Stress Responses Affecting Homeostasis of the Alveolar Capillary Unit

Rubin M. Tudor¹ and Toshinori Yoshida²

¹Program in Translational Lung Research, Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, University of Colorado School of Medicine, Aurora, Colorado; and ²The Institute of Environmental Toxicology, Ibaraki, Japan

The maintenance of the alveolar structure is required throughout life. To accomplish this goal, alveolar cells, including endothelial, epithelial, and fibroblastic cells, provide key molecules with broad survival and antiapoptotic effects. These complex interactions are disrupted by cigarette smoke, leading to emphysema. Smoke imposes an environmental stress to the lung with the activation of "sensor-like" molecular signaling. Activation of RTP801, leading to mTOR inhibition, is paradigmatic of these responses. The accumulation of cellular damage, with the generation of endogenous mediators of inflammation, may proceed toward an aging phenotype. These alterations may impose significant challenges to cell-based regenerative or pharmacological therapies.

Keywords: alveolus; emphysema; cigarette smoke; COPD; mTOR

The destruction of the alveolar structure is a key component of a large number of pulmonary diseases. A distinct pathological phenotype is the disappearance of alveolar septa, which is characteristic of emphysema, particularly in the setting of chronic obstructive pulmonary disease (COPD) caused by cigarette smoke. Adult-onset emphysema shares the morphological features present in the chronic stage of bronchopulmonary dysplasia (1), which results largely from a disruption of lung development and maturation and growth. These shared features suggest that similar molecular mechanisms may account for the emphysematous alveolar phenotype in both disease settings. Lung development and the expansion of alveolar surface areas require a complex set of coordinated molecular signals. Although alveolar formation ceases in the first decade of life, continuous maintenance of alveolar structure is required for proper preservation of gas exchange structures, including alveolar capillaries, later in life. We have proposed that disruption of the molecular signals involved in alveolar structural maintenance may play a central role in emphysema development due to cigarette smoke (2). Many of the molecular signals involved in lung development and growth are required to maintain the lung throughout adulthood, which therefore can explain the similar pathological manifestation of emphysema in COPD and bronchopulmonary dysplasia, with reduction of alveolar structures and decrease in overall gas exchange surface.

COPD has been considered a disease resulting from chronic inflammation, leading to extracellular matrix remodeling (3). We have recently summarized that the interplay between injurious agents may synergize with endogenously released danger-associated molecular patterns (DAMPs) in promoting pulmonary injury in several lung diseases (4) (Figure 1), many of which are also characterized by inflammation, such as COPD.

(Received in original form March 22, 2011; accepted in final form July 29, 2011)

Supported by COPD SCCOR P01-NHLBI 085609, R01ES016285, and the Flight Attendant Medical Research Institute (to R.M.T.).

Correspondence and requests for reprints should be addressed to Rubin M. Tudor, M.D., Anschutz Medical Campus Research 2, Room 9001, 12700 East 19th Avenue, Aurora, CO 80045. E-mail: Rubin.Tudor@ucdenver.edu

Proc Am Thorac Soc Vol 8. pp 485–491, 2011

DOI: 10.1513/pats.201103-029MW

Internet address: www.atsjournals.org

In fact, several DAMPs represent cellular components, such as high-mobility group box 1 proteins (HMGB-1) or mitochondria components, components of extracellular matrix molecules (e.g., collagen or elastin fragments), or signaling molecules involved in pathogen recognition (4). The conceptual advances derived from the identification of DAMPs in chronic inflammatory states have led to pathogenetic breakthroughs in the understanding of degenerative diseases, with the design of novel therapies based on these mechanisms (5). Moreover, aging may accelerate the development of self-sustained inflammatory responses. It is therefore conceivable to conclude that the generation of DAMPs may result from a global failure of cellular maintenance.

We present the concept that lung tissue injury by cigarette smoke, particularly in COPD, involves the following three stages: stage 1, initiation; stage 2, progression; and stage 3, propagation of cell damage (6). There is growing evidence that a fourth stage of "consolidation" of the pronounced cellular damage exists, leading to an aging phenotype, the ultimate manifestation of a global failure of lung maintenance (Figure 2) (7). Teleologically, the molecular players involved in the initial reaction of host stress responses caused by tobacco smoke, the direct organ damage causing progression of injury, and propagation of tissue injury are intimately involved in the process of aging. Previous reviews have emphasized the extent to which the pathology and molecular events underlying COPD emulate similar events underlying organ aging (8, 9). In accord with this concept, stresses, stress sensing, and activation of these endogenous molecular pathogenetic processes are intimately associated with the current paradigms of aging and senescence (10). These processes lead to inflammation, which becomes a consequence (rather than the cause) of all these destructive processes. This complex pathobiologic scenario may impart a formidable task to develop cell-based or pharmacological therapies once these processes are operational in the COPD lung.

This review highlights three specific aspects related to diseases that alter the steady-state homeostasis of the alveolar structure, with a particular focus on the impact of cigarette smoke on lung injury. We discuss (1) how the lung handles the stress imposed by cigarette smoke (i.e., initiation stage), leading to progression of injury; (2) the propagation of damage responses, with the activation of endogenous mediators of tissue injury, including DAMPs; and (3) the aging-related phenotype of COPD lungs (i.e., consolidation stage).

