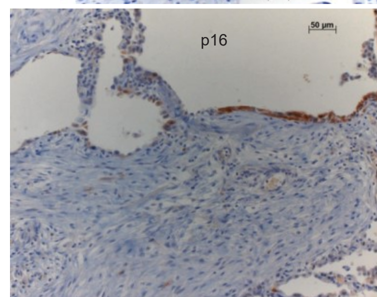
Introduction: Fibrosing pneumonia can present with the pattern of Usual Interstitial Pneumonia (UIP), fibrosing Non-Specific Interstitial Pneumonia, and as Organizing Pneumonia. Within the spectrum/pattern of UIP there is Idiopathic Pulmonary Fibrosis (IPF) but also chronic Autoimmune Diseases and chronic Hypersensitivity Pneumonia (cAD, cHP). Senescence was identified as a driving force in UIP of different aetiologies. Senescence is the state of cell cycle arrest, which is initiated by cyclin-dependent kinase inhibitors such as p16, p21 and p53. Senescent cells release various inflammatory mediators, e.g. IL-1, IL-6, IL-10, TGF-ß, which keeps the lung in an inflammatory state (inflammasome) and finally promotes fibrosis. Autophagy is important for cellular homeostasis. It might inhibit prolonged inflammation by degrading cellular debris by phagosomes. However, it also can help in hypoxic conditions by providing nutrients and oxygen out of recycled proteins, amino acids, etc. Conflicting results have been published for IPF: Markers for autophagy (LC3) were reduced in IPF lung cell lysates, thus dysfunctional autophagy leads to senescence and myofibroblast differentiation of epithelial cells. In other studies it was shown, that autophagy was increased and being necessary for TGF-ß induced fibrosis.

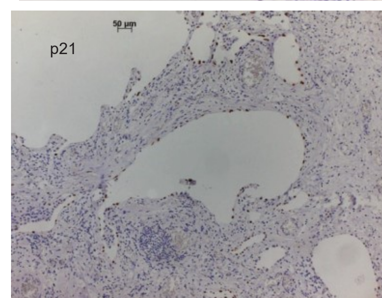
Methods and Materials: Patients were selected based on pathological reports from the Lung Archive of the Diagnostic and Research Institute of Pathology, Medical University of Graz. All together 23 cases of UIP/IPF were selected. All cases had a pattern of UIP and were clinically either classified as IPF or chronic AD.
Serial sections were produced and stained with H&E and incubated with antibodies for p16, p21, MAP1S, PAMPKα, LC3 and SIRT1 (See table below). Senescent cells were identified by the surrogate markers ß-Galactosidase, p21, and p16. Autophagy was demonstrated by immunohistochemical staining for phosphorylated Adenosin-5´ Monophosphate activated kinase (pAMPKα), microtubule-associated protein 1A/1B-light chain 3 (LC3), microtubule-associated protein 1S (MAP1S), and family of silent information regulator 1 (SIRT1). There are controversial studies about the protective effect of SIRT1 against senescence, some stating that SIRT1 may even reverse senescence, others observed that continuous SIRT1 stimulation leads to irreversible senescence.
Immunohistochemical staining was evaluated for percentage of positive cells. This was done separately for the normal bronchial epithelium and smooth muscle cells, vascular endothelium and smooth muscle cells, pneumocytes in unaffected lung areas, macrophages, myofibroblasts, and epithelial cells in areas of microcystic remodelling of lung parenchyma including peribronchiolar metaplasia. The evaluation was done independently by two authors (HP, LB). In rare discrepant cases slides were discussed on a multiheaded microscope and consensus was reached.

