Virtual Issue of *The Journal of Pathology*, February 2018

Recent Advances in Respiratory Pathology

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The Pathological Society of Great Britain and Ireland publishes two journals, *The Journal of Pathology* and *The Journal of Pathology; Clinical Research*. Both journals follow the mission of the Society – *Understanding Disease*, with *The Journal of Pathology* publishing translational research, whilst *The Journal of Pathology; Clinical Research* concentrates more on applied research with direct clinical relevance. In this Virtual Issue, I have compiled 29 recent papers, commentaries and reviews that discuss aspects of malignant, fibrotic and inflammatory diseases that occur in the lung and respiratory system, ranging from mechanistic studies to the identification of diagnostic or predictive biomarkers.

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Molecular characterisation of lung malignancies

Lung carcinomas are phenotypically diverse and their anatomical, histological and molecular characteristics depend to some extent on the cell(s) of origin. The molecular pathology of lung squamous carcinomas was recently reviewed, with a particular focus on the role of disrupted intercellular adhesion and cell polarity in this subtype [1]. Researchers from Vancouver and Toronto used a novel approach to identify nuclear factor I/B (NFIB) as commonly down-regulated in non-small-cell-lung carcinoma (NSCLC). The study began with a comprehensive miRNA profiling of human fetal lung and subsequent identification of “oncofetal” miRNAs that are re-expressed in NSCLC. Of these, many target NFIB mRNA and low levels of NFIB were associated with biologically aggressive tumours and poor survival in lung adenocarcinoma patients [2]. Around 5% of NSCLC contain anaplastic lymphoma kinase (ALK) rearrangements and whilst most of these respond to ALK inhibitors, some do not. An extensive molecular analysis of ALK-rearranged NSCLCs identified that EML4-ALK short forms correlate with poor prognosis and have lower sensitivity to ALK inhibitors [3]. The study also discovered four novel somatic ALK mutations that confer primary resistance and three novel fusion partners, showing different intracellular localisations. Together, the data highlight the importance of molecular profiling for predicting the effects of ALK inhibitors in lung carcinoma [3].

The human TP53 gene is commonly mutated in lung carcinomas. Lozano and colleagues used transgenic mice to investigate the effects of the p53 inhibitor, Mdm4 [4]. Amongst other findings, including evidence for p53-independent effects, of particular relevance to this Virtual Issue is that Mdm4 accelerated mutant K-Ras driven lung tumourigenesis, suggesting an important role in lung adenocarcinoma patients with mutant Ras and increased MDM4 copy number [4]. A recent study by Aldo Scarpa and colleagues across Italy investigated the genetic landscape of lung neuroendocrine tumours, including the four WHO classification categories, using whole-exome and targeted next generation sequencing. The data help to provide a molecular classification that complements histology and identifies both global and sub-type specific prognostic markers [5].

   Ester Bonastre, Elisabeth Brambilla and Montse Sanchez-Cespedes
   *The Journal of Pathology* 2016; **238**: 606-616.
Developmental transcription factor NFIB is a putative target of oncofetal miRNAs and is associated with tumour aggressiveness in lung adenocarcinoma.

Daiana D Becker-Santos, Kelsie L Thu, John C English, Larissa A Pikor, Victor D Martinez, May Zhang, Emily A Vucic, Margaret TY Luk, Anita Carraro, Jagoda Korbelik, Daniela Piga, Nicolas M Lhomme, Mike J Tsay, John Yee, Calum E MacAulay, Stephen Lam, William W Lockwood, Wendy P Robinson, Igor Jurisica and Wan L Lam


Ka-Won Noh, Mi-Sook Lee, Seung Eun Lee, Ji-Young Song, Hyun-Tae Shin, Yu Jin Kim, Doo Yi Oh, Kyungsoo Jung, Minjung Sung, Mengi Kim, Sungbin An, Joungho Han, Young Mog Shim, Jae Ill Zo, Jhingook Kim, Woong-Yang Park, Se-Hoon Lee and Yoon-La Choi


The p53 inhibitor Mdm4 cooperates with multiple genetic lesions in tumourigenesis.

Shunbin Xiong, Vinod Pant, Yun Zhang, Neeraj K Aryal, M James You, Donna Kusewitt and Guillermina Lozano


Lung neuroendocrine tumours: deep sequencing of the four World Health Organization histotypes reveals chromatin-remodelling genes as major players and a prognostic role for TERT, RB1, MEN1 and KMT2D.