INITIATION OF CIGARETTE SMOKE-INDUCED LUNG INJURY: RTP801/REDD1 AND LUNG INJURY DUE TO CIGARETTE SMOKE

Over the past 12 years, novel insights have provided support for the hypothesis that the disruption of alveolar structural and molecular maintenance programs by cigarette smoke contributes to the pathogenesis of emphysema (6). Based on observations that alveolar cell apoptosis interacts with oxidative stress in the causation of emphysema induced by vascular endothelial growth factor receptor (VEGFR) blockade (11) (and subsequently

Figure 1. Outline of the spectrum of airway-mediated injurious agents and activation of danger-associated molecular patterns (DAMPs) (see Reference 4) operative in multiple lung diseases, including chronic obstructive pulmonary disease. Many of injurious agents engage toll-like receptors and may also act as DAMPs. Some of these signaling events engage signaling systems involved in protection of the host from environmental stresses, including hypoxia and deprivation to nutrients. Progression and persistence of alveolar injury would involve the release of several endogenous mediators, which converge in promoting organ dysfunction and persistent inflammation (alveolar outline based on Reference 74). EC = extracellular; NLR = nucleotide oligomerization domain-like receptor; PGP = proline-glycine-proline; TLR = toll-like receptors.

confirmed by genetic deletion of lung VEGF [12]), we proposed that a feed-forward interaction among apoptosis, protease/antiprotease imbalance, and oxidative stress mediates alveolar destruction in emphysema (11).

The eventual disruption of organ maintenance by these processes is mediated by a prototypic organ response to the inhalation of cigarette smoke. Environmental stresses, such as those caused by cigarette smoke and pollutants, activate trigger molecules that govern harmful alveolar septal cell responses characterized by oxidative stress, apoptosis, and alveolar inflammation. In line with this overall paradigm, we undertook mechanistic studies targeting the cellular stress response gene RTP801 (also known as Redd1 for “regulated in development and DNA damage responses”), which was initially discovered based on a screening for genes up-regulated by hypoxia-inducible factor 1 α (HIF-1 α) and shown to induce oxidative stress and apoptosis (13, 14) (Figure 3).

In subsequent studies, however, the mutual interaction between RTP801 expression and oxidative stress was underscored by the documentation that arsenate induces RTP801 expression via activation of C/CAAT regions in the RTP801 promoter (15). This interaction was also supported by the report of induction of RTP801 expression in hypoxic neurons *in vivo* (13) and the protection of RTP801 KO mice from apoptosis in the neural retina and central vascular obliteration secondary to endothelial cell apoptosis in a model of retinopathy of prematurity (16). Conversely, forced overexpression of RTP801 in cultured cells and in the lung has been linked to apoptosis (13) and enhanced production of reactive oxygen species (14). We therefore hypothesized that RTP801 would function as a “switch,” integrating the lung’s reaction to cigarette smoke acting as an environmental stressor (Figure 3). Cell signaling involving RTP801 would provide a supplementary level of control of key cellular events, particularly inflammation, in addition to traditional inducers of inflammation and cell injury (e.g., toll-like receptors [TLRs] and the ensuing cytokines and chemokines).

We observed that smokers with mild COPD had increased lung levels of RTP801 mRNA when compared with normal individuals and patients with severe COPD (17). However, RTP801 protein levels were increased in this latter group of patients. RTP801 mRNA and protein were also augmented in lungs of

mice exposed acutely to cigarette smoke, preferentially in type II cells (Figure 2). Although the initial rise in lung transcript levels was followed by a decrease to baseline over 48 hours postexposure to cigarette smoke, RTP801 protein levels remained high. These findings agree with the observed RTP801 protein stabilization in cells exposed to arsenite or oxidants (18). We did not detect RTP801 in inflammatory cells retrieved by bronchopulmonary lavage. The up-regulation of RTP801 by cigarette smoke was dependent of oxidative stress *in vivo*, based on its blockade with N-acetyl cysteine supplementation, and *in vitro*, which required the C/CAAT response element (17).

RTP801 expression was necessary to activate inflammation due to cigarette smoke *in vivo* and *in vitro*. RTP801 knockout mice were remarkably protected against inflammation, apoptosis, and oxidative stress caused by short-term exposure to cigarette smoke; consistent with these observations, these mice did not develop emphysema after 6 months of exposure to cigarette smoke when compared with wild-type mice (17).

Figure 2. Endogenous signals that can propagate lung inflammation in emphysema.

Figure 3. RTP801 may act as a stress sensor, amplifying alveolar injury triggered by cigarette smoke with cellular processes of apoptosis, inflammation, and oxidative stress. Because these properties are dependent on RTP801-mediated inhibition of mTOR, the role of RTP801 can be reproduced by rapamycin, which phenocopies the role of RTP801 overexpression in wild-type mice when administered to RTP801 knockout mice.

