J Thorac Oncol. 2013 Aug;8(8):1032-42. doi: 10.1097/JTO.0b013e31829923c8.

Interleukin-22 is frequently expressed in small- and large-cell lung cancer and promotes growth in chemotherapy-resistant cancer cells.

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INTRODUCTION: In lung cancer, interleukin-22 (IL-22) expression within primary tissue has been demonstrated, but the frequency and the functional consequence of IL-22 signaling have not been addressed. This study aims at analyzing the cellular effects of IL-22 on lung carcinoma cell lines and the prognostic impact of IL-22 tissue expression in lung cancer patients.

METHODS: Biological effects of IL-22 signaling were investigated in seven lung cancer cell lines by Western blot, flow cytometry, real-time polymerase chain reaction, and proliferation assays. Tumor tissue specimens of two cohorts with a total of 2300 lung cancer patients were tested for IL-22 expression by immunohistochemistry. IL-22 serum concentrations were analyzed in 103 additional patients by enzyme-linked immunosorbent assay.

RESULTS: We found the IL-22 receptor 1 (IL-22-R1) to be expressed in six of seven lung cancer cell lines. However IL-22 signaling was functional in only four cell lines, where IL-22 induced signal transducer activator of transcription 3 phosphorylation and increased cell proliferation. Furthermore, IL-22 induced the expression of antiapoptotic B-cell lymphoma 2, but did not rescue tumor cells from carboplatin-induced apoptosis. Cisplatin-resistant cell lines showed a significant up-regulation of IL-22-R1 along with a stronger proliferative response to IL-22 stimulation. IL-22 was preferentially expressed in small- and large-cell lung carcinoma (58% and 46% of cases, respectively). However, no correlation between IL-22 expression by immunohistochemistry and prognosis was observed.

CONCLUSION: IL-22 is frequently expressed in lung cancer tissue. Enhanced IL-22-R1 expression and signaling in chemotherapy-refractory cell lines are indicative of a protumorigenic function of IL-22 and may contribute to a more aggressive phenotype.

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Gli1 mediates lung cancer cell proliferation and Sonic Hedgehog-dependent mesenchymal cell activation.

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Non-Small-Cell-Lung-Cancer (NSCLC) represents approximately 85% of all lung cancers and remains poorly understood. While signaling pathways operative during organ development, including Sonic Hedgehog (Shh) and associated Gli transcription factors (Gli1-3), have recently been found to be reactivated in NSCLC, their functional role remains unclear. Here, we hypothesized that Shh/Gli1-3 could mediate NSCLC autonomous proliferation and epithelial/stromal signaling in the tumoral tissue. In this context, we have investigated the activity of Shh/Gli1-3 signaling in NSCLC in both, cancer and stromal cells. We report here that inhibition of Shh signaling induces a significant decrease in the proliferation of NSCLC cells. This effect is mediated by Gli1 and Gli2, but not Gli3, through regulation of cyclin D1 and cyclin D2 expression. While exogenous Shh was unable to induce signaling in either A549 lung adenocarcinoma or H520 lung squamous carcinoma cells, both cells were found to secrete Shh ligand, which induced fibroblast proliferation, survival, migration, invasion, and collagen synthesis. Furthermore, Shh secreted by NSCLC mediates the production of proangiogenic and metastatic factors in lung fibroblasts. Our results thus provide evidence that Shh plays an important role in mediating epithelial/mesenchymal crosstalk in NSCLC. While autonomous Gli activity controls NSCLC proliferation, increased Shh expression by NSCLC is associated with fibroblast activation in tumor-associated stroma. Our study highlights the relevance of studying stromal-associated cells in the context of NSCLC regarding new prognosis and therapeutic options.

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Multiplex profiling of cellular invasion in 3D cell culture models.

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To-date, most invasion or migration assays use a modified Boyden chamber-like design to assess migration as single-cell or scratch assays on coated or uncoated planar plastic surfaces. Here, we describe a 96-well microplate-based, high-content, three-dimensional cell culture assay capable of assessing invasion dynamics and molecular signatures thereof. On applying our invasion assay, we were able to demonstrate significant effects on the invasion capacity of fibroblast cell lines, as well as primary lung fibroblasts. Administration of epidermal growth factor resulted in a substantial increase of cellular invasion, thus making this technique suitable for high-throughput pharmacological screening of novel compounds regulating invasive and migratory pathways of primary cells. Our assay also correlates cellular invasiveness to molecular events. Thus, we argue of having developed a powerful and versatile toolbox for an extensive profiling of invasive cells in a 96-well format. This will have a major impact on research in disease areas like fibrosis, metastatic cancers, or chronic inflammatory states.

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Cub domain-containing protein 1 negatively regulates TGF-β signaling and myofibroblast differentiation.

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Fibroblasts are thought to be the prime cell type for producing and secreting extracellular matrix (ECM) proteins in the connective tissue. The profibrotic cytokine transforming growth factor-β1 (TGF-β1) activates and transdifferentiates fibroblasts into α-smooth muscle actin (α-SMA)-expressing myofibroblasts, which exhibit increased ECM secretion, in particular collagens. Little information, however, exists about cell-surface molecules on fibroblasts that mediate this transdifferentiation process. We recently identified, using unbiased cell-surface proteome analysis, Cub domain-containing protein 1 (CDCP1) to be strongly downregulated by TGF-β1. CDCP1 is a transmembrane glycoprotein, the expression and role of which has not been investigated in lung fibroblasts to date. Here, we characterized, in detail, the effect of TGF-β1 on CDCP1 expression and function, using immunofluorescence, FACS, immunoblotting, and siRNA-mediated knockdown of CDCP1. CDCP1 is present on interstitial fibroblasts, but not myofibroblasts, in the normal and idiopathic pulmonary fibrosis lung. In vitro, TGF-β1 decreased CDCP1 expression in a time-dependent manner by impacting mRNA and protein levels. Knockdown of CDCP1 enhanced a TGF-β1-mediated cell adhesion of fibroblasts. Importantly, CDCP1-depleted cells displayed an enhanced expression of profibrotic markers, such as collagen V or α-SMA, which was found to be independent of TGF-β1. Our data show, for the very first time that loss of CDCP1 contributes to fibroblast to myofibroblast differentiation via a potential negative feedback loop between CDCP1 expression and TGF-β1 stimulation.

