June 19, 2009

# The Biology of Emphysema

Lance S. Terada, MD*
Internal Medicine Grand Rounds, June 19, 2009
University of Texas Southwestern Medical Center

***Lance S. Terada, MD,** has no financial interests or other relationships with commercial concerns related directly or indirectly to this presentation. He will not be discussing off-label drug uses.

Dr. Terada is a Professor of Internal Medicine, Division of Pulmonary and Critical Care Medicine. His research interests are in vascular cell signaling, specification of oxidant effects, cytoskeletal dynamics, and anoikis.

## Introduction

An estimated 3 million individuals in the United States suffer from pulmonary emphysema, a form of chronic obstructive pulmonary disease (COPD). COPD itself is now the 4th leading cause of death in North America. Alarmingly, it alone among the top six causes of death in America has been associated with a relentless increase in mortality, doubling between 1970 and 2002 [1]. In the same time period, cardiovascular mortality from heart disease and stroke and accidental death rates have decreased by more than 50%, while deaths related to cancer and diabetes have remained relatively constant. The problem is global, with tobacco-related deaths projected to skyrocket over the next 25 years worldwide.

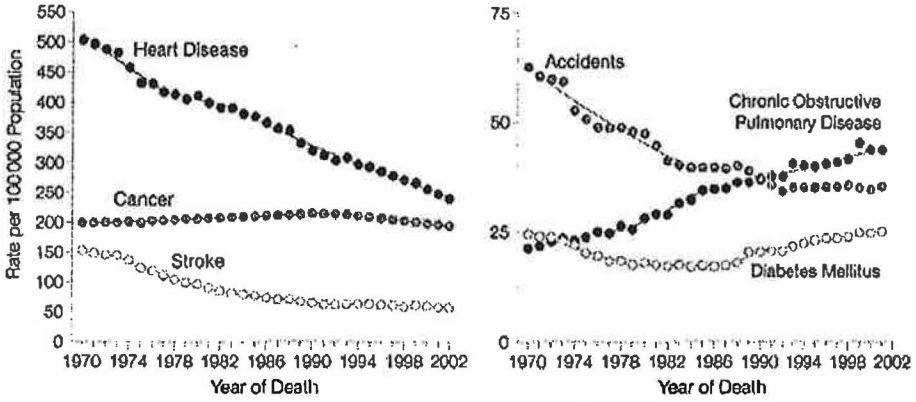



Figure 1. Mortality rates in the United States from 1970-2002. From [1].

Despite its longstanding recognition, high prevalence, and rising mortality, few studies into disease causation have been performed until relatively recently. Recent work has revealed mechanisms at work in the emphysematous lung which hold surprising general relevance for medicine and biology.

**The protease/antiprotease theory**

Emphysema per se is defined as permanent enlargement of the air spaces distal to the terminal bronchiole accompanied by destruction of their walls. Described as early as 1721 by the Dutch anatomist and botanist Frederick Ruysch and later by Rene Laennec in 1834, emphysema most commonly takes a centrilobular appearance. In this form, emphysema starts at the ends of the terminal bronchioles, affecting the respiratory bronchioles and adjacent alveolar structures. These structures are within the center of the acinus, or secondary lobule, hence the appearance of somewhat regularly spaced holes within the distal lung parenchyma. The reason for this distribution has still not been explained, but may be of potential importance for the failure of lung regeneration (see below). The organ response in emphysema is fairly unique, in that marked tissue regression occurs, leaving nothing behind. How does this occur, and what does this mean for the residual lung tissue?

The first real insight into the pathogenesis of emphysema came in 1963 with Laurell and Eriksson's reporting of five unrelated subjects with low levels of α1-antitrypsin (α1AT) [2]. The report was based on a simple electrophoretic separation of serum proteins, with assessment of the antitryptic activity in different fractions. Of these five subjects, three had emphysema, with one of the three also having significant bronchiectasis. Both of these pulmonary features are now known to be associated with α1AT deficiency. The following year, Eriksson suggested a familial element by reporting that one of the original subjects had two siblings with emphysema, one with low α1AT (the other was deceased) [3]. Based not on genotyping but on electrophoretic phenotyping, a Mendelian inheritance pattern was strongly suggested. A year later Eriksson characterized, within an extensive tour de force thesis on α1AT deficiency, 24 families with probands having either normal, ~60% normal, or <10% normal α1AT activity. All individuals with emphysema had <10% normal α1AT activity [4]. Thus within three years, Eriksson provided powerful clinical evidence that emphysema was linked to genetically insufficient antiprotease activity, setting the paradigm for what is still the predominant theory of the pathogenesis of emphysema.

Over the next two decades, neutrophil elastase was identified by Aaron Janoff, and was thought to be the principal substrate for α1AT [5]. In addition, the human genetics of α1AT was worked out, providing an explanation for the variations in electrophoretic mobility. Thus the theory which emerged involved activation of neutrophils, release of neutrophil elastase, and an overwhelming of antiprotease defense systems. This theory placed the acute inflammatory process squarely as the culprit in causing emphysema from cigarettes as well, since activated neutrophils released oxidants which inactivated α1AT through oxidation of the active site methionine. The general theory therefore held most forms of emphysema as representing an imbalance of the neutrophil elastase burden outweighing the antielastolytic capacity of the lung (Figure 2).

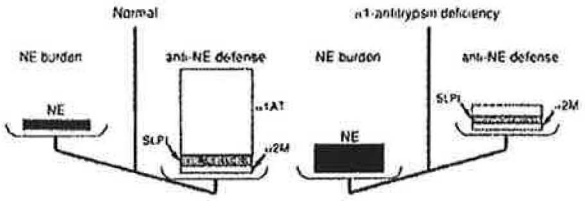



Figure 2. Balance of proteases, largely neutrophil elastase, and antiproteases, largely AAT, in maintaining lung health. From Crystal, R.G., α1-Antitrypsin Deficiency, in Update: Pulmonary Diseases and Disorders, Ed. A.P. Fishman, 1992.

There is little doubt about the veracity of this view, although in recent years it has become apparent that there are several shortcomings. First, neutrophil infiltration and activation is not a prominent feature of the distal airways in emphysema. Second, in conditions which are in fact characterized by such congregation and activation of lung parenchymal neutrophils (pneumonia, acute lung injury, pulmonary capillaritis), emphysema is not a common sequella. Finally, the fragmentation of elastin may in part explain the high lung compliance seen in the disease, but does not explain the peculiar septal tissue resorption that defines the pathology of emphysema. New ideas were needed.

