

Review

Epithelial Cell Apoptosis and Lung Remodeling

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Lung epithelium is the primary site of lung damage in various lung diseases. Epithelial cell apoptosis has been considered to be initial event in various lung diseases. Apoptosis signaling is classically composed of two principle pathways. One is a direct pathway from death receptor ligation to caspase cascade activation and cell death. The other pathway triggered by stresses such as drugs, radiation, infectious agents and reactive oxygen species is mediated by mitochondria. Endoplasmic reticulum has also been shown to be the organelle to mediate apoptosis. Epithelial cell death is followed by remodeling processes, which consist of epithelial and fibroblast activation, cytokine production, activation of coagulation pathway, neoangiogenesis, re-epithelialization and fibrosis. Epithelial and mesenchymal interaction plays important roles in these processes. Further understanding of apoptosis signaling and its regulation by novel strategies may lead to effective treatments against various lung diseases. We review the recent advances in the understanding of apoptosis signaling and discuss the involvement of apoptosis in lung remodeling. *Cellular & Molecular Immunology*. 2007;4(6):419-429.

Key Words: apoptosis, lung injury, pulmonary fibrosis, COPD, remodeling

Introduction

Apoptosis plays a major role in homeostasis to maintain a balance between cell survival and death. There are two principle-signaling pathways of apoptosis. One is a direct pathway from death receptor ligation to caspase cascade activation and cell death. Death receptor ligation triggers recruitment of the precursor form of caspase-8 to a death-inducing complex, through the adaptor protein Fas-associating protein with death domain (FADD), which leads to caspase-8 activation. The other pathway triggered by stimuli such as drugs, radiation, infectious agents and reactive oxygen species is initiated in mitochondria. After cytochrome c is released into the cytosol from the mitochondria, it binds to Apaf1 and ATP, which then activate caspase-9 (1). The activation of initiator caspase-8 and caspase-9 results in activation of effector caspases such as caspase-3. Recently, endoplasmic reticulum has also been shown to be the organelle to execute apoptosis. Various stresses can impair protein folding and induce endoplasmic

reticulum stress, and severe endoplasmic reticulum stress can transduce apoptotic signals (2). Active executioner caspases mediate the cleavage of protein substrates, resulting in morphological features of apoptosis.

Apoptosis may play important roles in lung diseases in two different ways. First, failure to clear unwanted cells by apoptosis will prolong the inflammation because of the release of their toxic contents, and also delay repair processes. Apoptotic cells should be quickly recognized and ingested by phagocytes before releasing their toxic contents, unlike accidental cell death or necrosis. Second, excessive apoptosis may cause diseases. Intratracheal instillation of agonistic anti-Fas antibody or recombinant Fas ligand (FasL) induces acute alveolar epithelial injury and lung inflammation (3, 4). Repeated inhalations of agonistic anti-Fas antibody induce epithelial cell apoptosis and lung inflammation, which subsequently leads to pulmonary fibrosis in mice (5).

As well as death receptors/ligands, death signals such as reactive oxygen species, nitrogen species, proinflammatory cytokines, chemokines and other signaling molecules of apoptosis are involved in the pathophysiology of various lung diseases. When the degree of lung injury is mild, damaged tissue will be normally repaired, whereas excess cell death may lead to unrepairable lung damage and remodeling process (Figure 1). The survival and recovery of epithelial and endothelial cells and the resolution of inflammatory cells appear to be important in the normal repair. The tissue remodeling is the pathological repair process accompanied with fibrosis. The degree of remodeling is closely associated with the patient's prognosis. Therefore, further understanding of roles of apoptosis in remodeling process may lead to the development of effective strategies against devastating lung