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Protease-mediated release of chemotherapeutics from mesoporous silica nanoparticles to ex vivo human and mouse lung tumors.

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Nanoparticles allow for controlled and targeted drug delivery to diseased tissues and therefore bypass systemic side effects. Spatiotemporal control of drug release can be achieved by nanocarriers that respond to elevated levels of disease-specific enzymes. For example, matrix metalloproteinase 9 (MMP9) is overexpressed in tumors, is known to enhance the metastatic potency of malignant cells, and has been associated with poor prognosis of lung cancer. Here, we report the synthesis of mesoporous silica nanoparticles (MSNs) tightly capped by avidin molecules via MMP9 sequence-specific linkers to allow for site-selective drug delivery in high-expressing MMP9 tumor areas. We provide proof-of-concept evidence for successful MMP9-triggered drug release from MSNs in human tumor cells and in mouse and human lung tumors using the novel technology of ex vivo 3D lung tissue cultures. This technique allows for translational testing of drug delivery strategies in diseased mouse and human tissue. Using this method we show MMP9-mediated release of cisplatin, which induced apoptotic cell death only in lung tumor regions of Kras mutant mice, without causing toxicity in tumor-free areas or in healthy mice. The MMP9-responsive nanoparticles also allowed for effective combinatorial drug delivery of cisplatin and proteasome inhibitor bortezomib, which had a synergistic effect on the (therapeutic) efficiency. Importantly, we demonstrate the feasibility of MMP9-controlled drug release in human lung tumors.

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FK506-Binding Protein 10, a Potential Novel Drug Target for Idiopathic Pulmonary Fibrosis.

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RATIONALE: Increased abundance and stiffness of the extracellular matrix, in particular collagens, is a hallmark of idiopathic pulmonary fibrosis (IPF). FK506-binding protein 10 (FKBP10) is a collagen chaperone, mutations of which have been indicated in the reduction of extracellular matrix stiffness (e.g., in osteogenesis imperfecta).
OBJECTIVES: To assess the expression and function of FKBP10 in IPF.
METHODS: We assessed FKBP10 expression in bleomycin-induced lung fibrosis (using quantitative reverse transcriptase-polymerase chain reaction, Western blot, and immunofluorescence), analyzed microarray data from 99 patients with IPF and 43 control subjects from a U.S. cohort, and performed Western blot analysis from 6 patients with IPF and 5 control subjects from a German cohort. Subcellular localization of FKBP10 was assessed by immunofluorescent stainings. The expression and function of FKBP10, as well as its regulation by endoplasmic reticulum stress or transforming growth factor-β1, was analyzed by small interfering RNA-mediated loss-of-function experiments, quantitative reverse transcriptase-polymerase chain reaction, Western blot, and quantification of secreted collagens in the lung and in primary human lung fibroblasts (phLF). Effects on collagen secretion were compared with those of the drugs nintedanib and pirfenidone, recently approved for IPF.
MEASUREMENTS AND MAIN RESULTS: FKBP10 expression was up-regulated in bleomycin-induced lung fibrosis and IPF. Immunofluorescent stainings demonstrated localization to interstitial (myo)fibroblasts and CD68(+) macrophages. Transforming growth factor-β1, but not endoplasmic reticulum stress, induced FKBP10 expression in phLF. The small interfering RNA-mediated knockdown of FKBP10 attenuated expression of profibrotic mediators and effectors, including collagens I and V and α-smooth muscle actin, on the transcript and protein level. Importantly, loss of FKBP10 expression significantly suppressed collagen secretion by phLF.
CONCLUSIONS: FKBP10 might be a novel drug target for IPF.

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Comprehensive genomic profiles of small cell lung cancer.

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We have sequenced the genomes of 110 small cell lung cancers (SCLC), one of the deadliest human cancers. In nearly all the tumours analysed we found bi-allelic inactivation of TP53 and RB1, sometimes by complex genomic rearrangements. Two tumours with wild-type RB1 had evidence of chromothripsis leading to overexpression of cyclin D1 (encoded by the CCND1 gene), revealing an alternative mechanism of Rb1 deregulation. Thus, loss of the tumour suppressors TP53 and RB1 is obligatory in SCLC. We discovered somatic genomic rearrangements of TP73 that create an oncogenic version of this gene, TP73Δex2/3. In rare cases, SCLC tumours exhibited kinase gene mutations, providing a possible therapeutic opportunity for individual patients. Finally, we observed inactivating mutations in NOTCH family genes in 25% of human SCLC. Accordingly, activation of Notch signalling in a pre-clinical SCLC mouse model strikingly reduced the number of tumours and extended the survival of the mutant mice. Furthermore, neuroendocrine gene expression was abrogated by Notch activity in SCLC cells. This first comprehensive study of somatic genome alterations in SCLC uncovers several key biological processes and identifies candidate therapeutic targets in this highly lethal form of cancer.

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Preclinical validation and imaging of Wnt-induced repair in human 3D lung tissue cultures.

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Chronic obstructive pulmonary disease (COPD) is characterised by a progressive loss of lung tissue. Inducing repair processes within the adult diseased lung is of major interest and Wnt/β-catenin signalling represents a promising target for lung repair. However, the translation of novel therapeutic targets from model systems into clinical use remains a major challenge.We generated murine and patient-derived three-dimensional (3D) ex vivo lung tissue cultures (LTCs), which closely mimic the 3D lung microenvironment in vivo. Using two well-known glycogen synthase kinase-3β inhibitors, lithium chloride (LiCl) and CHIR 99021 (CT), we determined Wnt/β-catenin-driven lung repair processes in high spatiotemporal resolution using quantitative PCR, Western blotting, ELISA, (immuno)histological assessment, and four-dimensional confocal live tissue imaging.Viable 3D-LTCs exhibited preserved lung structure and function for up to 5 days. We demonstrate successful Wnt/β-catenin signal activation in murine and patient-derived 3D-LTCs from COPD patients. Wnt/β-catenin signalling led to increased alveolar epithelial cell marker expression, decreased matrix metalloproteinase-12 expression, as well as altered macrophage activity and elastin remodelling. Importantly, induction of surfactant protein C significantly correlated with disease stage (per cent predicted forced expiratory volume in 1 s) in patient-derived 3D-LTCs.Patient-derived 3D-LTCs represent a valuable tool to analyse potential targets and drugs for lung repair. Enhanced Wnt/β-catenin signalling attenuated pathological features of patient-derived COPD 3D-LTCs.