RTP801 acts as an inhibitory switch of the serine–threonine kinase mTOR signaling in mammalian cells (19) and in *Drosophila* (20). Nutrients, particularly amino acids (e.g., glutamine or leucine), lipids, and glucose, activate mTOR, which drives hypertrophic and hyperplastic cell growth responses. Conversely, mTOR is suppressed by the complex TSC–1/TSC–2 under adverse conditions (including low energetic states), by hypoxia, and by cigarette smoke (21). mTOR is a central component of two well defined, evolutionary conserved complexes

named mammalian TORC1 and TORC2. mTORC1 involves the association of mTOR with Raptor (for “regulatory associated protein of TOR”), mSLT8/GβL, Deptor, and PRAS40 and is primarily involved in cell growth signals. mTORC2, a multi-protein complex containing mTOR and Rictor (for “rapamycin insensitive companion of TOR”), activates AKT and mediates the reorganization of the cytoskeleton.

In line with these observations, we noted that RTP801 knockout mice have increased baseline lung mTOR signaling when compared with wild-type littermates. The increased mTOR in the knockout mice mediated alveolar protection, as its blockade with rapamycin abrogated the protection against cigarette smoke and triggered alveolar inflammation and apoptosis in wild-type mice.

RTP801 overexpression in ambient air-exposed mice triggered alveolar inflammation, oxidative stress, and alveolar cell apoptosis (Figure 4). These effects were related to the mTOR inhibitory activity of RTP801 (and are in accord with the effects of rapamycin in the lung) because a RTP801 mutant protein (variant RPAA), unable to bind to TSC-2 and displace the 14–3–3 adaptor (22), did not cause the lung pathologies seen with the wild-type construct (17). These findings confirm the report that blockade of mTOR, such as that achieved by rapamycin or RTP801 overexpression, can be proinflammatory (23–25). In line with these findings, HIF-1 α , an inducer of RTP801 expression, activates NF- κ B under conditions of hypoxia (26). These observations agree with the reports that rapamycin causes lymphocytic pneumonitis in transplant recipients (27). However, the impact of mTOR activation on cigarette smoke-induced lung injury can be cell-, disease-, and organ-context specific. In contrast with the findings in the RTP801 knockout mice, we observed that rapamycin protected against acute cigarette smoke-induced inflammation in RTP801 wild-type mice, which might have been due to mTORC1-driven IKK β activation, particularly in inflammatory cells (28). In accord with these observations, rapamycin-induced protection was observed in experimental asthma (29).

The finding that reduced mTORC1 signaling accounts for most of the pathology of RTP801 deregulation (17) may have broader pathogenetic and potential therapeutic implications for COPD. Muscle wasting due to hypoxia (30), a major contributing factor on morbidity and mortality in advanced COPD, has also been linked to up-regulated RTP801 expression (30), cell

Figure 4. RTP801 expression in mouse lungs exposed to cigarette smoke for 3 days (red), costained with (A) pro-surfactant protein C (green, type II cells), (B) T1 α (green, type I cell), and (C) endothelial cells (green, thrombomodulin). (D–F) Expression of RTP801 (red) in type II cells (green) in vector control (D), mutant RTP801 unable to inhibit mTOR (RPAA) (E), and human RTP801 (F) in transduced mice lung (3 days postinoculation). (G–I) Lung macrophages (immunohistochemistry, brown) in vector control (G), RPAA- (H), and RTP801 transduced mouse lungs exposed to ambient air (I) (based on Ref. 17).

apoptosis, and oxidative stress (31). Furthermore, mTOR is a key blocker of autophagy, a process of cell defense due to accumulation of cellular compartments within lysosomal-like structures, through sensing the abundance of nutrients in the cellular microenvironment. In this setting, mTORC1 binds and inhibits the initial molecular activators of autophagy, involving ULK-1/2, Atg13, and FIP200. Rapamycin inhibits mTORC1 and therefore simulates a state similar to that caused by nutrient deprivation or stress responses (including hypoxia and oxidative stress), causing autophagy. The observation of excessive autophagy in the lungs of patients with emphysema (32) appears to be detrimental because blockade of autophagy in mice lacking Atg8 (microtubule-associated light chain 3) confers protection against cigarette smoke-induced emphysema (33). Taken together, these data may link baseline mTOR signaling in the protection against cigarette smoke-induced lung injury and its broad antiautophagic role.