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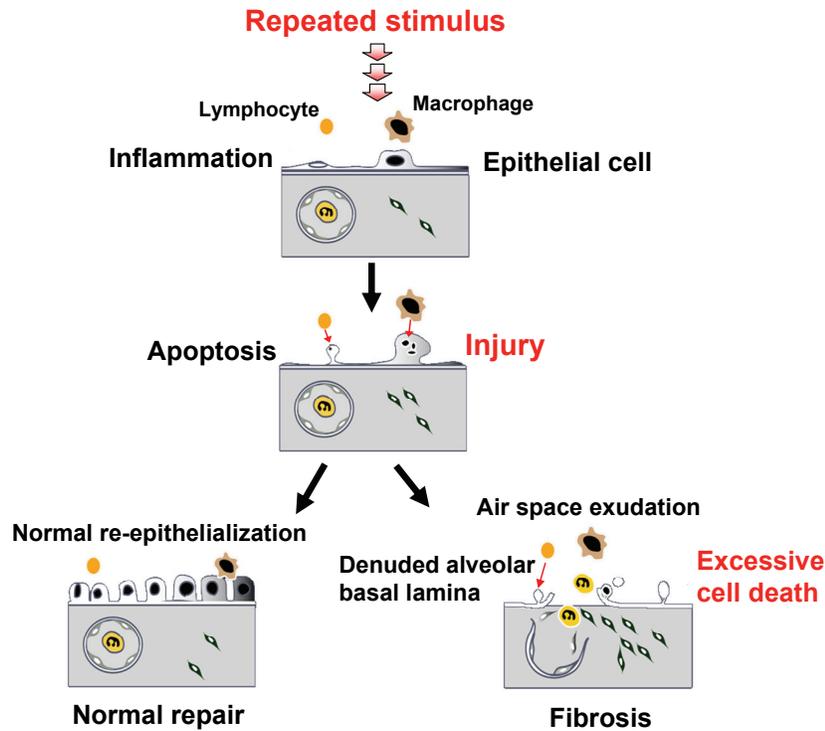


Figure 1. Excess cell death leads to fibrosis. When the degree of lung injury is mild, damaged tissue will be normally repaired, whereas excess cell death may lead to unreparable lung damage and remodeling process.

diseases.

Apoptosis in acute lung injury

Apoptosis of type I alveolar epithelial cells and endothelial cells are observed in lung tissues from patients with acute respiratory distress syndrome (ARDS) (6, 7). The expression of Bax and Bcl-2 protein are upregulated in alveolar epithelial cells and the number of epithelial cell apoptosis is associated with the prognosis of patients with diffuse alveolar damage (8, 9). Lipopolysaccharide (LPS) is an important factor in acute lung injury. LPS stimulates endothelial cells and induces the expression of pro-inflammatory mediators. Intravenous injection of LPS induces apoptosis of endothelial cells of systemic organs in mice (10, 11). Expression and activation of caspase-1 and caspase-3 were detected in lung epithelial cells in LPS-induced acute lung injury in mice (12). A broad-spectrum caspase inhibitor (Z-VAD.fmk) inhibited the intracellular activation of caspase-like proteases *in vivo*, and protected mice from LPS-induced acute lung injury (10, 12). These results suggest that apoptosis is essential in the development of acute lung injury in mice and ARDS in human, and also suggest that caspase cascade is involved in acute lung injury, and may be a novel treatment strategy against acute lung injury. In addition, epithelial cell apoptosis is associated with upregulation of Fas and FasL expression after LPS instillation, and suppressed by the treatment with

neutralizing antibody (13). These results suggest the significance of the Fas-FasL pathway in LPS-induced acute lung injury.

Normal repair after an acute lung injury requires the elimination of excessive mesenchymal and inflammatory cells from the alveolar airspace or alveolar wall (14). Clearance of apoptotic neutrophils by phagocytes has an important role in the resolution of inflammation (15). Phagocytosis of apoptotic neutrophils by macrophages not only suppresses the release of proinflammatory cytokines and reactive oxygen species, but also induces the production of transforming growth factor- β (TGF- β) and hepatocyte growth factor (HGF) from macrophages to regenerate damaged tissues (16). In fact, CDK inhibitor is important in tissue repair through enhancing the resolution of established inflammation by promoting apoptosis of inflammatory cells (17). Accordingly, the insufficiency of neutrophil apoptosis and the clearance of apoptotic cells by phagocytosis may lead to the prolongation of inflammation and the impairment of repair process.

Cytokines also participate in apoptosis. IL-6 attenuates hyperoxic lung injury and this protection is associated with a marked decrease of cell death probably through the induction of bcl-2 and tissue inhibitor of metalloproteinase (TIMP)-1 (18). IL-11 and GM-CSF reduce hyperoxia-induced epithelial cell apoptosis (19, 20). IL-15 overexpression can prevent TNF- α -induced apoptosis and protect against *E. coli*-induced shock (21). Considering these events, molecule specific

therapy is required instead of steroid treatment in order to regulate apoptosis.