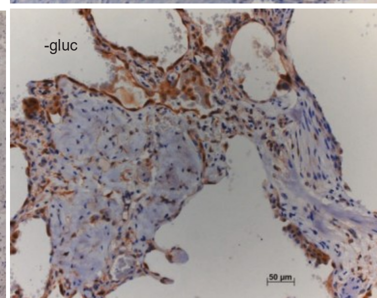
ANTIBODY	P16 INK4A	P21	MAPS1S	PAMPKA	LC3	SIRT1
PRODUCER	ENZO	DAKO	Novus	Cell Signaling	Nanotools	LSBio
CLONE	ENZ-ABS-377-0100	SX118	NBP2-47383	THr172	0231-100/LC3-5F10	LS-B1895
DILUTION	1:1000	1:30	1:1000	1:100	1:200	1:500
PRE-TREATMENT	CC1	MW9,0 (DAKO S2367)	CC1	CC1	MW9,0 (DAKO S2367)	MW9,0 (DAKO S2367)
DETECTION KIT	Ventana optiView + Amplification	Envision DAKO K5007	Ventana ultraView	Ventana ultraView	Envision DAKO K5007	Envision DAKO K5007











		Regenerative/ remodelled epithelium	Myofibro-blasts	Normal pneumocyte	Bronchial-epithelium	Endothelium	Smooth muscle cells	macrophage
Senescence								
P21	IPF	9/9	1/9	8/9	0/9	0/9	0/9	0/9
	UIP	13/13	0/13	13/13	6/13	0/13	0/13	0/13
P16	IPF	9/9	0/9	1/9	0/9	0/9	0/9	0/9
	UIP	13/13	1/13	0/13	0/13	0/13	0/13	1/13
Autophagy								
SIRT1	IPF	10/10	7/10	7/10	6/10	0/10	0/10	10/10
	UIP	13/13	12/13	11/13	9/13	3/13	0/13	12/13
MAP1S	IPF	10/10	4/10	8/10	7/10	4/10	0/10	10/10
	UIP	13/13	6/13	7/13	7/13	2/13	0/13	12/13
LC3	IPF	10/10	10/10	10/10	10/10	9/10	0/10	10/10
	UIP	13/13	12/13	13/13	13/13	8/13	3/13	13/13
pAMPK	IPF	10/10	9/10	9/10	10/10	2/10	2/10	10/10
	UIP	13/13	13/13	13/13	12/13	2/13	1/13	13/13

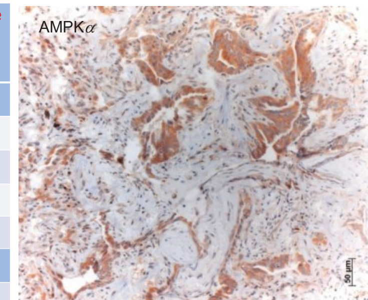
Results

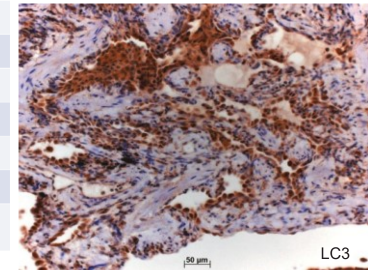
Senescence markers were investigated in cases of UIP/IPF and UIP/chronic immune diseases, predominantly autoimmune diseases. -Galactosidase despite several attempts with different protocols did not work in FFPE tissues. However, p16 and p21 could be demonstrated in all cases. We also evaluated b-Glucuronidase: However this enzyme was expressed in epithelia as well as stroma cells without any preferred expression pattern. The expression just signaled that all these cell are in an activated metabolic state. In contrast to some reports senescence markers were exclusively positive in epithelial cells; p16 was more specific staining only epithelia in remodelled areas, but not myofibroblasts. P21 stained also senescent cells, but in addition gave positive reactions also in normal pneumocytes and in few bronchial epithelia (basal cells). To evaluate autophagy we used four different markers. All of them showed positivity in epithelia within the remodeling areas, and partially also in normal pneumocytes and bronchial epithelia. Myofibroblasts were also positively stained, however, only young myofibroblastic lesions were positive, whereas older foci were negative. Macrophages showed some unspecific uptake of the molecules, probably by phagocytosis. In few cases and only focally positive reactions for autophagy markers were seen in endothelia and smooth muscle cells in areas of muscular cirrhosis.