Michele Simbolo, Andrea Mafficini, Katarzyna O Sikora, Matteo Fassan, Stefano Barbi, Vincenzo Corbo, Luca Mastracci, Borislav Rusev, Federica Grillo, Caterina Vicentini, Roberto Ferrara, Sara Pilotto, Federico Davini, Giuseppe Pelosi, Rita T Lawlor, Marco Chilosi, Giampaolo Tortora, Emilio Bria, Gabriella Fontanini, Marco Volante and Aldo Scarpa


Mechanistic studies and prognostic/predictive markers in lung carcinoma

As with other malignancies, lung carcinomas are thought to be maintained by a distinct population of cancer stem cells. A novel pathway for promoting lung cancer stem cells in NSCLC was recently reported, involving the action of Serine-arginine protein kinase 1 (SRPK1) and leading to hyperactive Wnt/β-catenin signalling [6]. Importantly, SRPK1 levels associate with the clinical features of human NSCLC and may represent a novel therapeutic target. These findings are placed into a broader context in an Invited Commentary, which discusses mechanisms and the pleiotropic effects of SRPK1 in other cancer types [7]. Activation of Wnt/β-catenin signalling is a known factor in many malignancies, including lung carcinogenesis. Recent data have identified links between Wnt and the Hippo pathway, which is involved in regulating organ size and whose inhibition promoted malignancy. A survey of 127 NSCLCs identified decreased levels of WWC3, a scaffolding protein that was shown to stimulate the Hippo pathway and inhibit Wnt signalling [8]. The reduced WWC3 levels correlated with poor differentiation, lymph node metastasis and poor prognosis, suggesting that WWC3 is an upstream link between Wnt and Hippo pathways that may be a therapeutic target in NSCLC [8]. Growth factor receptors are increasingly recognised as potential targets for biological therapy. In one recent report, fibroblast growth factor receptors (FGFR) 1-3 were analysed in 653 early stage NSCLC tissues using immunohistochemistry. FGFR1 was associated with adenocarcinomas and poor prognosis, and FGFR3 with squamous cell carcinomas. Translocations and amplification of FGFR3 were also found. Thus, FGFRs serve as prognostic markers and interesting therapeutic targets for the treatment of early stage NSCLC [9]. The MET receptor for hepatocyte growth factor (HGF) is also a potential target for treatment of lung carcinomas, but the lack of robust companion
diagnostics hampers the success of anti-HGF/MET drugs. A comprehensive study by investigators from Los Angeles, Halle, Maryland and Michigan used seven antibodies in the HGF/MET pathway on 20 representative cases of each of 18 human cancer types. They revealed cancer-type specific differences in the performance of antibodies and their data paves the way for improved analysis of the pathway in lung and other malignancies to predict drug response [10]. Tumour angiogenesis is a prognostic factor for many neoplasms, often measured as microvessel density. Combining nestin as a marker of newly formed vessels with Ki67 to identify proliferating vessels in 210 lung adenocarcinomas, the vascular proliferation index was found to be an independent prognostic factor for reduced cancer-specific survival, suggesting it should be explored in trials of anti-angiogenesis therapy [11]. Finally in this section, a novel effect of the endogenous angiogenesis inhibitor, endostatin, was identified in p53-deficient NCSLC. The mechanism involves endostatin interacting with DNA-dependent protein kinase, inhibiting its activity and leading to accumulation of unrepaired DNA lesions following genotoxic therapy. Thus, TP53 mutation is a potential biomarker for patients in whom endostatin may have dual beneficial effects by inhibiting angiogenesis and boosting the effects of genotoxic therapies [12].

6. **Serine-arginine protein kinase 1 promotes a cancer stem cell-like phenotype through activation of Wnt/β-catenin signalling in NSCLC.**
Liyun Gong, Junwei Song, Xi Lin, Fakai Wei, Cuicui Zhang, Zimei Wang, Jinrong Zhu, Shu Wu, Yu Chen, Jin Liang, XiaoYuan Fu, Junqiang Lu, Chunhui Zhou and Libing Song

7. **The many faces of SRPK1.**
Nicholas Bullock and Sebastian Oltean

8. **WWC3 regulates the Wnt and Hippo pathways via Dishevelled proteins and large tumour suppressor 1, to suppress lung cancer invasion and metastasis.**
Qiang Han, Xuyong Lin, Xiupeng Zhang, Guiyang Jiang, Yong Zhang, Yuan Miao, Xuezhu Rong, Xiaoying Zheng, Yong Han, Xu Han, Jingji Wu, Joachim Kremerskothen and Enhua Wang