PROGRESSION OF ALVEOLAR INJURY INTO EMPHYSEMA

Continuous lung injury leads to the destructive disappearance of alveolar structures, characteristic of emphysema. Although this destruction results from a chronic exposure to cigarette smoke, cigarette smoke also triggers acute inflammatory responses and episodes of oxidative stress. It is conceivable that emphysema would develop acutely if the influx of inflammatory cells and the ensuing protease/antiprotease imbalance were the sole trigger of alveolar destruction. Experimental data suggest that these events, though critical in the pathogenesis of the disease, may require chronic disruption of alveolar cell and molecular maintenance, overcoming protective mechanisms that allow preservation of alveolar architecture. This concept has been supported by the observations that (1) epithelial cell-specific deletion of VEGF coreceptor neuropilin-1 predisposes to accelerated cigarette smoke-induced alveolar cell death and emphysema (34), (2) mice lacking the master transcription factor nuclear erythroid-related factor 2 develop more severe and early-onset emphysema due to cigarette smoke exposure (35), and (3) mice prone to early aging have accelerated emphysema associated with alveolar cell death when compared with wild-type littermates (36). Chronic cigarette smoke may act on the lung to progressively impair its cellular and maintenance program (6), including down-regulation of VEGF and VEGF coreceptors (37). The down-regulation of VEGF receptor signaling or decreases in lung VEGF may then lead to apoptosis, with the potential activation of proinflammatory destructive mechanisms (12, 38, 39).

Based on data that showed the feedback interplay between oxidative stress and apoptosis in the VEGF receptor rat model of emphysema, we postulated that the mutual interaction among these destructive processes (11), in addition to the protease/antiprotease imbalance, might set in motion and amplify the alveolar tissue destruction (40). These interactions were also verified when endothelial cell apoptosis was directly targeted by a lung homing peptide linked to a mitochondria-pore forming peptide, in which apoptosis was accompanied by increased macrophage influx and oxidative stress (41). These experiments indicated that the disruption of alveolar integrity based on capillary endothelial cell death reproduced several of the key findings seen with cigarette smoke.

The vascular compartment within alveolar septa plays a central role in maintaining lung structure during the attack promoted by chronic cigarette smoke exposure. In opposition to the documented regenerative ability of type II cells, capillary endothelial cells may not be able to reconstitute the native

alveolar capillary network once cell death ensues. Whether either one of the cells coordinates the final fate of alveolar septa in emphysema is an important question that will require clear and stringent pursuit. Although the "vascular hypothesis" of emphysema was introduced more than 50 years ago (42), it has been recently reinforced by the finding of increased levels of apoptotic endothelial cell microparticles in the circulation of patients with COPD with no or minimal emphysema but with decreased DL_{CO} (43). Notwithstanding the evidence that type II cell apoptosis might also occur in emphysema (44), endothelial cells might represent the key cell driving the pathogenesis of destructive airspace enlargement. This interpretation might be supported by the evidence that type II cell apoptosis seen in other lung disease processes, such as acute lung injury (34), are not associated with emphysema development.

PROPAGATION OF ALVEOLAR INJURY: ROLE OF ENDOGENOUS AMPLIFICATION OF ALVEOLAR TISSUE DESTRUCTION

A vexing observation pertaining to the so-called inflammation-centric hypothesis of COPD is the lack of clear therapeutic responses with immunosuppressive drugs and persistent inflammation despite smoking cessation (45). An alternative hypothesis is that an autoimmune mechanism is engaged (46), notably in advanced stages of the disease (47). The authors propose that infectious, environmental, and endogenous mediators (released due to cell injury caused by cigarette smoking and known as DAMPS) may amplify lung inflammation by binding TLR or alternative signaling receptors, such as the receptor for advanced glycation end products (RAGE) (4) (Figure 1). Like the skin and the gastrointestinal tract, the lung serves as a conduit for several infectious and environmental agents that may trigger chronic illnesses. Infectious stimuli can activate shared inflammatory pathways via pathogen-associated molecular patterns (PAMPs). This infection-triggered paradigm is supported by several examples of chronic pulmonary diseases involving bacteria, viruses, and parasitic agents. Infections by bacteria (e.g., *Hemophilus influenza*) and viruses (e.g., influenza and parainfluenza) lead to significant disease worsening and increased mortality in COPD and in disease progression due to asthma (48); γ -herpes viruses have been linked to the pathogenesis of fibrotic lung diseases (49) and pulmonary hypertension (50), and the infection by the parasite *Schistosoma mansoni* has been associated with pulmonary hypertension (51).

The lung is uniquely affected by environmental agents, such as cigarette smoke and pollution, which can activate a series of molecular pathways involved in COPD pathogenesis (52). Shared inflammatory pathways have been proposed among infectious and environmental agents, particularly those initiated by the activation by PAMPs (53). Furthermore, infection and environmental triggers interact to promote tissue damage via the activation of several endogenous molecules and pathways. These processes enhance proinflammatory signals and increase inflammatory cell influx and expression of cytokines and chemokines (Figures 1 and 2). Products derived from dying cells acting as DAMPS elicit potent inflammatory stimuli, which synergize with infectious agents in the promotion of tissue injury. Indeed, many DAMPS can be triggered by infectious agents and environmental exposures, underscoring the potential for DAMPS or their downstream signaling events as attractive target(s) for disease modification and treatment.