## The TH1 adaptive immune system is activated in emphysema

As far back as 1957, it was noted that the predominant inflammatory cells associated with emphysema were lymphocytes. In an early morphometric study, peribronchiolar lymphocytes and plasma cells were noted in 86% of centrilobular lesions [6], suggesting a low grade, chronic inflammatory process rather than activation of acute inflammation. Indeed, clinical progression of COPD was much later found to be strongly associated with an increase in B cells and both CD4 and CD8 T cells, as well as lymphoid follicles [7], indicting stimulation of the adaptive immune response as part of COPD progression. For emphysema specifically, this pattern of inflammation also appears to be true. Inflammatory cells extracted from emphysematous lung tissue demonstrate that while neutrophils are not any more numerous than in normal lungs or lungs of smokers without emphysema, CD3+ and CD8+ cells predominate, again suggesting a response to antigenic stimulation [8].

Surprisingly, the adaptive immune response in emphysema does not take the form of a TH2-predominant reaction, setting it apart from immune mechanisms operating in asthma. Instead, T cells from emphysematous airways express CXCR3 and secrete IFN-γ and the CXCR3 ligand CXCL10, all characteristic of a TH1 response [9, 10]. Lymphocytes purified from resected lung tissue from ex-smokers with emphysema do not express TH2 receptors such as CCR3 and CCR4 differently from controls: in contrast, higher levels of lymphocytes with TH1 characteristics can be recovered from emphysema lungs (CXCR3+, CCR5+, secretion of IFN-γ, monokine induced by IFN-γ (MIG), and IP-10) [10]. Moreover, IP-10 and MIG induce the protease MMP12, a potent elastolytic enzyme, in lung macrophages. Even 4-7 years after smoking cessation, a TH1 response remains active in emphysematous tissue [10].

Such persistence of an adaptive immune response raises questions of whether emphysema formation is accompanied by specific antigenic stimulation. Two groups have addressed this possibility through examination of TCR repertoire biasing. In the first study, interstitial lymphocytes were obtained from eight emphysematous and six normal lungs [11]. Upon exposure to IL-2, a strong TH1 cytokine, blasting (proliferation) was observed with lymphocytes from emphysema and not controls, suggesting *in*

*vivo* priming of lymphocyte subsets. Further, the frequency distribution of the variable subunits of the β chain (Vβ) were skewed, suggesting oligoclonal expansion of certain lymphocyte subsets, and sequencing of the hypervariable junctional (J) segments confirmed clonal expansion. Interestingly, such clones were found to be expanded in lymphocytes from different lobes within the same individuals, suggesting diffuse response to a common antigen [11].

In a second study, PCR was used to assess Vβ family skewing from smokers [12]. Although it is not clear which individuals had emphysema, smoking itself appeared to induce oligoclonal expansion of lymphocyte subsets extracted directly from these lungs. In this latter study, oligoclonality was found in 81% of CD4+ and 100% of CD8+ subsets [12].

The nature of the specific antigens causing chronic inflammation are not clear, although reasonable candidates may be foreign antigens from bacteria known to colonize the upper airways of COPD patients or neoantigens arising from lung tissues exposed to cigarette smoke. An interesting hypothesis was recently put forth based on the observation that elastin fragmentation is common in emphysema. Indeed, levels of antibodies against elastin fragments are higher in serum of patients with emphysema, lymphocytes from these patients secrete higher levels of antibodies to elastin fragments, and stimulation of peripheral lymphocytes with elastin fragments cause MHC class II-dependent release of the TH1 cytokines IFN-γ and IL-10 [13]. Thus a positive feedback loop was invoked in which autoimmunity to elastin fragments may facilitate further degradation of intact elastin fibers [13]. This hypothesis has yet to be validated, although in theory it explains the propensity of smokers to develop a diffuse elastinopathy affecting skin and aortic media as well as lung disease.

## VEGF signaling is deranged in emphysema

While the primary target of chronic inflammation can be easily envisioned to be the lung matrix, the question of why and how septal tissue resorbs completely is more difficult to answer. Some light can be shed on what happens to the lung parenchymal cells by interrogating lung tissue for gene expression patterns. Four genome-wide expression profiling studies have been studied which seek to identify genes differentially regulated in emphysematous tissues [14-16]. While the inter-study concordance of the resultant gene sets is very low on a gene-per-gene basis, some information can be gleaned by comparing which cell and organ functions the various gene sets represent. This analysis can be performed easily using the Gene Ontogeny (GO) database. This analysis was recently performed on the gene sets derived from these four studies, revealing a propensity for upregulated genes to fall within such categories as cellular organization, stress response, organ development, and biological/ cell adhesion [17].

The latter two functional sets, organ development and cell adhesion, are of interest. The differential expression of developmental genes in emphysematous lungs is reminiscent of the fact that in some respects, emphysema appears to be a reversal of the terminal stage of lung development, alveolarization. In the penultimate stage, the saccular stage, the terminal bud mesenchyme and epithelium thins into simple blind ended sacs. During alveolarization, secondary septae form between the walls of the terminal sacs, eventually forming complex, smaller alveolar units. In emphysema, such secondary septae disappear, reforming simplified airsacs. It is possible, therefore, that terminal developmental events may continue to be important throughout

adulthood in order to maintain proper lung architecture. Such events may fail in emphysema and, as noted below, during senescence. The second functional gene set, including genes which control matrix and cell adhesion proteins, is of obvious significance to a disorder characterized by matrix degeneration. This will be considered below.

Notably, two soluble factors known to control the alveolarization stage of development are VEGF and TGFβ. Sputum levels of VEGF have been found to be low in subjects with emphysema compared with normal controls (Figure 3) [18]. Sputum VEGF levels are higher in emphysema patients than normals and in more active inflammatory diseases such as asthma and chronic bronchitis, setting emphysema apart from other chronic obstructive pulmonary diseases. In addition, levels of sputum VEGF correlate well with DLCO, a primary marker of severity for emphysema [18]. These findings are not an artifact related to sputum measurements, as VEGF levels extracted from emphysematous lung tissue has also been found to be lower than normal lung tissue [19]. In addition, *in situ* hybridization demonstrates reduced expression of both *VEGF* and *VEGFR2* in epithelium and endothelium of emphysematous compared to normal tissues.