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Multidimensional immunolabeling and 4D time-lapse imaging of vital ex vivo lung tissue.

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During the last decades, the study of cell behavior was largely accomplished in uncoated or extracellular matrix (ECM)-coated plastic dishes. To date, considerable cell biological efforts have tried to model in vitro the natural microenvironment found in vivo. For the lung, explants cultured ex vivo as lung tissue cultures (LTCs) provide a three-dimensional (3D) tissue model containing all cells in their natural microenvironment. Techniques for assessing the dynamic live interaction between ECM and cellular tissue components, however, are still missing. Here, we describe specific multidimensional immunolabeling of living 3D-LTCs, derived from healthy and fibrotic mouse lungs, as well as patient-derived 3D-LTCs, and concomitant real-time four-dimensional multichannel imaging thereof. This approach allowed the evaluation of dynamic interactions between mesenchymal cells and macrophages with their ECM. Furthermore, fibroblasts transiently expressing focal adhesions markers incorporated into the 3D-LTCs, paving new ways for studying the dynamic interaction between cellular adhesions and their natural-derived ECM. A novel protein transfer technology (FuseIt/Ibidi) shuttled fluorescently labeled α-smooth muscle actin antibodies into the native cells of living 3D-LTCs, enabling live monitoring of α-smooth muscle actin-positive stress fibers in native tissue myofibroblasts residing in fibrotic lesions of 3D-LTCs. Finally, this technique can be applied to healthy and diseased human lung tissue, as well as to adherent cells in conventional two-dimensional cell culture. This novel method will provide valuable new insights into the dynamics of ECM (patho)biology, studying in detail the interaction between ECM and cellular tissue components in their natural microenvironment.

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TGF-β-induced profibrotic signaling is regulated in part by the WNT receptor Frizzled-8.

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TGF-β is important in lung injury and remodeling processes. TGF-β and Wingless/integrase-1 (WNT) signaling are interconnected; however, the WNT ligand-receptor complexes involved are unknown. Thus, we aimed to identify Frizzled (FZD) receptors that mediate TGF-β-induced profibrotic signaling. MRC-5 and primary human lung fibroblasts were stimulated with TGF-β1, WNT-5A, or WNT-5B in the presence and absence of specific pathway inhibitors. Specific small interfering RNA was used to knock down FZD8. In vivo studies using bleomycin-induced lung fibrosis were performed in wild-type and FZD8-deficient mice. TGF-β1 induced FZD8 specifically via Smad3-dependent signaling in MRC-5 and primary human lung fibroblasts. It is noteworthy that FZD8 knockdown reduced TGF-β1-induced collagen Iα1, fibronectin, versican, α-smooth muscle (sm)-actin, and connective tissue growth factor. Moreover, bleomycin-induced lung fibrosis was attenuated in FZD8-deficient mice in vivo Although inhibition of canonical WNT signaling did not affect TGF-β1-induced gene expression in vitro, noncanonical WNT-5B mimicked TGF-β1-induced fibroblast activation. FZD8 knockdown reduced both WNT-5B-induced gene expression of fibronectin and α-sm-actin, as well as WNT-5B-induced changes in cellular impedance. Collectively, our findings demonstrate a role for FZD8 in TGF-β-induced profibrotic signaling and imply that WNT-5B may be the ligand for FZD8 in these responses.-Spanjer, A. I. R., Baarsma, H. A., Oostenbrink, L. M., Jansen, S. R., Kuipers, C. C., Lindner, M., Postma, D. S., Meurs, H., Heijink, I. H., Gosens, R., Königshoff, M. TGF-β-induced profibrotic signaling is regulated in part by the WNT receptor Frizzled-8.

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Surface proteome analysis identifies platelet derived growth factor receptor-alpha as a critical mediator of transforming growth factor-beta-induced collagen secretion.

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Fibroblasts are extracellular matrix-producing cells in the lung. Fibroblast activation by transforming growth factor-beta leads to myofibroblast-differentiation and increased extracellular matrix deposition, a hallmark of pulmonary fibrosis. While fibroblast function with respect to migration, invasion, and extracellular matrix deposition has been well-explored, little is known about the surface proteome of lung fibroblasts in general and its specific response to fibrogenic growth factors, in particular transforming growth factor-beta. We thus performed a cell-surface proteome analysis of primary human lung fibroblasts in presence/absence of transforming growth factor-beta, followed by characterization of our findings using FACS analysis, Western blot, and siRNA-mediated knockdown experiments. We identified 213 surface proteins significantly regulated by transforming growth factor-beta, platelet derived growth factor receptor-alpha being one of the top down-regulated proteins. Transforming growth factor beta-induced downregulation of platelet derived growth factor receptor-alpha induced upregulation of platelet derived growth factor receptor-beta expression and phosphorylation of Akt, a downstream target of platelet derived growth factor signaling. Importantly, collagen type V expression and secretion was strongly increased after forced knockdown of platelet derived growth factor receptor-alpha, an effect that was potentiated by transforming growth factor-beta. We therefore show previously underappreciated cross-talk of transforming growth factor-beta and platelet derived growth factor signaling in human lung fibroblasts, resulting in increased extracellular matrix deposition in a platelet derived growth factor receptor-alpha dependent manner. These findings are of particular importance for the treatment of lung fibrosis patients with high pulmonary transforming growth factor-beta activity.

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Impairment of Immunoproteasome Function by Cigarette Smoke and in Chronic Obstructive Pulmonary Disease.