A growing number of DAMPS have been linked to COPD (Figures 1 and 2). Elastin fragments attract monocytes (54); the tripeptide proline-glycine-proline, originated from degradation of collagen, activates the CXCR2 receptor (55). HMGB1

can activate TLR4 and RAGE (56). Many of these products are generated from dying cells, including apoptotic cells inefficiently cleared in the COPD lung (57). These processes may be amplified by ceramide, a lipid second messenger implicated in cigarette smoke-induced emphysema. We have highlighted the potential amplifying role of ceramide, a second messenger lipid signaling molecule, in emphysema because ceramide is generated during stresses (including cigarette smoke) (58). Recently, Petrache and collaborators showed that ceramide not only increases the rate of cell death but also disrupts apoptotic cell clearance (59). These data and the concept of accelerated aging underscore the alternative view that lung inflammation in COPD is driven from the “within” rather than from the stimulus originated from cigarette smoke (Figure 3).

CONSOLIDATION OF LUNG INJURY: SHARED MECHANISMS BETWEEN AGING AND COPD

Studies performed in rats with pancreatic elastase-induced emphysema demonstrated that all-trans retinoic acid (ATRA) rescued the alveolar destruction (60). These promising results were followed by clinical trials aimed at addressing whether ATRA would also provide a therapy for cigarette smoke-induced emphysema (61). Because these expectations were not realized (62), it is important to determine why ATRA did not work and, by extension, whether cell regenerative therapies might provide relief from the progressive nature of emphysema. Several studies have recently revealed that the emphysematous lung contains several features seen in aged organs and senescent cells (7), including shortened telomeres, activation of senescence-associated cyclin kinase inhibitors (such as p16^{Ink4a}, p19^{Arf}, and p21^{CIP1/WAF1/SDI1}), and macromolecular modifications indicative of oxidative stress (63, 64) (Figure 3). These molecular signatures of aging and senescence complement earlier observations of marked extracellular matrix modification, including exaggerated collagen deposition in advanced emphysema (65). We have recently reported increased DNA strand breaks in the promoter regions of the VEGF gene that might explain the decreased VEGF levels seen in COPD lungs (66). In light of these alterations, chronic cigarette smoking substantially modifies the lung structure, disrupts its maintenance program, and curtails its repair ability, suggesting that regenerative therapies might not be feasible or efficacious.

There are additional important implications as the lung becomes prematurely aged. Senescent cells assume a senescence-associated secretory phenotype with the production of cytokines, notably of IL-6 and IL-8, which are increased in patients with COPD. These cytokines may further drive the senescence phenotype in the emphysematous lungs (67). A recent observation has linked senescence, dysregulated cytokine production, Klotho (an anti-aging molecule that protects against development of emphysema [68]), and activation of viral PAMP-associated signaling. Klotho, which is decreased in aged cells, blocks the activation of retinoic-inducible helicase (RIG-I). RIG-I recognizes viral double-stranded RNA and activates a signaling process resulting in NF- κ B activation and NF- κ B-dependent cytokine IL-6 (69). Inappropriate RIG-I activation by poly I:C and influenza virus mediate accelerated emphysema in mice subjected for a short exposure of cigarette smoke (70), potentially providing additional insights into the link between cigarette smoke, viral PAMPs, and COPD-associated lung aging.

Finally, the recent demonstration that telomerase-deficient mice are more susceptible to cigarette smoke-induced lung injury provides compelling evidence that key molecular mechanisms involved in aging are also central in the emphysema pathogenesis (71). These data provide elegant mechanistic insights of erosion

of telomere lengths in alveolar cells in COPD lungs (72) and peripheral blood mononuclear cells (73).

CONCLUSIONS

Cigarette smoke may promote injury not only because of the generation of thousands of oxidants per puff but also because it activates powerful endogenous destructive mediators. These regulators of innate immunity, with the aid of acquired immunity targeted at lung antigens or to infectious agents, promote the relentless destruction of the lung. The overall paradigm imposes significant challenges for therapy development. Late-stage disease may have a multitude of pathogenetic processes that cannot be counteracted by single-target approaches. An alternative resides in fortifying antipathogenetic processes that limit these amplifying processes. The better understanding of which processes are activated and how they are activated may indicate how best to intervene to modify the course of the disease.

Author Disclosure: Neither of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