TNF- α causes inflammation by damaging tissues and inducing the expression of adhesion molecules and cytokines in epithelial and endothelial cells as well as in inflammatory cells. The cellular effects of TNF are mediated by two distinct cell surface receptors, termed TNF-receptor 1 (TNFR1) and TNF-receptor 2 (TNFR2) (22). Most of the cytotoxic effects of TNF are mediated by TNFR1 through interaction of its death domain with the TNFR-associated death domain (TRADD) protein (23). TRADD protein interacts with Fas-associated death domain protein (FADD) (24) to activate caspase-8, thereby initiating the apoptosis pathway. TNF also activates NF- κ B and induces the expression of inhibitor of apoptosis proteins (IAPs) (25). Therefore, TNF usually does not kill most types of cells without metabolic inhibitors. There are several studies demonstrating that TNF participates in epithelial cell apoptosis in lung injury (26, 27). Since the sensitivity to TNF-induced apoptosis is variable in various types of cells, it is required to understand the intracellular signaling molecules and microenvironment in order to regulate TNF-induced apoptosis in lung diseases.

The Fas-FasL pathway is a representative system of apoptosis-signaling receptor molecules. Fas antigen is expressed in various cells and tissues. Mice carrying the lymphoproliferative (lpr) mutation have defects in the Fas antigen gene (28). FasL, a cell surface molecule belonging to the tumor necrosis factor family, binds to its receptor Fas, thus inducing the apoptosis of Fas bearing cells (29). FasL is expressed predominantly in activated T lymphocytes and in tissues including the small intestines, kidney, testis and lung (29). Generalized lymphoproliferative disease (gld) mice have a point mutation in the FasL and develop autoimmune disease (30). The Fas-FasL pathway has been demonstrated to contribute to severe epithelial damage that occurs in ARDS. Soluble FasL can be released as a biologically active, death-inducing mediator capable of inducing apoptosis of epithelial cells during acute lung injury (31). Bronchoalveolar lavage fluid (BALF) from patients with ARDS could induce apoptosis on small airway epithelial cells, which are dependent on the Fas-FasL pathway (32). Therefore, inhibiting this pathway may be one of novel treatment strategies against acute lung injury.

Oxidative stress and lung injury

Lung epithelium is not only the primary site of lung damage but it also participates in inflammatory reaction through a number of mechanisms, including the release of inflammatory mediators and reactive oxygen species. Alterations in the structure and function of lung epithelial cells by oxidative stress may affect the expression of these molecules. Apoptosis plays a central role in hyperoxic lung injury (33). Type I alveolar epithelial cells and endothelial cells are susceptible to hyperoxia. Type II epithelial cells present DNA damage induced by hyperoxia (34). Hydrogen

peroxide induces Fas upregulation by promoting cytoplasmic transport of Fas to the cell surface in human airway epithelial cells. Fas-mediated apoptosis may accelerate hyperoxia-induced acute lung injury in *Legionella pneumonia* (35). Hyperoxia exaggerates ventilator-induced cytokine production, neutrophil influx, and apoptosis through activation of the JNK and ERK pathway (36). Hyperoxia induces epithelial cell apoptosis in the lungs of neonatal rats, in which the expressions of Bax, ceramide, and Bcl-2 were upregulated, whereas the rise in Bax and ceramide overcomes the anti-apoptotic effect of Bcl-2 (37). In contrast, anti-apoptotic protein FLICE-inhibitory protein (FLIP) prevented hyperoxia-induced trafficking of death-inducing signal complex, activation of caspases, Bid, and Bax in lung endothelial cells (38). *In vivo* activation of A2A adenosine receptor confers protection against reperfusion lung injury through decreased apoptosis associated with ERK activation (39). Thioredoxin-1 is an important radical scavenger. Thioredoxin-1 transgenic mice had decreased alveolar damage after exposure to hyperoxia through upregulation of Bcl-2 protein and mRNA in the lung (40). Although various molecules are associated with oxidative stress-induced apoptosis and its mechanisms are complicated, there are many promising targets to regulate epithelial cell apoptosis and lung injury.