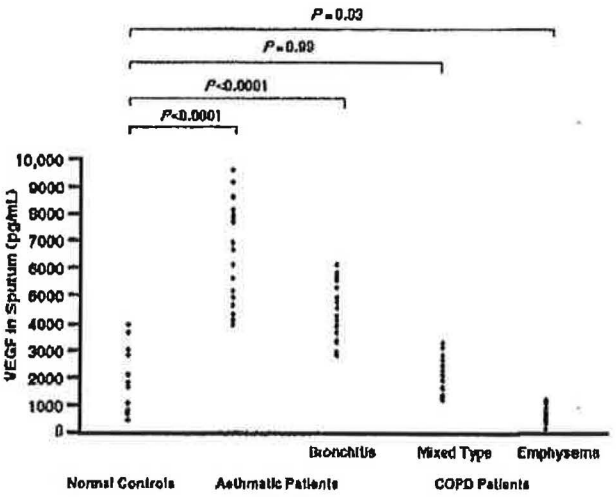



Figure 3. Sputum levels of VEGF were measured in various chronic lung conditions and normals. Levels were lowest in emphysema. From reference [18].

**Cell death, oxidative stress, and senescence are increased in emphysema**

Given that VEGF can act as a survival factor for the vascular endothelium, it is notable that emphysematous lungs harbor a surprisingly high density of parenchymal cells undergoing apoptosis. Both alveolar epithelial cells and capillary and small vessel endothelial cells display DNA fragmentation [19-21] and activation of caspase-3 [21], and increases in the apoptosis-related proteins PARP, Bax, Bad, and TRAIL [21, 22]. While the relevance of reduced VEGF levels to lung cell death is difficult to test in humans, rats given the VEGFR2 antagonist SU5416 develop pulmonary vascular attenuation, endothelial and epithelial cell death, and emphysema [23], suggesting a causal relationship. This conclusion was strengthened by a report of emphysema as a result of airway delivery of AAV/Cre to *VEGF* floxed mice (a technique resulting in specific loss of *VEGF* expression from lung epithelial cells) [24]. The mechanism of lung cell death following VEGF pathway inhibition is unclear, although SU5416-induced emphysema is blocked by suppressing synthesis of the proapoptotic lipid ceramide, and ceramide levels are found to be elevated in emphysematous human lungs [25].

The data implicating VEGF as important in the maintenance of lung structure suggests that at least one important pathway to lung tissue resorption is through pulmonary vascular regression, as opposed to most assumptions which hold that loss of pulmonary vasculature occurs secondary to loss of lung tissue in emphysema. A more direct proof of concept was recently

performed in mice, using phage peptide biopanning to identify a peptide, CGSPGWVRC, specific for lung endothelium [26]. When conjugated to the proapoptotic peptide $_D$(KLAKLAK)$_2$, the compound is taken up by mouse lung endothelium, induces endothelial apoptosis, and results in emphysema [26]. The reliance of lung structure on vascular integrity has also been suggested by studies in which emphysema results from immunization of rats with xenogenic human endothelial cells [27]. In these latter studies, passive immunization with antisera or adoptive transfer using CD4+ splenocytes also caused emphysema. Interestingly, the concept that emphysema may arise from vascular compromise was initially proposed by the great lung pathologist Averill Liebow, who in 1959 noted extreme capillary attenuation in the nearly avascular emphysematous alveolar septal walls [28].

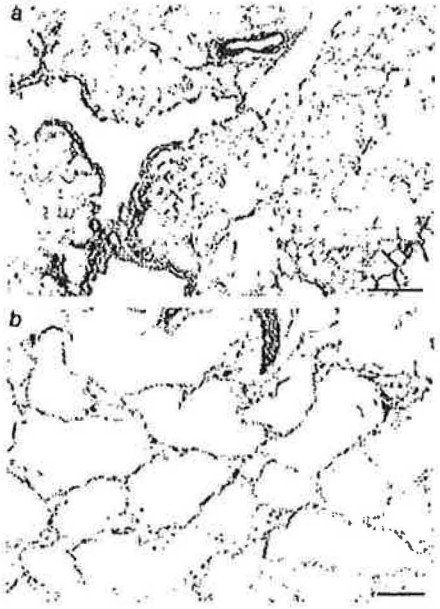
The finding of numerous lung parenchymal cells undergoing apoptotic cell death in emphysema is of importance for several reasons. First, apoptotic cells are normally removed by a highly efficient process recently named efferocytosis (to carry to the grave, to bury) [29]. This process is accomplished by professional phagocytes and is evolutionarily conserved. During initial contact and recognition of the corpse by a macrophage, anti-inflammatory pathways are initiated, to suppress an inflammatory response to dying cells expressing self-antigens [30]. Relevant to emphysema, both neutrophil elastase and MMP12 inhibit efferocytosis through cleavage of macrophage receptors. In fact, alveolar macrophages recovered from emphysema subjects have a diminished efferocytotic response to apoptotic airway epithelial cells, while having an intact phagocytic response to inert latex beads [31]. The implication is that in emphysema, numerous apoptotic cells are present in part from a failure of corpse clearance mechanisms.

Second, both human and experimental emphysematous lung cell death is accompanied by intense oxidative stress. Human emphysematous lungs have increased 4-hydroxy-2-nonenal, a product of lipid peroxidation, and demonstrate increases in oxidative stress genes [32, 33]. In addition, exhaled breath condensate from patients with emphysema have higher than normal levels of oxidants such as $H_2O_2$, nitrosothiols, and 8-isoprostane [34]. Oxidative stress markers also increase in experimental models of emphysema such as the lung endothelial cell targeted peptide model [26], various knockout models [35], and the VEGFR antagonist model [23]. Conversely, mice deficient in antioxidants develop age and cigarette smoke-induced emphysema with lung parenchymal cell death [36]. In the VEGFR antagonism model, antioxidants prevent emphysema formation, suggesting a link between oxidants and either cell death or cell replacement (regeneration).

Third, related to oxidative stress and failure of tissue regeneration, cell death in emphysema is also accompanied by signs of cellular senescence. Emphysematous lungs have increased expression of the cyclin kinase inhibitors $p16^{INK4a}$ and $p21^{CIP1/WAF1}$ [37], both in endothelial and epithelial cells. Sustained expression of $p16^{INK4a}$ in particular occurs in most if not all forms of cellular senescence. Fibroblasts grown from emphysematous lung explants have reduced proliferative capacity and increased senescence-associated β-galactosidase [38]. In addition, these cells express lower levels of the RecQ helicase WRN, whose loss of function is responsible for the premature senescence of Werner's syndrome [39]. A second fundamental anti-aging protein, the protein deacetylase SIRT1, is also severely reduced in emphysematous lung tissue, and becomes modified with nitrotyrosine and

carbonyl adducts, signs of protein oxidative stress [40]. Interestingly, telomere length is decreased in both circulating lymphocytes and in lungs of smokers with emphysema compared to normal lungs, but not compared to smokers without emphysema [37, 41]. It is known that oxidative stress itself may precipitate cellular senescence, independent of replicative senescence. Whether such mechanisms are at play in the emphysematous lung is not known.