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RATIONALE: Patients with chronic obstructive pulmonary disease (COPD) and in particular smokers are more susceptible to respiratory infections contributing to acute exacerbations of disease. The immunoproteasome is a specialized type of proteasome destined to improve major histocompatibility complex (MHC) class I-mediated antigen presentation for the resolution of intracellular infections.
OBJECTIVES: To characterize immunoproteasome function in COPD and its regulation by cigarette smoke.
METHODS: Immunoproteasome expression and activity were determined in bronchoalveolar lavage (BAL) and lungs of human donors and patients with COPD or idiopathic pulmonary fibrosis (IPF), as well as in cigarette smoke-exposed mice. Smoke-mediated alterations of immunoproteasome activity and MHC I surface expression were analyzed in human blood-derived macrophages. Immunoproteasome-specific MHC I antigen presentation was evaluated in spleen and lung immune cells that had been smoke-exposed in vitro or in vivo.
MEASUREMENTS AND MAIN RESULTS: Immunoproteasome and MHC I mRNA expression was reduced in BAL cells of patients with COPD and in isolated alveolar macrophages of patients with COPD or IPF. Exposure of immune cells to cigarette smoke extract in vitro reduced immunoproteasome activity and impaired immunoproteasome-specific MHC I antigen presentation. In vivo, acute cigarette smoke exposure dynamically regulated immunoproteasome function and MHC I antigen presentation in mouse BAL cells. End-stage COPD lungs showed markedly impaired immunoproteasome activities.
CONCLUSIONS: We here show that the activity of the immunoproteasome is impaired by cigarette smoke resulting in reduced MHC I antigen presentation. Regulation of immunoproteasome function by cigarette smoke may thus alter adaptive immune responses and add to prolonged infections and exacerbations in COPD and IPF.

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An ex vivo model to induce early fibrosis-like changes in human precision-cut lung slices.

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Idiopathic pulmonary fibrosis (IPF) is a devastating chronic interstitial lung disease (ILD) characterized by lung tissue scarring and high morbidity. Lung epithelial injury, myofibroblast activation, and deranged repair are believed to be key processes involved in disease onset and progression, but the exact molecular mechanisms behind IPF remain unclear. Several drugs have been shown to slow disease progression, but treatments that halt or reverse IPF progression have not been identified. Ex vivo models of human lung have been proposed for drug discovery, one of which is precision-cut lung slices (PCLS). Although PCLS production from IPF explants is possible, IPF explants are rare and typically represent end-stage disease. Here we present a novel model of early fibrosis-like changes in human PCLS derived from patients without ILD/IPF using a combination of profibrotic growth factors and signaling molecules (transforming growth factor-β, tumor necrosis factor-α, platelet-derived growth factor-AB, and lysophosphatidic acid). Fibrotic-like changes of PCLS were qualitatively analyzed by histology and immunofluorescence and quantitatively by water-soluble tetrazolium-1, RT-qPCR, Western blot analysis, and ELISA. PCLS remained viable after 5 days of treatment, and fibrotic gene expression (FN1, SERPINE1, COL1A1, CTGF, MMP7, and ACTA2) increased as early as 24 h of treatment, with increases in protein levels at 48 h and increased deposition of extracellular matrix. Alveolar epithelium reprogramming was evident by decreases in surfactant protein C and loss of HOPX In summary, using human-derived PCLS, we established a novel ex vivo model that displays characteristics of early fibrosis and could be used to evaluate novel therapies and study early-stage IPF pathomechanisms.

J Exp Med. 2017 Feb;214(2):565. doi: 10.1084/jem.2016067501052017c. Epub 2017 Jan

Correction: Noncanonical WNT-5A signaling impairs endogenous lung repair in COPD.

Baarsma HA, Skronska-Wasek W, Mutze K, Ciolek F, Wagner DE, John-Schuster G, Heinzelmann K, Günther A, Bracke KR, Dagouassat M, Boczkowski J, Brusselle GG, Smits R, Eickelberg O, Yildirim AÖ, Königshoff M.

Erratum for
J Exp Med. 2017 Jan;214(1):143-163.

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Noncanonical WNT-5A signaling impairs endogenous lung repair in COPD.

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Chronic obstructive pulmonary disease (COPD) is a leading cause of death worldwide. One main pathological feature of COPD is the loss of functional alveolar tissue without adequate repair (emphysema), yet the underlying mechanisms are poorly defined. Reduced WNT-β-catenin signaling is linked to impaired lung repair in COPD; however, the factors responsible for attenuating this pathway remain to be elucidated. Here, we identify a canonical to noncanonical WNT signaling shift contributing to COPD pathogenesis. We demonstrate enhanced expression of noncanonical WNT-5A in two experimental models of COPD and increased posttranslationally modified WNT-5A in human COPD tissue specimens. WNT-5A was increased in primary lung fibroblasts from COPD patients and induced by COPD-related stimuli, such as TGF-β, cigarette smoke (CS), and cellular senescence. Functionally, mature WNT-5A attenuated canonical WNT-driven alveolar epithelial cell wound healing and transdifferentiation in vitro. Lung-specific WNT-5A overexpression exacerbated airspace enlargement in elastase-induced emphysema in vivo. Accordingly, inhibition of WNT-5A in vivo attenuated lung tissue destruction, improved lung function, and restored expression of β-catenin-driven target genes and alveolar epithelial cell markers in the elastase, as well as in CS-induced models of COPD. We thus identify a novel essential mechanism involved in impaired mesenchymal-epithelial cross talk in COPD pathogenesis, which is amenable to therapy.

Eur Respir J. 2017 Aug 3;50(2). pii: 1602367. doi: 10.1183/13993003.02367-2016. Print 2017 Aug.

Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo.