As well as apoptosis, necrosis is also induced by hyperoxia. Ang2 is a mediator of epithelial necrosis. During hyperoxia, Ang2 expression is induced in lung epithelial cells, while hyperoxia-induced lung injury, cell death, and mortality are ameliorated in Ang2^{-/-} mice (41). In addition to necrosis, oncosis is reported. In ischemic-reperfusion lung injury, electron microscopy showed morphological findings compatible with oncotic cell death rather than apoptosis or necrosis, including mitochondrial swelling and cytoplasm disorganization in pulmonary epithelial cells (42). The significance of differences among three types of cell death remains to be investigated.

Heme oxygenase-1 (HO-1) confers protection against a variety of oxidant-induced cell death and tissue injury mechanisms. HO-1 overexpression using adenovirus exhibited attenuation of hyperoxia-induced neutrophil inflammation and apoptosis (43). CO, a major by-product of heme catalysis by HO-1, exhibited a marked attenuation of hyperoxia-induced neutrophil infiltration into the airways and total lung apoptotic index (44). CO utilizes p38 MAPK and caspase-3 in exerting its anti-apoptotic effect both *in vitro* and *in vivo* during ischemia-reperfusion injury (34, 45). CO inhibits death-inducing signaling complex trafficking from Golgi apparatus to the plasma membrane and downstream caspase-8, and also inhibits intrinsic apoptosis signaling pathways in endothelial cells (46). CO inhalation may be a clinically useful treatment against acute lung injury, in which excessive apoptosis is occurring.

Keratinocyte growth factor (KGF) prevents the induction of p53, Bax, and Bcl-x mRNAs during hyperoxia, and oxygen-induced damage of alveolar epithelium and of endothelium (47). KGF attenuates hydrogen peroxide-induced DNA strand breaks in cultured alveolar epithelial cells by mechanisms that involve tyrosine kinase, PKC, and

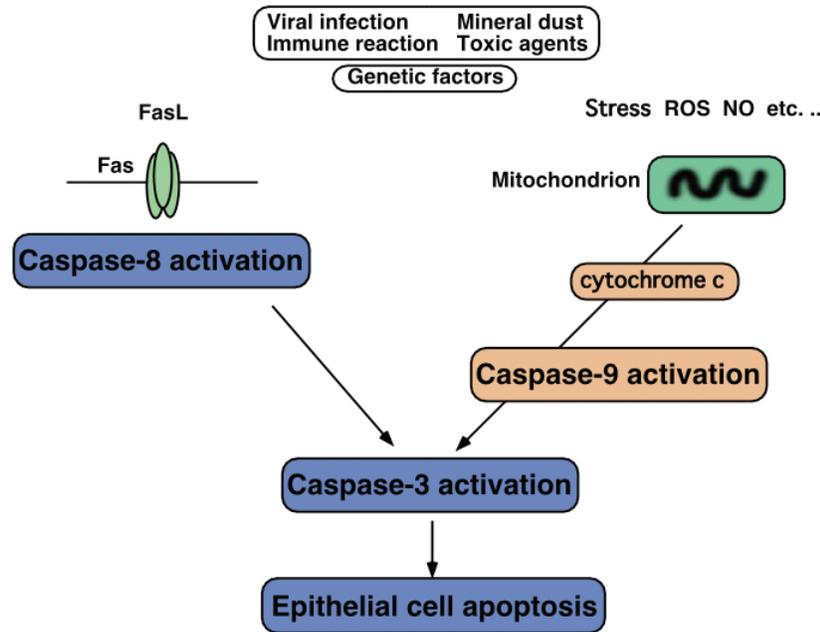


Figure 2. Activation of apoptosis signaling molecules in epithelial cells from patients with IPF. Death receptor-mediated and mitochondria-mediated apoptosis signaling molecules are upregulated and activated in epithelial cells of IPF.

DNA polymerases (48). KGF is able to activate the antiapoptotic Akt signaling (49). HGF inhibited hypoxia/reoxygenation-induced endothelial cell apoptosis through upregulation of FLIP and Bcl-xL, and inhibition of pro-apoptotic protein Bid and Bax (50). KGF and HGF may participate in maintaining and repairing the alveolar epithelium by regulating proapoptotic molecules and have the potential to become an effective agent against lung injury.