The direct relevance of senescence to emphysema is demonstrated by mouse models of premature aging. Mice lacking Senescence Marker Protein-30 (SMP-30) age prematurely, accumulate markers of oxidative stress, and develop age- and cigarette smoke-induced emphysema at a markedly accelerated rate [42]. Another mouse model, the klotho mouse, displays premature aging due to insertional mutagenesis of a gene of as yet unknown cellular function [43]. The klotho mouse is born with normal lungs but develops emphysema starting at 4 weeks and dies by 9 weeks [44]. The lungs, as with other models of emphysema, demonstrate significant increases in lung parenchymal cell death [45].




In humans, premature aging syndromes such as Progeria and Werner's syndrome are not known to be associated with emphysema. Physiologic aging, however, is accompanied by both histologic and physiologic evidence of significant emphysema (Figure 4) [46]. In concept, the appearance of senescence markers in emphysematous lungs may well explain the failure of normal tissue restorative programs to regenerate dying lung tissue. Epithelial stem and progenitor cell niches are not well studied in humans, but in mice, variant Clara cells are thought to represent the principal stem cells which repopulate the distal respiratory epithelium [47]. Such cells reside in a niche at the bronchoalveolar duct junction, a tenuous location given that this zone is the epicenter of centrilobular emphysema. Whether gradual loss of resident stem cells, by any one of a variety of means, is the cause of emphysema or not is currently not known.

**Local TGFβ activity and matrix integrity are compromised in emphysema**

Returning to lung development, TGFβ is also critical for alveolarization as well as earlier stages of lung development. Notably, TGFβ's actions are highly site specific within organs. Produced as a procytokine, the C-terminus is cleaved off, dimerizes, and becomes the soluble agonist, while the N-terminus (latency associated peptide, LAP) noncovalently associates the cytokine, rendering it latent. The complex then typically covalently binds a larger matrix protein of the LTBP subfamily (latent TGFβ-

Figure 4. Histologic sections of lungs from a 29 year old (upper panel) and a nonsmoking 100 year old man (lower panel) demonstrate senile emphysema. Note similar scale bars. From reference [46].

binding protein). The LTBPs are members of the fibrillin glycoprotein family, which are important structural elements of microfibrils. LTBPs associate with other fibrillin family proteins such as fibrillin-1 during the formation of microfibrils. Microfibrils, in turn, are the major organizational units of elastic fibers. Thus during development, TGFβ is targeted to the elastic lung matrix, in theory providing lung tissue with a rapidly inducible and highly localized TGFβ signal.

Given the importance of microfibrilar integrity to elastic fiber formation, it is notable that roughly 10% of individuals with Marfan Syndrome, a connective tissue disease caused by mutations in fibrillin-1, have emphysematous lung lesions [48]. Deficiency in fibrillin-1 in mice recapitulates Marfan Syndrome, with both aortic aneurysm formation and emphysema [49]. Complete loss of function mutants are born with mild developmental emphysema, which appears as simplified terminal airsacs with a paucity of alveolar septal tips [50]. This emphysema progresses and is associated with an abnormal and robust increase in TGFβ signaling. Through use of a TGFβ-responsive GFP transgenic strain, the increase in signaling was shown to be intense and diffuse throughout the lung, suggesting delocalization of active TGFβ activity, along with lung parenchymal cell death [50]. Neutralizing antibodies against TGFβ reversed the developmental emphysema, indicating that matrix abnormalities in this case appear to cause emphysema through derangements of TGFβ signaling.

Interestingly, loss of function mutants in various arms of the TGFβ pathway also result in emphysema. Interference with TGFβR1 function through mutation of the core fucosylation enzyme causes emphysema, in this case through induction of MMP12 and MMP13 [51]. LTBP-1 hypomorphs and LTBP-3 knockout mice also develop severe emphysema [52, 53]. In the former case, activation of downstream factors such as SMAD2 is diminished, suggesting a global loss of TGFβ signaling. Accordingly, SMAD3 deleted mice also develop emphysema [54]. Finally, mice lacking the αvβ6 integrin also develop emphysema [55]. In this case, the epithelial αvβ6 integrin binds the LAP fragment and causes release of active TGFβ. Loss of the integrin causes TGFβ to remain trapped in a latent form; thus, this model also causes emphysema through diminished TGFβ signaling. The mechanism for release of TGFβ was recently demonstrated to be integrin dependent traction on the LAP through cell contraction against a stiff matrix [56], mechanically opening the closed LAP. This model suggests an additional mechanism by which the compromised emphysema matrix may impair TGFβ release. The reason why both excessive and insufficient TGFβ signaling lead to emphysema is unclear, although presumably the effects of this cytokine must be spatially restricted in order to exert proper developmental instructions.

Not all matrix disorders cause emphysema through derangements in TGFβ signaling. Another microfibrillar component, fibulin-5, is a known gene target in human cutis laxa. This disorder is another elastinopathy more commonly associated with mutations in the gene for elastin, and presents with characteristic loss of skin elasticity but can also present with emphysema. Mice deficient in fibulin-5 also develop loose skin and emphysema, as well as aortic abnormalities (though not aneurysmal dilation) [57]. Mild emphysema is present on postnatal day 1, and thus starts as a developmental disorder; however, by 1.5 months the emphysema progresses to a marked extent. Fibulin-5 contains an RGD motif which binds αvβ3, αvβ5, and α9β1 integrins, all of which are expressed by vascular endothelial cells [58]. Indeed, fibulin-5 appears by immune-EM to be

positioned on the outer margins of elastic fibers, in contact with the abluminal surface of endothelial cells [57]. Thus, loss of fibulin-5 is expected to cause tissue disorganization at least in part through loss of normal anchorage signals from the matrix to lung parenchymal cells. One might anticipate that in cigarette-related emphysema, in which specific matrix proteins are not lost but rather the quaternary structure of the entire elastic fiber is disrupted, that loss of matrix anchorage signals may represent an important feature to explain the effect of elastin fragmentation on cell death. The actual impact of abnormal anchorage and mechanical signals on lung endothelium and epithelium in the context of emphysema is unknown.