9. **FGFR1, 2 and 3 protein overexpression and molecular aberrations of FGFR3 in early stage non-small cell lung cancer.**
Willemijn SME Theelen, Lorenza Mittempergher, Stefan M Willems, Astrid J Bosma, Dennis DGC Peters, Vincent van der Noort, Eva J Japenga, Ton Peeters, Koos Koole, Tonći Šuštić, JL Blaauwgeers, Carel J van Noesel, René Bernards and Michel M van den Heuvel

10. **Quantitative imaging for development of companion diagnostics to drugs targeting HGF/MET.**
Fangjin Huang, Zhaoxuan Ma, Sara Pollan, Xiaopu Yuan, Steven Swartwood, Arkadiusz Gertych, Maria Rodriguez, Jayati Mallick, Sanica Bhele, Maha Guindi, Deepti Dhall, Ann E Walts, Shikha Bose, Mariza de Peralta Venturina, Alberto M Marchevsky, Daniel J Luthringer, Stephan M Feller, Benjamin Berman, Michael R Freeman, W Gregory Alvord, George Vande Woude, Mahul B Amin and Beatrice S Knudsen

11. **Microvascular proliferation is associated with aggressive tumour features and reduced survival in lung adenocarcinoma.**
Maria Ramnøffjell, Christina Aamelfot, Sura Aziz, Lars Helgeland and Lars A Akslen
Pulmonary fibrosis

Lung fibrosis is the result of dysfunctional repair mechanisms involving epithelial cells, fibroblasts and innate immunity. Using a TGFβ-induced model, fibroblast growth factor-1 (FGF1) was found to inhibit myofibroblast differentiation and induce epithelial cell proliferation whilst reducing TGFβ signalling in epithelial and myofibroblast cells [13]. These data were further analysed in an accompanying Invited Commentary by Jeffrey Horowitz and colleagues [14], placing their relevance in the context of nintedanib and perfenidone, the first drugs approved for idiopathic pulmonary fibrosis (IPF) treatment. The type 2 cytokine, interleukin-13 (IL-13), contributes to tissue repair and the development of fibrosis, as well as inhibiting type 1 inflammatory cytokines such as IFN-γ that in turn inhibit type 2 cytokine production. Using murine transgenic models deficient for IL-13 and/or IFN-γ, Thomas Wynn and colleagues demonstrated that suppression of IL-13 reduces fibrosis by an IFNγ-independent mechanism and blocking both IL-13 and IFN-γ simultaneously confers greater protection from progressive fibrosis [15]. Fibrosis is also affected by the infiltration of monocytes into sites of tissue injury. These cells are generally considered as a source of tissue macrophages, but new work indicates that monocytes may retain myeloid characteristics without differentiating into macrophages during lung fibrosis [16]. These monocytic myeloid-derived suppressor cells contribute to collagen deposition by expressing TGFβ-1 and inhibiting remodelling enzymes through tissue inhibitor of matrix metalloproteinase-1 (TIMP1). Fibroblasts in pulmonary fibrosis are known to be resistant to apoptosis and a mechanistic study of this property revealed a role for Decoy Receptor 3 (DcR3), which is increased by the interaction of IPF fibroblasts with collagen and protects them from FasL-induced cell death [17]. Thus, inhibition of DcR3 function may limit the progression of lung fibrosis. Macrophage activation is another key component of lung fibrosis. Using transgenic mice models, it was found that endoplasmic reticulum stress and the associated unfolded protein response are activated in macrophages in fibrotic lung and associate directly with macrophage apoptosis [18]. The whole area of lung fibrosis (and other respiratory disorders) was the subject of an Invited Review, concentrating on the use of spontaneous and artificially induced animal models of these diseases [19]. One conclusion of this review is that domestic animals suffer from similar respiratory diseases to humans, and may serve as useful models in the future.

12. **Endostatin sensitizes p53-deficient non-small-cell lung cancer to genotoxic chemotherapy by targeting DNA-dependent protein kinase catalytic subunit.**
   Lin Jia, Xin-an Lu, Guanghua Liu, Shan Wang, Min Xu, Yang Tian, Shaosen Zhang, Yan Fu and Yongzhang Luo

13. **Fibroblast growth factor-1 attenuates TGF-β1-induced lung fibrosis.**
    Chiko Shimbori, Pierre-Simon Bellaye, Jiaji Xia, Jack Gauldie, Kjetil Ask, Carlos Ramos, Carina Becerril, Annie Pardo, Moises Selman and Martin Kolb

14. **Fibroblast growth factors and pulmonary fibrosis: it's more complex than it sounds.**
    Kevin K Kim, Thomas H Sisson and Jeffrey C Horowitz