Apoptosis and interstitial lung diseases

Alveolar epithelial damage is an important initial event in pulmonary fibrosis. When the degree of lung injury is mild, damaged tissue will normally be repaired, whereas excess cell death may lead to unrepairable lung damage and pulmonary fibrosis. Epithelial cell damage and cell death during alveolitis induce the formation of gaps in the epithelial basement membranes. The migration of fibroblasts through these gaps into the alveolar space leads to intra-alveolar fibrosis. Interstitial fibrosis and the subsequent relining of intra-alveolar fibrosis by alveolar and bronchiolar epithelial cells result in structural remodeling after lung injury. Maintaining normal function and repair of parenchymal cells are important to improve the prognosis of patients. The fibrosing process is common to all interstitial lung diseases, including IPF, interstitial pneumonia associated with collagen vascular diseases, drug-induced pneumonitis, and sarcoidosis, as well as radiation pneumonitis, pneumoconiosis, asbestosis, and chronic hypersensitivity pneumonitis.

Hermansky-Pudlak syndrome is a recessive disorder

associated with pulmonary inflammation and fibrosis. Hermansky-Pudlak mice are susceptible to bleomycin-induced type II cell apoptosis and fibrosis (51). The incidence of epithelial cell apoptosis has been demonstrated using TUNEL method and electron microscopy in idiopathic pulmonary fibrosis (IPF) (52-54). The Fas-FasL mediated pathway and mitochondria-mediated apoptotic pathway are also activated in IPF (55) (Figure 2).

Bleomycin-induced pulmonary fibrosis is an animal model for lung injury and fibrosis. In this model, FasL mRNA is upregulated in infiltrating lymphocytes, and Fas is upregulated in bronchiolar and alveolar epithelial cells, in which excessive apoptosis is detected (56). The neutralization of FasL by Fas-Ig fusion protein or neutralizing anti-FasL antibody could prevent the development of this model, and Fas- or FasL-deficient mice are resistant to the induction of this model (57). The repeated inhalation of anti-Fas antibody mimicking Fas-FasL cross-linking induced excessive apoptosis of epithelial cells and inflammation, which resulted in pulmonary fibrosis in mice (5). Fas ligation induced not only apoptosis but also IL-8 expression *via* NF- κ B activation in bronchiolar epithelial cells *in vitro* (58). Expression and activation of caspase-1 and caspase-3 were detected in lung epithelial cells in bleomycin-induced pulmonary fibrosis in mice. A broad-caspase inhibitor Z-VAD.fmk attenuates bleomycin-induced pulmonary fibrosis in mice (59, 60). These results suggest that the Fas-mediated apoptotic pathway is essential in this model, and also suggest that inhibition of caspases may be a novel strategy against pulmonary fibrosis.

Recently, FasL molecules are reported to be expressed on

α -smooth muscle actin positive cells in mice with bleomycin-induced pulmonary fibrosis, and in humans with IPF (61). Myofibroblast cytotoxicity may cause epithelial cell death and inhibit reepithelialization in remodeling process. MMP-12 is essential for the fibrotic phenotype induced by Fas ligation in the lung. MMP-12 is required for the activation of the profibrotic genes *egr-1* and *cyr61* (62). To prevent the release of proapoptotic molecules from mesenchymal cells and epithelial cells may be effective against pulmonary fibrosis.

Upregulation of p53 and p21 in lung epithelial cells has been demonstrated in lung tissues from patients with IPF (52). The wild-type p53 normally acts to suppress cell growth while the cell attempts DNA repair. It also promotes apoptosis in those cells, which have irreparably damaged DNA or continue to proliferate (63, 64). Expression of p53 is upregulated in response to a variety of stresses. Apoptosis of type II alveolar epithelial cells is associated with upregulation of p53 and p21 expression in diffuse alveolar damage (9). DNA damage to alveolar epithelial cells occurs in response to bleomycin, and p53 and p21 were overexpressed within these cells (65, 66). Mice expressing dominant negative p53 in the lung epithelium have decreased induction of p21 expression, and impaired recovery from bleomycin-induced pneumopathy (67). p53 knockout mice present more severe inflammation and fibrosis after bleomycin instillation compared with wild-type mice (68). In addition, alveolar macrophage apoptosis and TNF- α secretion rather than p53 expression contribute to the difference in murine strain response to bleomycin (69). Whether p53 induces apoptosis or promotes repair in lung epithelial cells is likely to be tightly regulated by complex mechanisms including PUMA and NOXA within the cell.