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Idiopathic pulmonary fibrosis (IPF) is a devastating lung disease with poor prognosis and limited therapeutic options. The incidence of IPF increases with age, and ageing-related mechanisms such as cellular senescence have been proposed as pathogenic drivers. The lung alveolar epithelium represents a major site of tissue injury in IPF and senescence of this cell population is probably detrimental to lung repair. However, the potential pathomechanisms of alveolar epithelial cell senescence and the impact of senolytic drugs on senescent lung cells and fibrosis remain unknown. Here we demonstrate that lung epithelial cells exhibit increased P16 and P21 expression as well as senescence-associated β-galactosidase activity in experimental and human lung fibrosis tissue and primary cells.Primary fibrotic mouse alveolar epithelial type (AT)II cells secreted increased amounts of senescence-associated secretory phenotype (SASP) factors in vitro, as analysed using quantitative PCR, mass spectrometry and ELISA. Importantly, pharmacological clearance of senescent cells by induction of apoptosis in fibrotic ATII cells or ex vivo three-dimensional lung tissue cultures reduced SASP factors and extracellular matrix markers, while increasing alveolar epithelial markers.These data indicate that alveolar epithelial cell senescence contributes to lung fibrosis development and that senolytic drugs may be a viable therapeutic option for IPF.

Am J Physiol Lung Cell Mol Physiol. 2018 May 1;314(5):L708-L723. doi: 10.1152/ajplung.00408.2017. Epub 2018 Jan 18.

Distinct niches within the extracellular matrix dictate fibroblast function in (cell free) 3D lung tissue cultures.

Burgstaller G(1), Sengupta A(1), Vierkotten S(1), Preissler G(1)(2), Lindner M(1)(3), Behr J(1)(4), Königshoff M(1)(5), Eickelberg O(5).

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Cues from the extracellular matrix (ECM) and their functional interplay with cells play pivotal roles for development, tissue repair, and disease. However, the precise nature of this interplay remains elusive. We used an innovative 3D cell culture ECM model by decellularizing 300-µm-thick ex vivo lung tissue scaffolds (d3D-LTCs) derived from diseased and healthy mouse lungs, which widely mimics the native (patho)physiological in vivo ECM microenvironment. We successfully repopulated all d3D-LTCs with primary human and murine fibroblasts, and moreover, we demonstrated that the cells also populated the innermost core regions of the d3D-LTCs in a real 3D fashion. The engrafted fibroblasts revealed a striking functional plasticity, depending on their localization in distinct ECM niches of the d3D-LTCs, affecting the cells' tissue engraftment, cellular migration rates, cell morphologies, and protein expression and phosphorylation levels. Surprisingly, we also observed fibroblasts that were homing to the lung scaffold's interstitium as well as fibroblasts that were invading fibrotic areas. To date, the functional nature and even the existence of 3D cell matrix adhesions in vivo as well as in 3D culture models is still unclear and controversial. Here, we show that attachment of fibroblasts to the d3D-LTCs evidently occurred via focal adhesions, thus advocating for a relevant functional role in vivo. Furthermore, we found that protein levels of talin, paxillin, and zyxin and phosphorylation levels of paxillin Y118, as well as the migration-relevant small GTPases RhoA, Rac, and CDC42, were significantly reduced compared with their attachment to 2D plastic dishes. In summary, our results strikingly indicate that inherent physical or compositional characteristics of the ECM act as instructive cues altering the functional behavior of engrafted cells. Thus, d3D-LTCs might aid to obtain more realistic data in vitro, with a high relevance for drug discovery and mechanistic studies alike.

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Quality assessment of tissue samples stored in a specialized human lung biobank.

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Human sample, from patients or healthy donors, are a valuable link between basic research and clinic. Especially in translational research, they play an essential role in understanding development and progression of diseases as well as in developing new diagnostic and therapeutic tools. Stored in biobanks, fast access to appropriate material becomes possible. However, biobanking in a clinical context faces several challenges. In practice, collecting samples during clinical routine does not allow to strictly adhere to protocols of sample collection in all aspects. This may influence sample quality to variable degrees. Time from sample draw to asservation is a variable factor, and influences of prolonged storage at ambient temperature of tissues are not well understood. We investigated whether delays between 5 minutes and 3 hours, and the use of RNAlater RNA-preserving reagent would lead to a relevant drop in sample quality, measured by quantitative mRNA expression analysis. Our findings suggest that even under ambient conditions, delays up to 3 hours do not have a major impact on sample quality as long as the tissue remains intact.

J Thorac Dis. 2019 May;11(5):1963-72. doi:10.21037/jtd.2019.04.93.

Pleurectomy/decortication and hyperthermic intrathoracic chemoperfusion using cisplatin and doxorubicin for malignant pleural mesothelioma.

Klotz, Laura V.(1), Lindner, Michael (1) Eichhorn, Martin E. (2), Grützner, Uwe (1), Koch, Ina (1), Winter, Hauke (2)
Kauke, Teresa (1), Duell, Thomas (1), Hatz, Rudolf A. (1)

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Background: Malignant pleural mesothelioma (MPM) is an aggressive malignancy with few long-term survivors. Despite the dismal prognosis, hyperthermic intrathoracic chemoperfusion (HITHOC) was shown to improve survival in a selective group of patients. We analyzed the influence of HITHOC following pleurectomy and decortication on postoperative morbidity and overall survival for patients suffering from localized mesothelioma.
Methods: From 2009 until 2013, 71 patients with localized pleural mesothelioma underwent pleurectomy and decortication followed by HITHOC with cisplatin and doxorubicin. We analyzed postoperative morbidity, age, overall survival and influence of macroscopic resection on survival.
Results: Median patient age was 70 years (range, 65–73 years). Patients having the sarcomatoid subtype of mesothelioma showed a poor median survival of 9.2 months. In contrast, patients having the epithelioid subtype had a median survival of 17.9 months. Patients following macroscopic complete resection had a significantly better survival with 28.2 months compared to 13.1 months in patients with incomplete resection of the mesothelioma (P<0.0001). HITHOC was performed in all patients after tumor resection using cisplatin and doxorubicin.
Conclusions: Taken together, HITHOC following pleurectomy and decortication is supposed to be a safe therapeutic option for selected patients with localized epithelial pleural mesothelioma.

Cancer Med. 2019 Feb 25;8(4):1486-99. doi:10.1002/cam4.2031.

Comprehensive clinical profiling of the Gauting locoregional lung adenocarcinoma donors.