15. **Enhanced protection from fibrosis and inflammation in the combined absence of IL-13 and IFN-γ.**
    Thirumalai R Ramalingam, Richard L Gieseck, Thomas H Acciani, Kevin M Hart, Allen W Cheever, Margaret M Mentink-Kane, Kevin M Vannella and Thomas A Wynn
16. **CCR2**+ monocytic myeloid-derived suppressor cells (M-MDSCs) inhibit collagen degradation and promote lung fibrosis by producing transforming growth factor-β1.
Astrid Lebrun, Sandra Lo Re, Mathilde Chantry, Xavier Izquierdo Carerra, Francine Uwambayinema, Doriana Ricci, Raynal Debosse, Saloua Ibouraadaten, Lisa Brombin, Mihaly Palmai-Pallag, Yousof Yakoub, Manolis Pasparakis, Dominique Lison and François Huaux

17. **Idiopathic pulmonary fibrosis fibroblasts become resistant to Fas ligand-dependent apoptosis via the alteration of decoy receptor 3.**
Jintaek Im, Kyutae Kim, Polla Hergert and Richard Seonghun Nho

18. **GRP78 and CHOP modulate macrophage apoptosis and the development of bleomycin-induced pulmonary fibrosis.**

19. **Studying human respiratory disease in animals – role of induced and naturally occurring models.**
Kurt Williams and Jesse Roman

**Allergic and infectious conditions**

In addition to the review above that discusses asthma and emphysema alongside fibrosis [19], another recent review focusses on the changes in extracellular matrix components in a range of pulmonary diseases [20]. The review provides a comprehensive and integrative discussion of the alterations in the composition, folding or rigidity of extracellular matrix proteins in asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), highlighting potential avenues for therapeutic intervention. There are also a number of primary articles in relation to these and other respiratory system diseases. Emphysema is a major consequence of COPD and is known to be associated with altered TGFβ1/SMAD signalling and altered matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (TIMP) activities. A recent study demonstrates a role for prothrombin α in regulating SMAD7 acetylation and activity, both in animal models and in human patients [21]. In asthma, a chronic allergic airway disease, the extracellular matrix protein Fibulin 1c is increased in serum and airway fluids. In a multi-institute study involving researchers from the USA, Australia and The Netherlands, transgenic knockout mice, pharmacologic inhibition and human epithelial cells were used to show a role for Fibulin 1c in airway collagen deposition, airway hyper-responsiveness and inflammatory cells during allergic airway disease, suggesting a potential therapeutic avenue for asthma patients [22]. Bronchopulmonary dysplasia is a chronic lung disease of prematurely born infants. Using a hyperoxia-induced model, lack of fibroblast growth factor 10 (FGF10) exacerbated hyperoxic lung injury and altered the formation of alveolar epithelial type II (AEC II) cells. Thus, a deficiency in AEC II cells may be an additional complication for infants with bronchopulmonary dysplasia [23]. Hypoxic acute lung injury is a complication in patients ventilated with high oxygen concentrations and leads to pulmonary oedema as a consequence of leakage from blood vessels. Using transgenic mice and clinical samples, VEGF-D and its receptor, VEGFR-3, were found to promote vascular leakage and oedema after hypoxia [24]. The pathology and mechanisms involved in pulmonary arterial hypertension (PAH), also termed World Health Organisation (WHO)
group 1 pulmonary hypertension, were recently reviewed by Lijiang Ma and Wendy Chung, emphasising the diversity of PAH and with a particular focus on the role of genetics [25].

Pulmonary dysfunction is an early complication of acute pancreatitis and accounts for over half of deaths in the first week after onset of this disease. A recent study showed that circulating exosomes are increased in pancreatitis and reach the alveoli, where they are internalised by pulmonary macrophages, polarising them to a pro-inflammatory M1 phenotype. Interestingly, proteomic analysis suggested the liver as a major source of these exosomes and tracking experiments revealed that the liver retains the majority of exosomes from the peritoneal cavity [26]. Human respiratory syncytial virus (RSV) is the most common cause of severe lower respiratory tract disease in young children, causing around 200,000 deaths and more than 3 million hospitalisations each year worldwide. A recent study showed that activated neutrophils in RSV infections cause the formation of neutrophil extracellular traps (NETs), which capture RSV to limit infection, but their exaggerated formation contributes to the pathogenesis of airway obstruction and associated morbidity [27]. Pneumonia is another infectious disease of the lower respiratory tract with a huge morbidity worldwide. A recent study highlights a role for endoplasmic chaperoning of Toll-like receptors (TLR2 and TLR4) by macrophages in their response to K. pneumoniae infection. A potential role for integrin signaling in these response was shown to be unlikely, placing macrophage TLR signalling at the centre of an adequate host defence [28]. Finally in this Virtual Issue, I draw attention to a recent paper in our Open Access journal, The Journal of Pathology: Clinical Research, that investigated lung allograft dysfunction, the major obstacle to long-term survival following lung transplantation. A compartment-specific study using morphology, molecular and immunostaining approaches revealed an absence of classical fibrinolytic enzymes and an alternative macrophage-mediated process that could represent a therapeutic target. The study also provides diagnostically relevant data and helps predict the clinical course of respiratory dysfunction [29].