**Emphysema *per se* constitutes a pro-malignancy risk**

To summarize recent findings, emphysema arises in the distal airspaces within a field of chronic inflammation, oxidative stress, accelerated cellular senescence, and abnormal anchorage. These factors, along with aberrant soluble signals, appear to mediate cell death as the proximate cause of septal regression. This construct fits well with current theories of aging. The disposable soma hypothesis, for instance, holds that aging results from the stochastic interaction between injury and repair as the result of energy devoted by an individual to maintain organ integrity and protect DNA against oxidative injury. In this model, aging results from the interaction between intrinsic characteristics of the organism and the environment, and is therefore a lifelong accumulation of molecular damage (Figure 5). This view correlates well with current notions of how emphysema forms as "...the cumulative response of susceptible lungs to the total burden of inhaled particles and gases over a lifetime." [59].

An unfortunate general consequence of tissue senescence is the propensity to undergo malignant transformation. Indeed, one of the chief risk factors for cancer is aging itself. While the molecular theories explaining this link are numerous, it is clear overall that proteins which safeguard against cancer can promote aging ($p16^{INK4a}$, p53), while proteins which prevent aging are activated in cancer cells (telomerase) [60, 61]. Though it has been proposed that whatever endogenous factors constitute a risk for COPD also place one at risk for lung cancer, it is less clear whether these risks act independently on the two diseases. The model of emphysema which has emerged over the past several years would suggest that the same factors which drive emphysematous changes also force transformation, but that the latter may occur because of the former. Put another way, do patients with COPD get lung cancer from smoking or do they get it from emphysema?

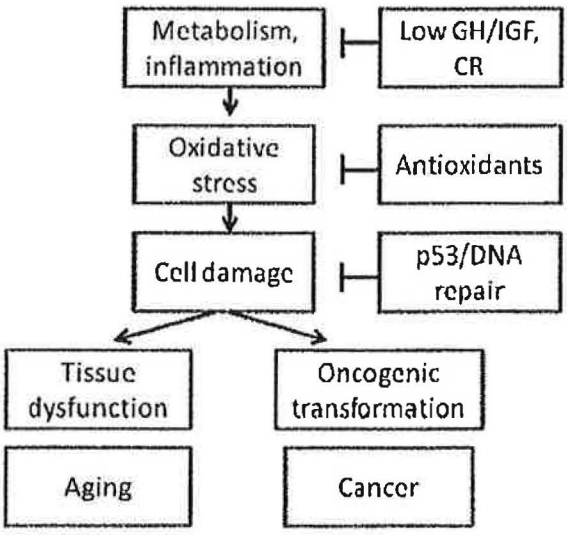



Figure 5. Overview of forces which drive cellular senescence and organism aging, and link to cancer. Adapted from reference [61].

Although only 20-40% of smokers develop COPD and 10-15% develop lung cancer, the majority of individuals who have lung cancer also have COPD, supporting a link. In one study, two cohorts (n=113 each) of subjects with similar pack-year histories (one with COPD as defined by FEV1<70%, the other with normal spirometry) were followed for over 10 years [62]. Here, the cumulative probability of developing lung cancer was 8.8% in the group with COPD, 2.0% in the non-COPD group. Similar results have been obtained by other groups. In a separate study, the odds ratio of developing lung cancer for subjects with decreased FEV1 compared with normal airflow was 6.43 (3.23-12.95), and diminished only to 6.07 when adjusted for smoking [63].

A reduction in FEV1 is used to classify COPD severity, but this parameter does not necessarily track with emphysema per se. Two recent studies have addressed the independent contribution of emphysema to lung cancer risk. In the first, 1,166 current and former smokers were followed for 5 years, and 23 developed lung cancer [64]. While both airflow limitation and radiographic emphysema predicted an increased risk for developing lung cancer (3.33 and 4.83, respectively), subjects with emphysema without airflow obstruction continued to have an elevated risk (4.33). Using a Poisson regression to adjust for obstruction, the presence of emphysema conferred a significant risk for lung cancer (2.51, 1.01-6.23) whereas obstruction adjusted for the degree of emphysema did not (2.10, 0.79-5.58). While the overall rate of cancer was low, the same conclusion was reached by a subsequent study of 3,638 smokers and ex-smokers. When stratified by severity of airflow and presence or absence of emphysema, the presence of emphysema was shown to be a strong independent risk factor for the subsequent development of lung cancer [65]. In addition, when stratified by radiographic severity of emphysema, there did not appear to be a threshold: that is, even a trace amount of emphysema elevated one's risk for lung cancer.

Finally, the risk of lung cancer from emphysema, independent of smoking, was examined using the large Cancer Prevention Study II cohort, which was initiated in 1982. Out of this group, 448,600 never-smokers were followed for 20 years [66]. Estimated hazard ratios were adjusted for a variety of exposures, including second-hand smoke, asbestos, chemical/acid/solvent exposure, coal or stone dust, coal tar/pitch/asphalt exposure, formaldehyde, diesel exhaust, alcohol, and vegetable/fruit/fiber/fat intake. Emphysema, in this case physician-diagnosed, comprised an elevated risk for the development of lung cancer (1.66, 1.06-2.59), whereas chronic bronchitis did not (0.96, 0.72-1.28). Thus emphysema constitutes a clinical risk for lung cancer, independent of airflow obstruction or smoking history.

These studies even in aggregate do not definitively answer the question of what role emphysema per se plays in malignant transformation. Importantly, lung cancer is not a single disease, and thus "single arrow" hypotheses are likely to fail. However, an important concept which emerges is that malignant transformation can and frequently does arise in the context of diseased tissues. If one again considers the features of emphysema, being oxidative stress, premature senescence and cell death, vascular regression, and abnormal matrix, it is reasonable to guess that at least one potential effect of the diseased tissue is to impose a severe selection pressure on cells to bypass these stresses. The successful cell which emerges from this selection process, one may infer, would be resistant to oxidative stress, immortal, able to invoke angiogenesis, and be attachment independent. These, of

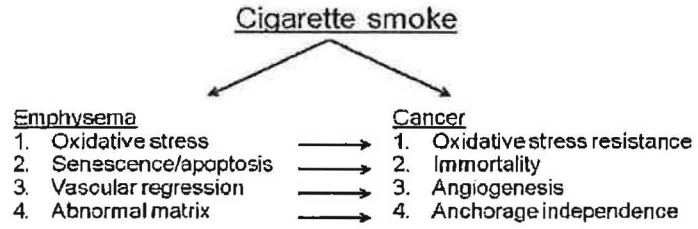



Figure 6. Proposed effect of emphysema in forcing selection of transformation characteristics.

course, are some of the key characteristics of cancer cells. The real impact that diseased tissues such as emphysematous lungs have on malignant transformation is unknown, however, and in fact, is poorly studied.