1. Abman SH. Bronchopulmonary dysplasia: “a vascular hypothesis.” *Am J Respir Crit Care Med* 2003;164:1755–1756.
2. Tuder RM, Voelkel NF. Pathobiology of chronic bronchitis and emphysema. In: Voelkel NF, McNee W, editors. *Chronic obstructive lung disease*, 1st ed. BC Decker: Montreal, Quebec, Canada; 2001. pp. 63–75.
3. Shapiro SD. COPD unwound. *N Engl J Med* 2005;352:2016–2019.
4. Schmidt EP, Tuder RM. Role of apoptosis in amplifying inflammatory responses in lung diseases. *J Cell Death* 2010;3:41–53.
5. Kono H, Rock KL. How dying cells alert the immune system to danger. *Nat Rev Immunol* 2008;8:279–289.
6. Tuder RM, Yoshida T, Fijalkowka I, Biswal S, Petrache I. Role of lung maintenance program in the heterogeneity of lung destruction in emphysema. *Proc Am Thorac Soc* 2006;3:673–679.
7. MacNee W, Tuder RM. New paradigms in the pathogenesis of chronic obstructive pulmonary disease I. *Proc Am Thorac Soc* 2009;6:527–531.
8. Tuder RM. Aging and cigarette smoke: fueling the fire. *Am J Respir Crit Care Med* 2006;174:490–491.
9. Tuder RM, Yoshida T, Arap W, Pasqualini R, Petrache I. Cellular and molecular mechanisms of alveolar destruction in emphysema: an evolutionary perspective. *Proc Am Thorac Soc* 2006;3:503–510.
10. Kirkwood TBL. Understanding the odd science of aging. *Cell* 2005;120:437–447.
11. Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, Flores SC. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 2003;29:88–97.
12. Tang K, Rossiter HB, Wagner PD, Breen EC. Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. *J Appl Physiol* 2004;97:1559–1566.
13. Shoshani T, Faerman A, Mett I, Zelin E, Tenne T, Gorodin S, Moshel Y, Elbaz S, Budanov A, Chajut A, et al. Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. *Mol Cell Biol* 2002;22:2283–2293.
14. Ellison LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, Oliner JD, McKeon F, Haber DA. REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. *Mol Cell* 2002;10:995–1005.
15. Jin HO, Seo SK, Woo SH, Kim ES, Lee HC, Yoo DH, An S, Choe TB, Lee SJ, Hong SI, et al. Activating transcription factor 4 and CCAAT/enhancer-binding protein- β negatively regulate the mammalian target of rapamycin via Redd1 expression in response to oxidative and endoplasmic reticulum stress. *Free Radic Biol Med* 2009;46:1158–1167.
16. Brafman A, Mett I, Shafir M, Gottlieb H, Damari G, Gozlan-Kelner S, Vishnevskia-Dai V, Skaliter R, Einat P, Faerman A, et al. Inhibition of oxygen-induced retinopathy in RTP801-deficient mice. *Invest Ophthalmol Vis Sci* 2004;45:3796–3805.