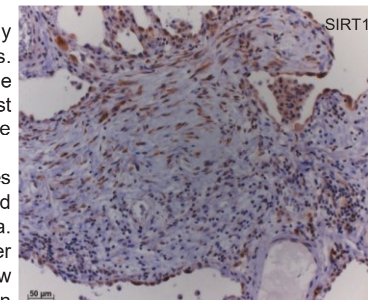
Conclusions

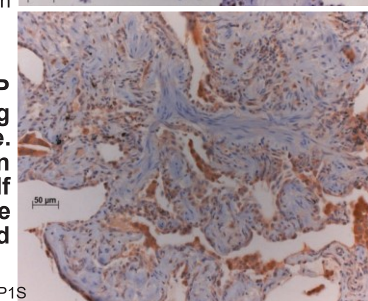
Senescence was equally activated in IPF as well as chronic immune diseases with UIP pattern. The positive reaction was exclusively confined to the epithelia within remodelling areas (this corresponds to honeycomb lesions by CT scan). Myofibroblasts were negative. This means that the proliferation of myofibroblasts is most likely maintained by the secretion of an inflammasome by the epithelial cells and myofibroblasts do not undergo senescence. If there are rare mesenchymal cells from the bone marrow cannot be answered, as none were present in our samples. Autophagy was upregulated in epithelia within remodelled areas, and in young myofibroblastic foci. This was unexpected: This means inflammation and proliferation is not maintained by cellular debris not degraded by autophagy mechanisms. Therefore the function of autophagy in UIP seems not to be directed towards downregulation of the inflammation, but more likely autophagy functions to help the proliferating cells to keep up their nutrition functioning under a hypoxic condition, which is well known for the remodeled areas of the lung.

Bibliography
1.Araya J, Kojima J, Takasaka N, *et al.* Insufficient autophagy in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2013;304:L56-69.
2.Naikawadi RP, Disayabutr S, Mallavia B, *et al.* Telomere dysfunction in alveolar epithelial cells causes lung remodeling and fibrosis. JCI insight 2016;1:e86704.
3.Schafer MJ, White TA, Iijima K, *et al.* Cellular senescence mediates fibrotic pulmonary disease. Nat Commun 2017;8:14532.
4. Alvarez D, Cardenes N, Sellares J, *et al.* IPF lung fibroblasts have a senescent phenotype. Am J Physiol Lung Cell Mol Physiol 2017;313:L1164-l73.
5. Lehmann M, Korfei M, Mutze K, *et al.* Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. Eur Respir J 2017;50.
6.Tian Y, Li H, Qiu T, *et al.* Loss of PTEN induces lung fibrosis via alveolar epithelial cell senescence depending on NF-kappaB activation. Aging Cell 2019;18:e12858.









MAP1S

What cells are replacing the epithelium in cystic remodeling in UIP and COPD

Florian Gallob, Tamas Zombori, Luca Brcic, Sylvia Eidenhammer, Wim Timens, Helmut Popper
Institute of Pathology, Medical University of Graz, Austria, University of Szeged, Dept. of Pathology, Hungary, University of Groningen, University Medical Center, Dept. Pathology and Medical Biology, Groningen, The Netherlands

Medical University of Graz

Remodeling in usual interstitial pneumonia (UIP) results in the repopulation of alveolar remnants by cuboidal cells. There are speculations these cells might migrate from the proximal bronchial airways, facilitated by the secretion of chemokines from senescent cells. Senescent cells have not only been identified in UIP, but also in chronic obstructive lung disease (COPD), where remodeling might also occur. The aim of the study was to characterize the origin of the regenerating cells in the remodeled cystic areas.

Well characterized tissues from cases with UIP pattern composed of IPF, chronic autoimmune disease (CAD) and chronic hypersensitivity pneumonia (CHP), and cases of COPD were chosen. Sections were incubated with antibodies for cytokeratins 5/6, 7, 8, 9. 14, 17, 18, 19, and p40, TTF1, and Clara cell protein 10/uroplakin (CC10).