p21 is induced in wild-type p53-containing cells following exposure to DNA-damaging agents. p21 inhibits cyclin-Cdk complex kinase activity and is a critical downstream effector in the p53-specific pathway of growth control in mammalian cells (70). p21 directly inhibits PCNA-dependent DNA replication in the absence of a cyclin/Cdk, but does not inhibit DNA repair (71). Forced p21 expression has been shown to have a protective effect against cell death caused by genotoxic stresses such as radiation or cytotoxic agents (72, 73). p21 enhanced survival either by promoting DNA repair or by modifying cell death caused by exposure to hyperoxia (74). The absence of p21 results in rapid necrotic alveolar cell death and mortality and also results in proliferating fibroblasts after oxidant injury (75). Adenovirus-mediated transfer of p21 gene to epithelial cells attenuates bleomycin-induced pulmonary fibrosis in mice (76). Interestingly, activation of caspase-3 is regulated by p21, and procaspase-3-p21 complex formation is an essential system for cell survival (77, 78). These findings suggest that p21 may be a key regulator of DNA replication and repair after lung injury and may be a promising molecule in the treatment of lung injury and fibrosis.

Angiotensin converting enzyme (ACE) levels in BALF and serum are increased in fibrosing lung diseases, including sarcoidosis, IPF, asbestosis, silicosis and ARDS. Angiotensin

II concentrations increase during radiation-induced pulmonary fibrosis (79). Angiotensin II and angiotensinogen induce apoptosis in alveolar epithelial cells *in vitro* (80). Furthermore, angiotensin II induces human lung fibroblast proliferation *in vitro* via activation of the angiotensin type I (AT1) receptor and the autocrine action of TGF- β (81). ACE inhibitors inhibit Fas- and TNF-induced apoptosis of human lung epithelial cells *in vitro* (82, 83), and also inhibit the accumulation of collagens and mast cells in the irradiated rat lung (84). The ACE inhibitor captopril ameliorates pulmonary fibrosis induced by monocrotaline in rats (85). Captopril also attenuates ventilator-induced lung injury in rats (86). The angiotensin receptor AT1 antagonist ameliorates apoptosis and pulmonary fibrosis induced by bleomycin (87). Angiotensinogen protein and mRNA are expressed in alveolar epithelial cells and myofibroblast in bleomycin-induced pulmonary fibrosis in mice and also in human with IPF (88). Additionally, intratracheal instillation of antisense oligonucleotide against angiotensinogen mRNA attenuates bleomycin-induced pulmonary fibrosis in rats (89). Angiotensin may be one of promising strategies against pulmonary fibrosis.

TGF- β is the most potent promoter of extra cellular matrix production, and also a strong chemotactic factor for monocytes and macrophages. In addition, TGF- β 1 can induce apoptosis directly in various cells (90-93). The mechanism of TGF- β 1-mediated apoptosis varies among cell types, although caspase activation, upregulation of p21, and downregulation of Bcl-xL expression are commonly observed (94-96). However, TGF- β 1 is a potent inducer of apoptosis through the caspase-3 activation and the downregulation of p21 and is also an enhancer of Fas-mediated apoptosis of lung epithelial cells (97). Semaphorin 7A and its receptors are induced by TGF- β 1 and play a central role in PI3K/PKB/AKT dependent pathway that contributes to TGF- β 1-induced apoptosis and remodeling (98). TGF- β 1 is a potent stimulator of Bax, Bid, and MMP-12. Bax, Bid and MMP-12 play key roles in the pathogenesis of TGF- β 1-induced fibrosis and apoptosis (99). TGF- β 1 overexpression in lung epithelial cells induced fibrosis in mice, in which a caspase inhibitor could attenuate apoptosis and fibrosis when it was administered from day 0 but not from day 5 after TGF- β 1 overexpression (99). These results indicate that TGF- β 1-induced epithelial cell apoptosis is critical early event in pulmonary fibrosis. This novel function of TGF- β 1 in apoptosis of lung epithelial cells should be considered in the treatment of lung injury and fibrosis.

HGF is known to act not only as a mitogen but also as a motogen or a morphogen for many kinds of epithelial cells. The receptor for HGF is the c-Met proto-oncogene product, which is predominantly expressed in various types of epithelial cells. As well as other epithelial cells, HGF promotes DNA synthesis in alveolar type II cells *in vitro* (100). A simultaneous or delayed administration of HGF equally represses apoptosis and pulmonary fibrosis in murine lung injury induced by bleomycin (101). HGF administration

may be a novel strategy in the effort to inhibit apoptosis and to promote repair processes in lung injury and fibrosis.