## Conclusions

From a bird's eye view, the present synthesis of what happens to lung tissue during the formation of emphysema involves concepts derived from a variety of fields, including developmental biology, oxidative stress, mechanotransduction, aging, and cell transformation. Therapeutically, the challenge ahead lies in determining first of all whether it is possible to reverse or halt the drastic tissue remodeling which occurs with emphysema, and secondly how to address each of the fundamental defects outlined above.

## REFERENCES

[1] Jemal, A.; Ward, E.; Hao, Y.; Thun, M. Trends in the leading causes of death in the United States, 1970-2002. *JAMA* **294:**1255-1259; 2005.

[2] Laurell, C. B.; Eriksson, S. The electrophoretic alpha1 globulin pattern of serum in alpha1-antitrypsin deficiency. *Scand.J.Clin.Lab.Invest.* **15:**132-140; 1963.

[3] Eriksson, S. Pulmonary Emphysema and Alpha1-Antitrypsin Deficiency. *Acta Med Scand* **175:**197-205; 1964.

[4] Eriksson, S. Studies in alpha 1-antitrypsin deficiency. *Acta Med Scand Suppl* **432:**1-85; 1965.

[5] Janoff, A. Neutrophil proteases in inflammation. *Annu Rev Med* **23:**177-190; 1972.

[6] Leopold, J. G.; Gough, J. The centrilobular form of hypertrophic emphysema and its relation to chronic bronchitis. *Thorax* **12:**219-235; 1957.

[7] Hogg, J. C.; Chu, F.; Utokaparch, S.; Woods, R.; Elliott, W. M.; Buzatu, L.; Cherniack, R. M.; Rogers, R. M.; Sciurba, F. C.; Coxson, H. O.; Pare, P. D. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N. Engl. J. Med.* **350:**2645-2653; 2004.

[8] Majo, J.; Ghezzo, H.; Cosio, M. G. Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *Eur Respir J* **17:**946-953; 2001.

[9] Saetta, M.; Mariani, M.; Panina-Bordignon, P.; Turato, G.; Buonsanti, C.; Baraldo, S.; Bellettato, C. M.; Papi, A.; Corbetta, L.; Zuin, R.; Sinigaglia, F.; Fabbri, L. M. Increased expression of the chemokine receptor CXCR3 and its ligand CXCL10 in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **165:**1404-1409; 2002.

[10] Grumelli, S.; Corry, D. B.; Song, L. Z.; Song, L.; Green, L.; Huh, J.; Hacken, J.; Espada, R.; Bag, R.; Lewis, D. E.; Kheradmand, F. An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema. *PLoS Med* **1:**e8; 2004.

[11] Sullivan, A. K.; Simonian, P. L.; Falta, M. T.; Mitchell, J. D.; Cosgrove, G. P.; Brown, K. K.; Kotzin, B. L.; Voelkel, N. F.; Fontenot, A. P. Oligoclonal CD4+ T cells in the lungs of patients with severe emphysema. *Am J Respir Crit Care Med* **172:**590-596; 2005.

[12] Korn, S.; Wiewrodt, R.; Walz, Y. C.; Becker, K.; Mayer, E.; Krummenauer, F.; Buhl, R. Characterization of the interstitial lung and peripheral blood T cell receptor repertoire in cigarette smokers. *Am J Respir Cell Mol Biol* **32:**142-148; 2005.

[13] Lee, S. H.; Goswami, S.; Grudo, A.; Song, L. Z.; Bandi, V.; Goodnight-White, S.; Green, L.; Hacken-Bitar, J.; Huh, J.; Bakaeen, F.; Coxson, H. O.; Cogswell, S.; Storness-Bliss, C.; Corry, D. B.; Kheradmand, F. Antielastin autoimmunity in tobacco smoking-induced emphysema. *Nat. Med.* **13:**567-569; 2007.

[14] Wang, I. M.; Stepaniants, S.; Boie, Y.; Mortimer, J. R.; Kennedy, B.; Elliott, M.; Hayashi, S.; Loy, L.; Coulter, S.; Cervino, S.; Harris, J.; Thornton,

M.; Raubertas, R.; Roberts, C.; Hogg, J. C.; Crackower, M.; O'Neill, G.; Pare, P. D. Gene expression profiling in patients with chronic obstructive pulmonary disease and lung cancer. *Am J Respir Crit Care Med* **177**:402-411; 2008.

[15] Spira, A.; Beane, J.; Pinto-Plata, V.; Kadar, A.; Liu, G.; Shah, V.; Celli, B.; Brody, J. S. Gene expression profiling of human lung tissue from smokers with severe emphysema. *Am J Respir Cell Mol Biol* **31**:601-610; 2004.

[16] Ning, W.; Li, C. J.; Kaminski, N.; Feghali-Bostwick, C. A.; Alber, S. M.; Di, Y. P.; Otterbein, S. L.; Song, R.; Hayashi, S.; Zhou, Z.; Pinsky, D. J.; Watkins, S. C.; Pilewski, J. M.; Sciurba, F. C.; Peters, D. G.; Hogg, J. C.; Choi, A. M. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. *Proc. Natl. Acad. Sci. USA* **101**:14895-14900; 2004.

[17] Zeskind, J. E.; Lenburg, M. E.; Spira, A. Translating the COPD transcriptome: insights into pathogenesis and tools for clinical management. *Proc Am Thorac Soc* **5**:834-841; 2008.

[18] Kanazawa, H.; Asai, K.; Hirata, K.; Yoshikawa, J. Possible effects of vascular endothelial growth factor in the pathogenesis of chronic obstructive pulmonary disease. *Am J Med* **114**:354-358; 2003.

[19] Kasahara, Y.; Tuder, R. M.; Cool, C. D.; Lynch, D. A.; Flores, S. C.; Voelkel, N. F. 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* **163**:737-744; 2001.

[20] Segura-Valdez, L.; Pardo, A.; Gaxiola, M.; Uhal, B. D.; Becerril, C.; Selman, M. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest* **117**:684-694; 2000.