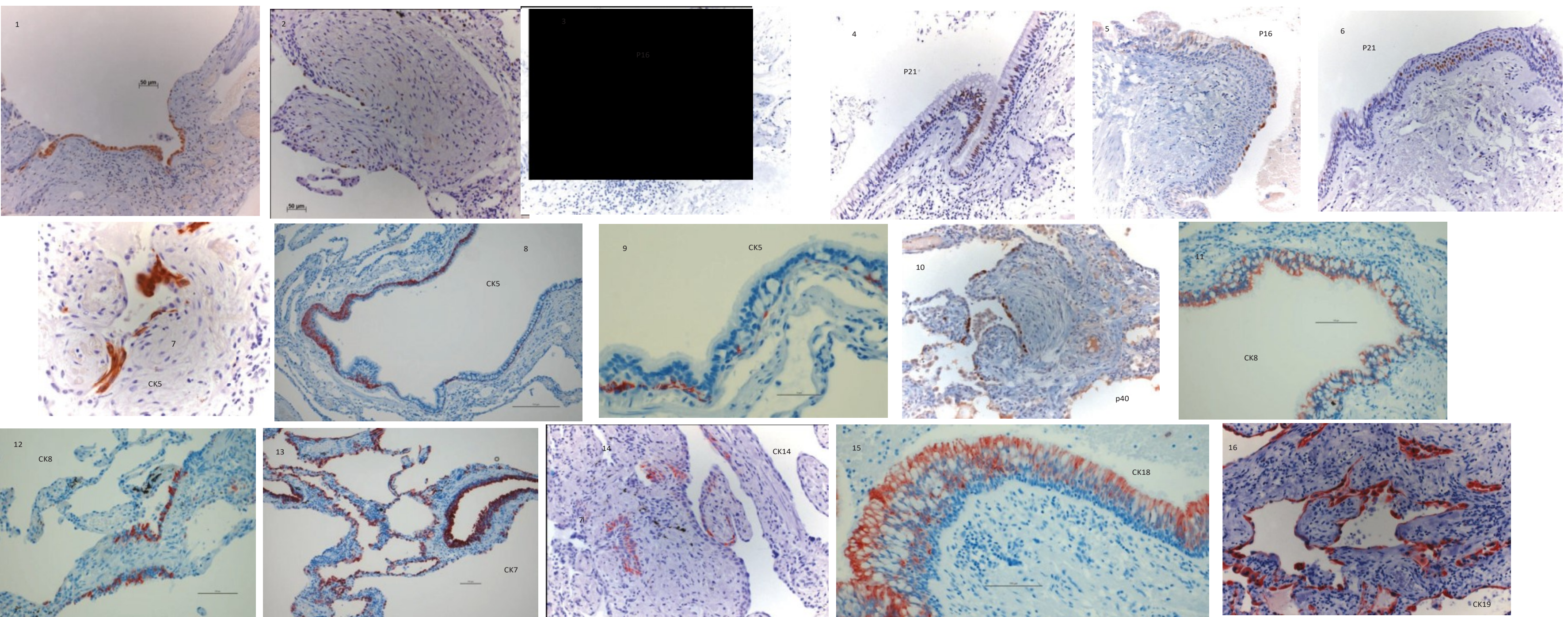
Cells in remodeling areas of IPF, CAD, CHP were positively stained by cytokeratin 5, in a few cases single cells also acquired CK14; almost all cells stained for CK8/18. The cells were positive for TTF1 and p40. The reaction for CC10 was seen in only few cells. Cells of the basal layer of bronchioles including the terminal ones stained for CK5/6 and P40, whereas the differentiated cells in the upper layer were positive for CK8/18 and CC10. Cells from the basal layer of bronchioles migrate into the denuded alveolar region and repopulate the remodeled cystic areas. Interestingly at this position they acquire TTF1, initially not expressed in basal cells. The expression of the so-called squamous cell marker p40 in basal cells of bronchioles and in the cystic areas was unexpected, as squamoid morphology was absent; if these cells give rise to squamous metaplasia cannot be answered. A coexpression with CK14 is rarely found, other cytokeratins associate with CK5. Also in COPD CK5/6 positive cells increase in number and replace pneumocytes in emphysematous areas and increase within bronchi.