Klotz, Laura V. (1), (2)Courty, Yves, Lindner, Michael (2), Petit-Courty, Agnès (2), Stowasser, Anja (1), Koch, Ina (1),

Eichhorn, Martin E. (1), Lilis, Ioannis (3), Morresi-Hauf, Alicia (1), Arendt, Kristina A. M. (4), Pepe, Mario (4), Giopanou, Ioanna (3), Ntaliarda, Giannoula (3), Behrend, Sabine J. (4), Oplopoiou, Maria (3), Gissot, Valérie (2), Guyetant, Serge (2), Marchand-Adam, Sylvain (2), Behr, Jürgen (1), Kaiser, Jan-Christian (5), Hatz, Rudolf A. (1), Lamort, Anne-Sophie (4), Stathopoulos, Georgios T. (4)

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A comprehensive characterization of lung adenocarcinoma (LADC) clinical features is currently missing. We prospectively evaluated Caucasian patients with early-stage LADC. Patients with LADC diagnosed between 2011 and 2015 were prospectively assessed for lung resection with curative intent. Fifty clinical, pathologic, radiologic, and molecular variables were recorded. Patients were followed till death/study conclusion. The main findings were compared to a separate cohort from France. Of 1943 patients evaluated, 366 were enrolled (18.8%; 181 female; 75 never-smokers; 28% of registered Bavarian cases over the study period). Smoking and obstruction were significantly more prevalent in GLAD compared with adult Bavarians ($P < 0.0001$). Ever-smoker tumors were preferentially localized to the upper lobes. We observed 120 relapses and 74 deaths over 704 cumulative follow-up years. Median overall and disease-free survival were >7.5 and 3.6 years, respectively. Patients aged <45 or >65 years, resected >60 days postdiagnosis, with abnormal FVC/DLCOVA, N2/N3 stage, or solid histology had significantly decreased survival estimates. These were fit into a weighted locoregional LADC death risk score that outperformed pTNM7 in predicting survival in the GLAD and in our second cohort. We define the clinical gestalt of locoregional LADC and provide a new clinical tool to predict survival, findings that may aid future management and research design.

Clin Proteomics. 2015 Jul 31;12(1):. doi:10.1186/s12014-015-9093-6.

Blood-sampling collection prior to surgery may have a significant influence upon biomarker concentrations measured.

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Background: Biomarkers can be subtle tools to aid the diagnosis, prognosis and monitoring of therapy and disease progression. The validation of biomarkers is a cumbersome process involving many steps. Serum samples from lung cancer patients were collected in the framework of a larger study for evaluation of biomarkers for early detection of lung cancer. The analysis of biomarker levels measured revealed a noticeable difference in certain biomarker values that exhibited a dependence of the time point and setting of the sampling. Biomarker concentrations differed significantly if taken before or after the induction of anesthesia and if sampled via venipuncture or arterial catheter. Methods: To investigate this observation, blood samples from 13 patients were drawn 1–2 days prior to surgery (T1), on the same day by venipuncture (T2) and after induction of anesthesia via arterial catheter (T3). The biomarkers Squamous Cell Carcinoma antigen (CanAG SCC EIA, Fujirebio Diagnostics, Malvern, USA), Carcinoembrionic Antigen (CEA), and CYFRA 21-1 (Roche Diagnostics GmbH, Mannheim, Germany) were analyzed. Results: SCC showed a very strong effect in relation to the sampling time and procedure. While the first two points in time (T1; T2) were highly comparable (median fold-change: 0.84; $p = 0.7354$; correlation $\rho = 0.883$), patients showed a significant increase (median fold-change: 4.96; $p = 0.0017$; correlation $\rho = -0.036$) in concentration when comparing T1 with the sample time subsequent to anesthesia induction (T3). A much weaker increase was found for CYFRA 21-1 at T3 (median fold-change: 1.40; $p = 0.0479$). The concentration of CEA showed a very small, but systematic decrease (median fold-change: 0.72; $p = 0.0039$).

Conclusions: In this study we show the unexpectedly marked influence of blood withdrawal timing (before vs. after anesthesia) and procedure (venous versus arterial vessel puncture) has on the concentration of the protein biomarker SCC and to a less extent upon CYFRA21-1. The potential causes for these effects remain to be elucidated in subsequent studies, however these findings highlight the importance of a standardized, controlled blood collection protocol for biomarker detection.

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Distinct niches within the extracellular matrix dictate fibroblast function in (cell free) 3D lung tissue cultures.

Burgstaller G(1), Sengupta A(1), Vierkotten S(1), Preissler G(1)(2), Lindner M(1)(3), Behr J(1)(4), Königshoff M(1)(5), Eickelberg O(5).

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A comprehensive characterization of lung adenocarcinoma (LADC) clinical features is currently missing. We prospectively evaluated Caucasian patients with early-stage LADC. Patients with LADC diagnosed between 2011 and 2015 were prospectively assessed for lung resection with curative intent. Fifty clinical, pathologic, radiologic, and molecular variables were recorded. Patients were followed till death/study conclusion. The main findings were compared to a separate cohort from France. Of 1943 patients evaluated, 366 were enrolled (18.8%; 181 female; 75 never-smokers; 28% of registered Bavarian cases over the study period). Smoking and obstruction were significantly more prevalent in GLAD compared with adult Bavarians ($P < 0.0001$). Ever-smoker tumors were preferentially localized to the upper lobes. We observed 120 relapses and 74 deaths over 704 cumulative follow-up years. Median overall and disease-free survival were >7.5 and 3.6 years, respectively. Patients aged <45 or >65 years, resected >60 days postdiagnosis, with abnormal FVC/DLCOVA, N2/N3 stage, or solid histology had significantly decreased survival estimates. These were fit into a weighted locoregional LADC death risk score that outperformed pTNM7 in predicting survival in the GLAD and in our second cohort. We define the clinical gestalt of locoregional LADC and provide a new clinical tool to predict survival, findings that may aid future management and research design.