[21] Imai, K.; Mercer, B. A.; Schulman, L. L.; Sonett, J. R.; D'Armiento, J. M. Correlation of lung surface area to apoptosis and proliferation in human emphysema. *Eur Respir J* **25**:250-258; 2005.

[22] Morissette, M. C.; Vachon-Beaudoin, G.; Parent, J.; Chakir, J.; Milot, J. Increased p53 level, Bax/Bcl-x(L) ratio, and TRAIL receptor expression in human emphysema. *Am J Respir Crit Care Med* **178**:240-247; 2008.

[23] Kasahara, Y.; Tuder, R. M.; Taraseviciene-Stewart, L.; Le Cras, T. D.; Abman, S.; Hirth, P. K.; Waltenberger, J.; Voelkel, N. F. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J. Clin. Invest.* **106**:1311-1319; 2000.

[24] Tang, K.; Rossiter, H. B.; Wagner, P. D.; Breen, E. C. Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. *J. Appl. Physiol.* **97**:1559-1566; discussion 1549; 2004.

[25] Petrache, I.; Natarajan, V.; Zhen, L.; Medler, T. R.; Richter, A. T.; Cho, C.; Hubbard, W. C.; Berdyshev, E. V.; Tuder, R. M. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. *Nat. Med.* **11**:491-498; 2005.

[26] Giordano, R. J.; Lahdenranta, J.; Zhen, L.; Chukwueke, U.; Petrache, I.; Langley, R. R.; Fidler, I. J.; Pasqualini, R.; Tuder, R. M.; Arap, W. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J. Biol. Chem.* **283**:29447-29460; 2008.

[27] Taraseviciene-Stewart, L.; Scerbavicius, R.; Choe, K. H.; Moore, M.; Sullivan, A.; Nicolls, M. R.; Fontenot, A. P.; Tuder, R. M.; Voelkel, N. F. An animal model of autoimmune emphysema. *Am J Respir Crit Care Med* **171**:734-742; 2005.

[28] Liebow, A. A. Pulmonary emphysema with special reference to vascular changes. *Am Rev Respir Dis* **80**:67-93; 1959.

[29] Henson, P. M.; Vandivier, R. W.; Douglas, I. S. Cell death, remodeling, and repair in chronic obstructive pulmonary disease? *Proc Am Thorac Soc* **3**:713-717; 2006.

[30] Savill, J.; Dransfield, I.; Gregory, C.; Haslett, C. A blast from the past: clearance of apoptotic cells regulates immune responses. *Nature Rev. Immunol.* **2**:965-975; 2002.

[31] Hodge, S.; Hodge, G.; Scicchitano, R.; Reynolds, P. N.; Holmes, M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol* **81**:289-296; 2003.

[32] Rahman, I.; van Schadewijk, A. A.; Crowther, A. J.; Hiemstra, P. S.; Stolk, J.; MacNee, W.; De Boer, W. I. 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* **166**:490-495; 2002.

[33] Pierrou, S.; Broberg, P.; O'Donnell, R. A.; Pawlowski, K.; Virtala, R.; Lindqvist, E.; Richter, A.; Wilson, S. J.; Angco, G.; Moller, S.; Bergstrand, H.; Koopmann, W.; Wieslander, E.; Stromstedt, P. E.; Holgate, S. T.; Davies, D. E.; Lund, J.; Djukanovic, R. Expression of genes involved in oxidative stress responses in airway epithelial cells of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **175**:577-586; 2007.

[34] Kluchova, Z.; Petrasova, D.; Joppa, P.; Dorkova, Z.; Tkacova, R. The association between oxidative stress and obstructive lung impairment in patients with COPD. *Physiol Res* **56**:51-56; 2007.

[35] Zhang, X.; Shan, P.; Jiang, G.; Cohn, L.; Lee, P. J. Toll-like receptor 4 deficiency causes pulmonary emphysema. *J. Clin. Invest.* **116**:3050-3059; 2006.

[36] Rangasamy, T.; Cho, C. Y.; Thimmulappa, R. K.; Zhen, L.; Srisuma, S. S.; Kensler, T. W.; Yamamoto, M.; Petrache, I.; Tuder, R. M.; Biswal, S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke-induced emphysema in mice. *J. Clin. Invest.* **114**:1248-1259; 2004.

[37] Tsuji, T.; Aoshiba, K.; Nagai, A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med* **174**:886-893; 2006.

[38] Holz, O.; Zuhlke, I.; Jaksztat, E.; Muller, K. C.; Welker, L.; Nakashima, M.; Diemel, K. D.; Branscheid, D.; Magnussen, H.; Jorres, R. A. Lung fibroblasts from patients with emphysema show a reduced proliferation rate in culture. *Eur Respir J* **24**:575-579; 2004.

[39] Nyunoya, T.; Monick, M. M.; Klingelhutz, A. L.; Glaser, H.; Cagley, J. R.; Brown, C. O.; Matsumoto, E.; Aykin-Burns, N.; Spitz, D. R.; Oshima, J.; Hunninghake, G. W. Cigarette smoke induces cellular senescence via Werner's syndrome protein down-regulation. *Am J Respir Crit Care Med* **179**:279-287; 2009.

[40] Rajendrasozhan, S.; Yang, S. R.; Kinnula, V. L.; Rahman, I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **177**:861-870; 2008.

[41] Morla, M.; Busquets, X.; Pons, J.; Sauleda, J.; MacNee, W.; Agusti, A. G. Telomere shortening in smokers with and without COPD. *Eur Respir J* **27**:525-528; 2006.

[42] Sato, T.; Seyama, K.; Sato, Y.; Mori, H.; Souma, S.; Akiyoshi, T.; Kodama, Y.; Mori, T.; Goto, S.; Takahashi, K.; Fukuchi, Y.; Maruyama, N.; Ishigami, A. Senescence marker protein-30 protects mice lungs from oxidative stress, aging, and smoking. *Am J Respir Crit Care Med* **174**:530-537; 2006.

[43] Kuro-o, M.; Matsumura, Y.; Aizawa, H.; Kawaguchi, H.; Suga, T.; Utsugi, T.; Ohyama, Y.; Kurabayashi, M.; Kaname, T.; Kume, E.; Iwasaki, H.; Iida, A.; Shiraki-Iida, T.; Nishikawa, S.; Nagai, R.; Nabeshima, Y. I. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature* **390**:45-51; 1997.

[44] Suga, T.; Kurabayashi, M.; Sando, Y.; Ohyama, Y.; Maeno, T.; Maeno, Y.; Aizawa, H.; Matsumura, Y.; Kuwaki, T.; Kuro, O. M.; Nabeshima, Y.; Nagai, R. 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* **22**:26-33; 2000.