Material and Methods

We investigated 23 cases of clinically proven UIP/IPF, 29 cases of clinically proven COPD, and 3 cases of bronchial asthma for the expression of senescence markers p16 and p21, and also for the expression of different cytokeratins. The IPF cases all presented with an UIP pattern, the COPD cases showed emphysema and chronic bronchitis. All COPD patients were current smokers, all presented with decreased lung function tests. Two asthma patients presented with reduced lung function tests, one tissue was from a transplant donor.
Immunohistochemistry was done for cytokeratin polypeptides (CK) 5/6, 7, 8, 9. 14, 17, 18, 19; a staining for CK 1-4, 10-12, and CK20 was omitted because these are not expressed in lung tissues, antibodies. Details for the other CKs are in table 1.

Table1: Antibodies

Antibody for	Company	clone	dilution	pretreatment	visualization
Cytokeratin 5	Epitomics		1:100	WB6,0	Envision DAKO K5007
Cytokeratin 7	DAKO M7018	OV-TL12/30	1:100	Protease XXIV	Envision DAKO K5007
Cytokeratin 8	Leica NCL-L-CK8-TS1	TS1	1:50	MW 9,0 (DAKO S2367)	Envision DAKO K5007
Cytokeratin 9	Progen		1:20	WB 6,0	Envision DAKO K5007
Cytokeratin 14	Leica NCL-L-LL002	LL002	1:50	WB 6,0	Envision DAKO K5007
Cytokeratin 17	DAKO	E3	1:20	MW 9,0 (DAKO S2367)	Envision DAKO K5007
Cytokeratin 18	Thermo scientific MS-142	DC10	1:50	MW 9,0 (DAKO S2367)	Envision DAKO K5007
Cytokeratin 19	DAKO M0888	RCK108	1:20	MW 9,0 (DAKO S2367)	Envision DAKO K5007
P40	Biocare ACiI3030A		1:100	CC1	Ventana ultraview
TTF1	DAKO M3575	8G7G3/1	1:100	MW 9,0 (DAKO S2367)	Envision DAKO K5007
CC10/uroplakin	Biovendor		1:2000	CC1	Ventana ultraview
P16/ INK4A	Enzo	ENZ-ABS-377-0100	1:1000	CC1	Ventana optiView + Amplification
P21	Dako	SX118	1:30	MW 9,0 (DAKO S2367)	Envision DAKO K5007



Results and Conclusions

Senescent cells were seen within the cystic remodeling in UIP/IPF (fig.1/2) and in the bronchial mucosa in COPD (fig.3/4) as well as in asthma (fig.5/6)
Cytokeratin 5 was expressed in basal cells of the bronchi and bronchioli. CK5 positive cells were seen in the cystic remodeling structures in UIP (fig.7) and a increase was seen in bronchi and bronchioli of COPD patients (fig.8/9), but not in asthma cases. CK5 positive cells in UIP also stained positively for p40 (fig.10)
The reaction for CK 8 and 9 was negative in UIP/IPF, whereas a positive reactivity for CK 8 was seen in high columnar cells including goblet cells in COPD, but not in asthma (fig.11/12)
Positivity for CK 7 was seen in all cases (fig.13), whereas CK14 was only positive in few cells in remodelled areas of UIP/IPF (fig.14). No positive reactions were seen for CK17, however, CK 18 was positive in COPD (fig.15) and CK19 in UIP (fig.16) exclusively.
As in UIP senescecent cells play a similar role in COPD maintaning inflammation. Basal cells expressing cytokeratin 5 act as a cell pool for regeneration in UIP as well as in COPD, however, in UIP this is restricted to the basal cells of the bronchioles, which very likely move to the remodelled cystic areas, whereas in COPD regeneration happens in bronchi and bronchioli. Whereas CK 7 is expressed in almost every compartment of the lung, the basic CKs are assembled differently in UIP and COPD: CK18 in COPD, CK19 in UIP; in COPD a second acidic CK, CK8 is expressed in bronchi and bronchioles.