17. Yoshida T, Mett I, Bhunia AK, Bowman J, Perez M, Zhang L, Gandjeva A, Zhen L, Chukwueke U, Mao T, *et al.* Rtp801, a suppressor of mTOR signaling, is an essential mediator of cigarette smoke-induced pulmonary injury and emphysema. *Nat Med* 2010;16:767–773.
18. Katiyar S, Liu E, Knutzen CA, Lang ES, Lombardo CR, Sankar S, Toth JJ, Petroski MD, Ronai Z, Chiang GG. REDD1, an inhibitor of mTOR signalling, is regulated by the CUL4A–DDB1 ubiquitin ligase. *EMBO Rep* 2009;10:866–872.
19. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin WG Jr. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev* 2004;18:2893–2904.
20. Reiling JH, Hafen E. The hypoxia-induced paralogs Scylla and Charibdis inhibit growth by down-regulating S6K activity upstream of TSC in *Drosophila*. *Genes Dev* 2004;18:2879–2892.
21. Inoki K, Ouyang H, Li Y, Guan KL. Signaling by target of rapamycin proteins in cell growth control. *Microbiol Mol Biol Rev* 2005;69:79–100.
22. DeYoung MP, Horak P, Sofer A, Sgroi D, Ellisen LW. Hypoxia regulates TSC1/2–mTOR signaling and tumor suppression through REDD1-mediated 14–3-3 shuttling. *Genes Dev* 2008;22:239–251.
23. Weichhart T, Costantino G, Poglitsch M, Rosner M, Zeyda M, Stuhlmeier KM, Kolbe T, Stulnig TM, Hörl WH, Hengstschläger M, *et al.* The TSC–mTOR signaling pathway regulates the innate inflammatory response. *Immunity* 2008;29:565–577.
24. Shah OJ, Wang Z, Hunter T. Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. *Curr Biol* 2004;14:1650–1656.
25. Bhaskar PT, Nogueira V, Patra KC, Jeon SM, Park Y, Robey RB, Hay N. mTORC1 hyperactivity inhibits serum deprivation-induced apoptosis via increased hexokinase II and GLUT1 expression, sustained Mcl-1 expression, and glycogen synthase kinase 3 β inhibition. *Mol Cell Biol* 2009;29:5136–5147.
26. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M. NF- κ B links innate immunity to the hypoxic response through transcriptional regulation of HIF-1 α . *Nature* 2008;453:807–811.
27. Morelon E, Stern M, Kreis H. Interstitial pneumonitis associated with sirolimus therapy in renal-transplant recipients. *N Engl J Med* 2000;343:225–226.
28. Lee DF, Kuo HP, Chen CT, Hsu JM, Chou CK, Wei Y, Sun HL, Li LY, Ping B, Huang WC, *et al.* IKK[β] suppression of TSC1 links inflammation and tumor angiogenesis via the mTOR pathway. *Cell* 2007;130:440–455.
29. Fujitani Y, Trifilieff A. In vivo and in vitro effects of SAR 943, a rapamycin analogue, on airway inflammation and remodeling. *Am J Respir Crit Care Med* 2003;167:193–198.
30. Favier FB, Costes F, Defour A, Bonnefoy R, Lefai E, Bauge S, Peinnequin A, Benoit H, Freyssen DG. Down-regulation of Akt/mammalian target of rapamycin pathway in skeletal muscle is associated with increased REDD1 expression in response to chronic hypoxia. *Am J Physiol Regul Integr Comp Physiol* 2010;298:R1659–R1666.
31. Agustí AG, Saulea J, Miralles C, Gomez C, Togores B, Sala E, Batle S, Busquets X. Skeletal muscle apoptosis and weight loss in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;166:485–489.
32. Ryter SW, Chen ZH, Kim HP, Choi AM. Autophagy in chronic obstructive pulmonary disease: homeostatic or pathogenic mechanism? *Autophagy* 2009;5:235–237.
33. Chen ZH, Lam HC, Jin Y, Kim HP, Cao J, Lee SJ, Ifedigbo E, Parameswaran H, Ryter SW, Choi AM. Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke-induced emphysema. *Proc Natl Acad Sci USA* 2010;107:18880–18885.
34. Le A, Zielinski R, He C, Crow MT, Biswal S, Tudor RM, Becker PM. Pulmonary epithelial neuropilin-1 deletion enhances development of cigarette smoke-induced emphysema. *Am J Respir Crit Care Med* 2009;180:396–406.
35. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tudor RM, Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J Clin Invest* 2004;114:1248–1259.
36. Sato T, Seyama K, Sato Y, Mori H, Souma S, Akiyoshi T, Kodama Y, Mori T, Goto S, Takahashi K, *et al.* Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. *Am J Respir Crit Care Med* 2006;174:530–537.
37. Marwick JA, Stevenson CS, Giddings J, MacNee W, Butler K, Rahman I, Kirkham PA. Cigarette smoke disrupts VEGF165–VEGFR-2 receptor signaling complex in rat lungs and patients with COPD: morphological impact of VEGFR-2 inhibition. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L897–L908.
38. Petrache I, Fijalkowska I, Zhen L, Medler TR, Brown E, Cruz P, Choe KH, Taraseviciene-Stewart L, Scerbavicius R, Shapiro L, *et al.* A novel antiapoptotic role for alpha1-antitrypsin in the prevention of pulmonary emphysema. *Am J Respir Crit Care Med* 2006;173:1222–1228.
39. Kasahara Y, Tudor RM, Taraseviciene-Stewart L, Le Cras TD, Abman SH, Hirth P, Waltenberger J, Voelkel NF. Inhibition of vascular endothelial growth factor receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000;106:1311–1319.
40. Tudor RM, Petrache I, Elias JA, Voelkel NF, Henson PM. Apoptosis and emphysema: the missing link. *Am J Respir Cell Mol Biol* 2003;28:551–554.
41. Giordano RJ, Edwards JK, Tudor RM, Arap W, Pasqualini R. Combinatorial ligand-directed lung targeting. *Proc Am Thorac Soc* 2009;6:411–415.
42. Liebow A. Pulmonary emphysema with special emphasis to vascular changes. *Am Rev Respir Dis* 1959;80:67–93.
43. Gordon C, Gudi K, Krause A, Sackrowitz R, Harvey BG, Strulovici-Barel Y, Mezey JG, Crystal RG. Circulating endothelial microparticles as a measure of early lung destruction in cigarette smokers. *Am J Respir Crit Care Med* 2011;184:224–232.
44. Zheng T, Kang MJ, Crothers K, Zhu Z, Liu W, Lee CG, Rabach LA, Chapman HA, Homer RJ, Aldous D, *et al.* Role of cathepsin S-dependent epithelial cell apoptosis in ifn- γ -induced alveolar remodeling and pulmonary emphysema. *J Immunol* 2005;174:8106–8115.
45. Hogg JC. Why does airway inflammation persist after the smoking stops? *Thorax* 2006;61:96–97.
46. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med* 2009;360:2445–2454.
47. Grumelli S, Corry DB, Song LZ, Song L, Green L, Huh J, Hacken J, Espada R, Bag R, Lewis DE, *et al.* An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. *PLoS Med* 2004;1:e8.
48. Anzueto A, Sethi S, Martinez FJ. Exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2007;4:554–564.
49. Vannella KM, Luckhardt TR, Wilke CA, van Dyk LF, Toews GB, Moore BB. Latent herpesvirus infection augments experimental pulmonary fibrosis. *Am J Respir Crit Care Med* 2010;181:465–477.
50. Cool CD, Rai MD, Yeager ME, Hernandez-Saavedra D, Serls AE, Bull TM, Geraci MW, Brown KK, Routes JM, Tudor RM, *et al.* Expression of human herpesvirus 8 in primary pulmonary hypertension. *N Engl J Med* 2003;349:1113–1122.
51. Graham BB, Bandeira AP, Morrell NW, Butrous G, Tudor RM. Schistosomiasis-associated pulmonary hypertension: pulmonary vascular disease: the global perspective. *Chest* 2010;137:20S–29S.
52. Yoshida T, Tudor RM. Pathobiology of cigarette smoke-induced chronic obstructive pulmonary disease. *Physiol Rev* 2007;87:1047–1082.
53. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nat Rev Immunol* 2009;9:377–384.
54. Houghton AM, Quintero PA, Perkins DL, Kobayashi DK, Kelley DG, Marconini LA, Mecham RP, Senior RM, Shapiro SD. Elastin fragments drive disease progression in a murine model of emphysema. *J Clin Invest* 2006;116:753–759.
55. Weatherington NM, van Houwelingen AH, Noerager BD, Jackson PL, Kraneveld AD, Galin FS, Folkerts G, Nijkamp FP, Blalock JE. A novel peptide CXCR ligand derived from extracellular matrix degradation during airway inflammation. *Nat Med* 2006;12:317–323.
56. Ferhani N, Letuve S, Kozhich A, Thibaudau O, Grandsaigne M, Maret M, Dombret MC, Sims GP, Kolbeck R, Coyle AJ, *et al.* Expression of high-mobility group box 1 and of receptor for advanced glycation end products in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;181:917–927.
57. Richens TR, Linderman DJ, Horstmann SA, Lambert C, Xiao YQ, Keith RL, Boe DM, Morimoto K, Bowler RP, Day BJ, *et al.* Cigarette smoke impairs clearance of apoptotic cells through oxidant-dependent activation of RhoA. *Am J Respir Crit Care Med* 2009;179:1011–1021.