20. The extracellular matrix – the under-recognized element in lung disease?
Janette K Burgess, Thais Mauad, Gavin Tjin, Jenny C Karlsson and Gunilla Westergren-Thorsson

21. Over-expression of prothymosin-α antagonizes TGFβ signalling to promote the development of emphysema.
Bing-Hua Su, Yau-Lin Tseng, Gia-Shing Shieh, Yi-Cheng Chen, Pensee Wu, Ai-Li Shiau and Chao-Liang Wu
The Journal of Pathology 2016; 238: 412-422.

22. Airway remodelling and inflammation in asthma are dependent on the extracellular matrix protein fibulin-1c.
Gang Liu, Marion A Cooley, Prema M Nair, Chantal Donovan, Alan C Hsu, Andrew G Jarnicki, Tatt Jhong Haw, Nicole G Hansbro, Qi Ge, Alexandra C Brown, Hock Tay, Paul S Foster, Peter A Wark, Jay C Horvat, Jane E Bourke, Chris L Grainge, W Scott Argraves, Brian G Oliver, Darryl A Knight, Janette K Burgess and Philip M Hansbro

23. Fgf10 deficiency is causative for lethality in a mouse model of bronchopulmonary dysplasia.
Cho-Ming Chao, Faady Yahya, Alena Moiseenko, Caterina Tiozzo, Amit Shrestha, Negah Ahmadvand, Elie El Agha, Jennifer Quantius, Salma Dilai, Vahid Kheirollahi, Matthew Jones, Jochen Wilhem, Gianni Carraro, Harald Ehrhardt, Klaus-Peter Zimmer, Guillermo Barreto, Katrin Ahlbrecht, Rory E Morty, Susanne Herold, Rosanna G Abellar, Werner Seeger, Ralph Schermuly, Jin-San Zhang, Parviz Minoo and Saverio Bellusci
24. **VEGF-D promotes pulmonary oedema in hyperoxic acute lung injury.**
   Teruhiko Sato, Sophie Paquet-Fifield, Nicole C Harris, Sally Roufail, Debra J Turner, Yinan Yuan, You-Fang Zhang, Stephen B Fox, Margaret L Hibbs, Jennifer L Wilkinson-Berka, Richard A Williams, Steven A Stacker, Peter D Sly and Marc G Achen

25. **The role of genetics in pulmonary arterial hypertension.**
   Lijiang Ma and Wendy K Chung

26. **Involvement of exosomes in lung inflammation associated with experimental acute pancreatitis.**
   Laia Bonjoch, Vanessa Casas, Montserrat Carrascal and Daniel Closa

27. **Neutrophil extracellular traps cause airway obstruction during respiratory syncytial virus disease.**
   Bart Cortjens, Onno J de Boer, Rineke de Jong, Adriaan FG Antonis, Yanaika S Sabogal Piñeros, René Lutter, Job BM van Woensel and Reinout A Bem
   *The Journal of Pathology* 2016; **238**: 401-411.

28. **Endoplasmic reticulum chaperone gp96 in macrophages is essential for protective immunity during Gram-negative pneumonia.**
   Adam A Anas, Alex F de Vos, Arie J Hoogendijk, Miriam HP van Lieshout, Jeroen WJ van Heijst, Sandrine Florquin, Zihai Li, Cornelis van 't Veer and Tom van der Poll
   *The Journal of Pathology* 2016; **238**: 74-84.

29. **Comparative analysis of morphological and molecular motifs in bronchiolitis obliterans and alveolar fibroelastosis after lung and stem cell transplantation.**
   Danny Jonigk, Berenice Rath, Paul Borchert, Peter Braubach, Lavinia Maegel, Nicole Izykowski, Gregor Warnecke, Wiebke Sommer, Hans Kreipe, Robert Blach, Adrian Anklamm, Axel Haverich, Matthias Eder, Michael Stadler, Tobias Welte, Jens Gottlieb, Mark Kuehnel and Florian Laenger

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