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Genome-Wide DNA Methylation Profiling in Early Stage I Lung Adenocarcinoma Reveals Predictive Aberrant Methylation in the Promoter Region of the Long Noncoding RNA PLUT: An Exploratory Study.

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Abstract:
INTRODUCTION: Surgical procedure is the treatment of choice in early stage I lung adenocarcinoma. However, a considerable number of patients experience recurrence within the first 2 years after complete resection. Suitable prognostic biomarkers that identify patients at high risk of recurrence (who may probably benefit from adjuvant treatment) are still not available. This study aimed at identifying methylation markers for early recurrence that may become important tools for the development of new treatment modalities.
METHODS: Genome-wide DNA methylation profiling was performed on 30 stage I lung adenocarcinomas, comparing 14 patients with early metastatic recurrence with 16 patients with a long-term relapse-free survival period using methylated-CpG-immunoprecipitation followed by high-throughput next-generation sequencing. The differentially methylated regions between the two subgroups were validated for their prognostic value in two independent cohorts using the MassCLEAVE assay, a high-resolution quantitative methylation analysis.
RESULTS: Unsupervised clustering of patients in the discovery cohort on the basis of differentially methylated regions identified patients with shorter relapse-free survival (hazard ratio: 2.23; 95% confidence interval: 0.66-7.53; $p = 0.03$). In two validation cohorts, promoter hypermethylation of the long noncoding RNA PLUT was significantly associated with shorter relapse-free survival (hazard ratio: 0.54; 95% confidence interval: 0.31-0.93; $p < 0.026$) and could be reported as an independent prognostic factor in the multivariate Cox regression analysis.
CONCLUSIONS: Promoter hypermethylation of the long noncoding RNA PLUT is predictive in patients with early stage I adenocarcinoma at high risk for early recurrence. Further studies are needed to validate its role in carcinogenesis and its use as a biomarker.

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Clinical Course of Three Postoperative Symptomatic COVID-19 Cases in Patients After Lung Lobectomy.

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Abstract:
The novel coronavirus disease 2019 is a highly contagious viral infection caused by the severe acute respiratory syndrome coronavirus 2 virus. Its rapid spread and severe clinical presentation influence patient management in all specialties including thoracic surgery. We report 3 cases of coronavirus disease 2019 occurring in patients shortly after thoracotomy and thoracoscopy procedures, illustrating the imminent threat of severe acute respiratory syndrome coronavirus 2 infection for thoracic surgery patients.

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Versatile workflow for cell type–resolved transcriptional and epigenetic profiles from cryopreserved human lung

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Abstract

Complexity of lung microenvironment and changes in cellular composition during disease make it exceptionally hard to understand molecular mechanisms driving development of chronic lung diseases. Although recent advances in cell type–resolved approaches hold great promise for studying complex diseases, their implementation relies on local access to fresh tissue, as traditional tissue storage methods do not allow viable cell isolation. To overcome these hurdles, we developed a versatile workflow that allows storage of lung tissue with high viability, permits thorough sample quality check before cell isolation, and befits sequencing-based profiling. We demonstrate that cryopreservation enables isolation of multiple cell types from both healthy and diseased lungs. Basal cells from cryopreserved airways retain their differentiation ability, indicating that cellular identity is not altered by cryopreservation. Importantly, using RNA sequencing and EPIC Array, we show that gene expression and DNA methylation signatures are preserved upon cryopreservation, emphasizing the suitability of our workflow for omics profiling of lung cells. Moreover, we obtained high-quality single-cell RNA-sequencing data of cells from cryopreserved human lungs, demonstrating that cryopreservation empowers single-cell approaches. Overall, thanks to its simplicity, our workflow is well suited for prospective tissue collection by academic collaborators and biobanks, opening worldwide access to viable human tissue.

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Infrared molecular fingerprinting of blood-based liquid biopsies for the detection of cancer

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Abstract:
Recent omics analyses of human biofluids provide opportunities to probe selected species of biomolecules for disease diagnostics. Fourier-transform infrared (FTIR) spectroscopy investigates the full repertoire of molecular species within a sample at once. Here, we present a multi-institutional study in which we analysed infrared fingerprints of plasma and serum samples from 1639 individuals with different solid tumours and carefully matched symptomatic and non-symptomatic reference individuals. Focusing on breast, bladder, prostate, and lung cancer, we find that infrared molecular fingerprinting is capable of detecting cancer: training a support vector machine algorithm allowed us to obtain binary classification performance in the range of 0.78–0.89 (area under the receiver operating characteristic curve [AUC]), with a clear correlation between AUC and tumour load. Intriguingly, we find that the spectral signatures differ between different cancer types. This study lays the foundation for high-throughput onco-IR-phenotyping of four common cancers, providing a cost-effective, complementary analytical tool for disease recognition

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Single-cell RNA sequencing reveals ex vivo signatures of SARS-CoV-2-reactive T cells through 'reverse phenotyping'

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Abstract
The in vivo phenotypic profile of T cells reactive to severe acute respiratory syndrome (SARS)-CoV-2 antigens remains poorly understood. Conventional methods to detect antigen-reactive T cells require in vitro antigenic re-stimulation or highly individualized peptide-human leukocyte antigen (pHLA) multimers. Here, we use single-cell RNA sequencing to identify and profile SARS-CoV-2-reactive T cells from Coronavirus Disease 2019 (COVID-19) patients. To do so, we induce transcriptional shifts by antigenic stimulation in vitro and take advantage of natural T cell receptor (TCR) sequences of clonally expanded T cells as barcodes for 'reverse phenotyping'. This allows identification of SARS-CoV-2-reactive TCRs and reveals phenotypic effects introduced by antigen-specific stimulation. We characterize transcriptional signatures of currently and previously activated SARS-CoV-2-reactive T cells, and show correspondence with phenotypes of T cells from the respiratory tract of patients with severe disease in the presence or absence of virus in independent cohorts. Reverse phenotyping is a powerful tool to provide an integrated insight into cellular states of SARS-CoV-2-reactive T cells across tissues and activation states.