58. Petrache I, Natarajan V, Zhen L, Medler TR, Richter AT, Cho C, Hubbard WC, Berdyshev EV, Tuder RM. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. *Nat Med* 2005;11:491–498.
59. Petrusca DN, Gu Y, Adamowicz JJ, Rush NI, Hubbard WC, Smith PA, Berdyshev EV, Birukov KG, Lee CH, Tuder RM, *et al.* Sphingolipid-mediated inhibition of apoptotic cell clearance by alveolar macrophages. *J Biol Chem* 2010;285:40322–40332.
60. Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat Med* 1997;3:675–677.
61. Hogg JC. A stimulating treatment for emphysema. *Nat Med* 1997;3:603–604.
62. Roth MD, Connett JE, D'Armiento JM, Foronjy RF, Friedman PJ, Goldin JG, Louis TA, Mao JT, Muindi JR, O'Connor GT, *et al.* Feasibility of retinoids for the treatment of emphysema study. *Chest* 2006;130:1334–1345.
63. Rahman I, van Schadewijk AAM, Crowther AJL, Hiemstra PS, Stolk J, MacNee W, De Boer WI. 4-Hydroxy-2-Nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;166:490–495.
64. Deslee G, Woods JC, Moore C, Conradi SH, Gierada DS, Atkinson JJ, Battaile JT, Liu L, Patterson GA, Adair-Kirk TL, *et al.* Oxidative damage to nucleic acids in severe emphysema. *Chest* 2009;135:965–974.
65. Finlay GA, O'Donnell MD, O'Connor CM, Hayes JP, FitzGerald MX. Elastin and collagen remodeling in emphysema: a scanning electron microscopy study. *Am J Pathol* 1996;149:1405–1415.
66. Kasahara Y, Tuder RM, Cool CD, Lynch DA, Flores SC, Voelkel NF. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. *Am J Respir Crit Care Med* 2001;163:737–744.
67. Acosta JC, O'Loughlin A, Banito A, Guijarro MV, Augert A, Raguz S, Fumagalli M, Da CM, Brown C, Popov N, *et al.* Chemokine signaling via the CXCR2 receptor reinforces senescence. *Cell* 2008;133:1006–1018.
68. Suga T, Kurabayashi M, Sando Y, Ohyama Y, Maeno T, Maeno Y, Aizawa H, Matsumura Y, Kuwaki T, Kuro O, *et al.* Disruption of the klotho gene causes pulmonary emphysema in mice: defect in maintenance of pulmonary integrity during postnatal life. *Am J Respir Cell Mol Biol* 2000;22:26–33.
69. Liu F, Wu S, Ren H, Gu J. Klotho suppresses RIG-I-mediated senescence-associated inflammation. *Nat Cell Biol* 2011;13:254–262.
70. Kang MJ, Lee CG, Lee JY, Dela Cruz CS, Chen ZJ, Enelow R, Elias JA. Cigarette smoke selectively enhances viral PAMP and virus-induced pulmonary innate immunity and remodeling responses. *J Clin Invest* 2008;118:2771–2784.
71. Alder JK, Guo N, Kembou F, Parry EM, Anderson CJ, Gorgy AI, Walsh MF, Sussan T, Biswal S, Mitzner W, *et al.* Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med* (In press)
72. Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in pulmonary emphysema patients. *Am J Respir Crit Care Med* 2006;173:886–893.
73. Morla M, Busquets X, Pons J, Saucedo J, MacNee W, Agusti AG. Telomere shortening in smokers with and without COPD. *Eur Respir J* 2006;27:525–528.
74. Sirianni FE, Chu FSF, Walker DC. Human alveolar wall fibroblasts directly link epithelial type 2 cells to capillary endothelium. *Am J Respir Crit Care Med* 2003;168:1532–1537.