[45] Ishii, M.; Yamaguchi, Y.; Yamamoto, H.; Hanaoka, Y.; Ouchi, Y. Airspace enlargement with airway cell apoptosis in klotho mice: a model of aging lung. *J Gerontol A Biol Sci Med Sci* **63**:1289-1298; 2008.

[46] Janssens, J. P.; Pache, J. C.; Nicod, L. P. Physiological changes in respiratory function associated with ageing. *Eur Respir J* **13**:197-205; 1999.

[47] Kim, C. F.; Jackson, E. L.; Woolfenden, A. E.; Lawrence, S.; Babar, I.; Vogel, S.; Crowley, D.; Bronson, R. T.; Jacks, T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* **121**:823-835; 2005.

[48] Wood, J. R.; Bellamy, D.; Child, A. H.; Citron, K. M. Pulmonary disease in patients with Marfan syndrome. *Thorax* **39**:780-784; 1984.

[49] Pereira, L.; Andrikopoulos, K.; Tian, J.; Lee, S. Y.; Keene, D. R.; Ono, R.; Reinhardt, D. P.; Sakai, L. Y.; Biery, N. J.; Bunton, T.; Dietz, H. C.; Ramirez, F. Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. *Nat Genet* **17**:218-222; 1997.

[50] Neptune, E. R.; Frischmeyer, P. A.; Arking, D. E.; Myers, L.; Bunton, T. E.; Gayraud, B.; Ramirez, F.; Sakai, L. Y.; Dietz, H. C. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet* **33**:407-411; 2003.

[51] Wang, X.; Inoue, S.; Gu, J.; Miyoshi, E.; Noda, K.; Li, W.; Mizuno-Horikawa, Y.; Nakano, M.; Asahi, M.; Takahashi, M.; Uozumi, N.; Ihara, S.; Lee, S. H.; Ikeda, Y.; Yamaguchi, Y.; Aze, Y.; Tomiyama, Y.; Fujii, J.; Suzuki, K.; Kondo, A.; Shapiro, S. D.; Lopez-Otin, C.; Kuwaki, T.; Okabe, M.; Honke, K.; Taniguchi, N. Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. *Proc. Natl. Acad. Sci. USA* **102**:15791-15796; 2005.

[52] Sterner-Kock, A.; Thorey, I. S.; Koli, K.; Wempe, F.; Otte, J.; Bangsow, T.; Kuhlmeier, K.; Kirchner, T.; Jin, S.; Keski-Oja, J.; von Melchner, H. Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Dev.* **16**:2264-2273; 2002.

[53] Colarossi, C.; Chen, Y.; Obata, H.; Jurukovski, V.; Fontana, L.; Dabovic, B.; Rifkin, D. B. Lung alveolar septation defects in Ltbp-3-null mice. *Am J Pathol* **167**:419-428; 2005.

[54] Bonniaud, P.; Kolb, M.; Galt, T.; Robertson, J.; Robbins, C.; Stampfli, M.; Lavery, C.; Margetts, P. J.; Roberts, A. B.; Gauldie, J. Smad3 null mice develop airspace enlargement and are resistant to

TGF-beta-mediated pulmonary fibrosis. *J. Immunol.* **173**:2099-2108; 2004.

[55] Morris, D. G.; Huang, X.; Kaminski, N.; Wang, Y.; Shapiro, S. D.; Dolganov, G.; Glick, A.; Sheppard, D. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. *Nature* **422**:169-173; 2003.

[56] Wipff, P. J.; Rifkin, D. B.; Meister, J. J.; Hinz, B. Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J. Cell Biol.* **179**:1311-1323; 2007.

[57] Yanagisawa, H.; Davis, E. C.; Starcher, B. C.; Ouchi, T.; Yanagisawa, M.; Richardson, J. A.; Olson, E. N. Fibulin-5 is an elastin-binding protein essential for elastic fibre development in vivo. *Nature* **415**:168-171; 2002.

[58] Nakamura, T.; Lozano, P. R.; Ikeda, Y.; Iwanaga, Y.; Hinek, A.; Minamisawa, S.; Cheng, C. F.; Kobuke, K.; Dalton, N.; Takada, Y.; Tashiro, K.; Ross Jr, J.; Honjo, T.; Chien, K. R. Fibulin-5/DANCE is essential for elastogenesis in vivo. *Nature* **415**:171-175; 2002.

[59] Buist, A. S. Consequences and comorbidities of cigarette-induced lung injury: lung cancer, COPD, and cardiovascular disease. *Proc. Am. Thoracic Soc.* **5**:796-799; 2008.

[60] Pawelec, G.; Solana, R. Are cancer and ageing different sides of the same coin? Conference on Cancer and Ageing. *EMBO Rep* **9**:234-238; 2008.

[61] Serrano, M.; Blasco, M. A. Cancer and ageing: convergent and divergent mechanisms. *Nature Rev. Mol. Cell Biol.* **8**:715-722; 2007.

[62] Skillrud, D. M.; Offord, K. P.; Miller, R. D. Higher risk of lung cancer in chronic obstructive pulmonary disease. A prospective, matched, controlled study. *Ann Intern Med* **105**:503-507; 1986.

[63] Tockman, M. S.; Anthonisen, N. R.; Wright, E. C.; Donithan, M. G. Airways obstruction and the risk for lung cancer. *Ann Intern Med* **106**:512-518; 1987.

[64] de Torres, J. P.; Bastarrika, G.; Wisnivesky, J. P.; Alcaide, A. B.; Campo, A.; Seijo, L. M.; Pueyo, J. C.; Villanueva, A.; Lozano, M. D.; Montes, U.; Montuenga, L.; Zulueta, J. J. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. *Chest* **132**:1932-1938; 2007.

[65] Wilson, D. O.; Weissfeld, J. L.; Balkan, A.; Schragin, J. G.; Fuhrman, C. R.; Fisher, S. N.; Wilson, J.; Leader, J. K.; Siegfried, J. M.; Shapiro, S. D.; Sciurba, F. C. Association of radiographic emphysema and airflow obstruction with lung cancer. *Am J Respir Crit Care Med* **178**:738-744; 2008.

[66] Turner, M. C.; Chen, Y.; Krewski, D.; Calle, E. E.; Thun, M. J. Chronic obstructive pulmonary disease is associated with lung cancer mortality in a prospective study of never smokers. *Am J Respir Crit Care Med* **176**:285-290; 2007.