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KRAS signaling in malignant pleural mesothelioma

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Abstract

Malignant pleural mesothelioma (MPM) arises from mesothelial cells lining the pleural cavity of asbestos-exposed individuals and rapidly leads to death. MPM harbors loss-of-function mutations in BAP1, NF2, CDKN2A, and TP53, but isolated deletion of these genes alone in mice does not cause MPM and mouse models of the disease are sparse. Here, we show that a proportion of human MPM harbor point mutations, copy number alterations, and overexpression of KRAS with or without TP53 changes. These are likely pathogenic, since ectopic expression of mutant KRASG12D in the pleural mesothelium of conditional mice causes epithelioid MPM and cooperates with TP53 deletion to drive a more aggressive disease form with biphasic features and pleural effusions. Murine MPM cell lines derived from these tumors carry the initiating KRASG12D lesions, secondary Bap1 alterations, and human MPM-like gene expression profiles. Moreover, they are transplantable and actionable by KRAS inhibition. Our results indicate that KRAS alterations alone or in accomplice with TP53 alterations likely play an important and underestimated role in a proportion of patients with MPM, which warrants further exploration.

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Prevention of COVID-19 in Thoracic Surgery Patients: Lessons Learned during the First Pandemic Wave

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Abstract

Background: The aim of this retrospective study was to investigate the implementation of measures to prevent perioperative COVID-19 in thoracic surgery during the first wave of the COVID-19 pandemic 2020 allowing a continued surgical treatment of patients.

Methods: The implemented preventive measures in patient management of the thoracic surgery department of the Asklepios Lung Clinic Munich-Gauting, Germany were retrospectively analyzed. Postoperative COVID-19 incidence before and after implementation of preventive measures was investigated. Patients admitted for thoracic surgical procedures between March and May 2020 were included in the study. Patient characteristics were analyzed. For the early detection of putative postoperative COVID-19 symptoms, typical post-discharge symptomatology of thoracic surgery patients was compared to non-surgical patients hospitalized for COVID-19.

Results: Thirty-five surgical procedures and fifty-seven surgical procedures were performed before and after implementation of the preventive measures, respectively. Three patients undergoing thoracic surgery before implementation of preventive measures developed a COVID-19 pneumonia post-discharge. After implementation of preventive measures, no postoperative COVID-19 cases were identified. Fever, dyspnea, dry cough and diarrhea were significantly more prevalent in COVID-19 patients compared to normally recovering thoracic surgery patients, while anosmia, phlegm, low energy levels, body ache and nausea were similarly frequent in both groups.

Conclusions: Based on the lessons learned during the first pandemic wave, we here provide a blueprint for successful easily implementable preventive measures minimizing SARS-CoV-2 transmission to thoracic surgery patients perioperatively. While symptoms of COVID-19 and the normal postoperative course of thoracic surgery patients substantially overlap, we found dyspnea, fever, cough, and diarrhea significantly more prevalent in COVID-19 patients than in normally recovering thoracic surgery patients. These symptoms should trigger further diagnostic testing for postoperative COVID-19 in thoracic surgery patients.

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Prognostic phenotypes of early-stage lung adenocarcinoma

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Abstract

Background: Survival after curative resection of early-stage lung adenocarcinoma (LUAD) varies and prognostic biomarkers are urgently needed.

Methods: Large-format tissue samples from a prospective cohort of 200 patients with resected LUAD were immunophenotyped for cancer hallmarks TP53, NF1, CD45, PD-1, PCNA, TUNEL, and FVIII, and were followed for median (95%CI)=2.34 (1.71-3.49) years.

Results: Unsupervised hierarchical clustering revealed two patient subgroups with similar clinicopathologic features and genotype, but with markedly different survival: "proliferative" patients (60%) with elevated TP53, NF1, CD45, and PCNA expression had 50% 5-year overall survival while "apoptotic" patients (40%) with high TUNEL had 70% 5-year survival [HR95%CI=2.23 (1.33-3.80); p=0.0069]. Cox regression and machine learning algorithms including random forests built clinically useful models: a score to predict overall survival and a formula and nomogram to predict tumour phenotype. The distinct LUAD phenotypes were validated in TCGA and KMplotter data and showed prognostic power supplementary to IASLC TNM stage and WHO histologic classification.

Conclusions: Two molecular subtypes of LUAD exist and their identification provides important prognostic information.

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Automated quantitative thin slice volumetric low dose CT analysis predicts disease severity in COVID-19 patients

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Abstract

Purpose: This study aimed to identify predictive (bio-)markers for COVID-19 severity derived from automated quantitative thin slice low dose volumetric CT analysis, clinical chemistry and lung function testing.

Methods: Seventy-four COVID-19 patients admitted between March 16th and June 3rd 2020 to the Asklepios Lung Clinic Munich-Gauting, Germany, were included in the study. Patients were categorized in a non-severe group including patients hospitalized on general wards only and in a severe group including patients requiring intensive care treatment. Fully automated quantification of CT scans was performed via IMBIO CT Lung Texture analysis™ software. Predictive biomarkers were assessed with receiver-operator-curve and likelihood analysis.

Results: Fifty-five patients (44% female) presented with non-severe COVID-19 and 19 patients (32% female) with severe disease. Five fatalities were reported in the severe group. Accurate automated CT analysis was possible with 61 CTs (82%). Disease severity was linked to lower residual normal lung (72.5% vs 87%, $p = 0.003$), increased ground glass opacities (GGO) (8% vs 5%, $p = 0.031$) and increased reticular pattern (8% vs 2%, $p = 0.025$). Disease severity was associated with advanced age (76 vs 59 years, $p = 0.001$) and elevated serum C-reactive protein (CRP, 92.2 vs 36.3 mg/L, $p < 0.001$), lactate dehydrogenase (LDH, 485 vs 268 IU/L, $p < 0.001$) and oxygen supplementation ($p < 0.001$) upon admission. Predictive risk factors for the development of severe COVID-19 were oxygen supplementation, LDH >313 IU/L, CRP >71 mg/L, <70% normal lung texture, >12.5% GGO and >4.5% reticular pattern.

Conclusion: Automated low dose CT analysis upon admission might be a useful tool to predict COVID-19 severity in patients.