Bronchial asthma

Bronchial asthma is characterized by allergic airway inflammation, airway obstruction, hyperresponsiveness, desquamation of bronchial epithelial cells, thickening of basement membrane, and inflammatory cell infiltration to submucosal tissues. The loss of columnar epithelial cells is one characteristic feature of asthma. Asthmatic bronchial epithelium is susceptible to hydrogen peroxide (H_2O_2)-induced apoptosis (102). Cyclin-dependent kinase inhibitor p21 is overexpressed in bronchiolar epithelial cells of asthmatics, and its expression is upregulated by TGF- β and low concentration of H_2O_2 (103). It is possible that abnormal tissue damage and repair responses may be involved in airway remodeling. Mucous cell metaplasia is developed by differentiation of epithelial cells and maintained by Th2 milieu in asthmatics through inhibiting mucous cell apoptosis (104). T cells and eosinophils induce apoptosis in bronchiolar epithelial cells through interferon- α and TNF- α . Eosinophil cationic protein induces necrosis in bronchial epithelial cells (105). It is also suggested that corticosteroids may induce epithelial cell death in chronic asthma and animal models (106). Despite steroid therapy, patients with severe asthma develop progressive airway obstruction. In severe asthma, there is a greater level of apoptotic activity and increased cellular proliferation in the airway epithelium (107). Since the remodeling processes of bronchial epithelium are associated with various molecules which are involved in epithelial cell apoptosis, some patients with severe asthma may be resistant to steroid treatment. Novel treatments targeting epithelial cell apoptosis may be considered in these patients.

Peroxisome proliferator activated receptor γ (PPAR γ) is a nuclear hormone receptor involved in cell proliferation, differentiation and apoptosis (108). PPAR γ expression is upregulated in airway epithelium, bronchial submucosa, and smooth muscle cells, which is correlated with the decline of FEV₁ values. PPAR γ expression on epithelium correlates with the subepithelial membrane thickening. Corticosteroids downregulate PPAR γ expression on these cells, and decrease the thickening of subepithelial membrane and collagen deposition, and also increase the number of apoptotic cells in submucosa. In contrast, PPAR γ is produced by eosinophils, and its agonists inhibit IL-5-stimulated eosinophil survival (109). Additionally, PPAR γ agonists regulate human cultured airway smooth muscle proliferation (110). Therefore, the therapeutic modality of PPAR γ agonist in asthma should be examined more precisely.

The increase of number of smooth muscle cells in airway walls in asthma depends on the interaction of $\alpha v \beta 1$ integrin as a receptor on smooth muscle cells and extracellular matrix such as fibronectin, laminin, and collagens I and IV as anti-apoptotic factors (111). The proliferation and apoptosis of smooth muscle cells is one of promising targets for the

novel treatment of asthma.

Chronic obstructive pulmonary disease (COPD)

Increased numbers of apoptotic alveolar, bronchiolar and endothelial cells in lung tissues from patients with COPD were demonstrated in several reports. Particles, xenobiotics, and oxidants contained in cigarette smoke induce inflammation, oxidative stress, and protease activation. Protease-antiprotease imbalance and oxidative stress amplifies alveolar destruction (112). Since a large number of lymph follicles are found in lung tissues from patients with COPD, T-cell mediated immune response is also considered to have a role in inducing apoptosis (113). Additionally, impaired phagocytosis also called "efferocytosis" can cause increasing number of apoptotic cells. Defective efferocytosis leads to prolongation of inflammation. Therefore, apoptosis is involved in initial injury and defective repair in COPD (114).

Pulmonary emphysema is characterized by the enlargement of distal air spaces due to the destruction and loss of alveolar structures. Recently, endothelial and epithelial cell apoptosis have been implicated as one of important mechanisms of pulmonary emphysema. Intratracheal injection of activated caspase-3 induces epithelial cell apoptosis, enhances elastolytic activity, and subsequently induces emphysematous changes in mice (115). In addition, overexpression of α -1 antitrypsin attenuated endothelial cell death, alveolar wall destruction and oxidative stress caused by caspase-3 instillation (116). In TNF- α and IL-1 β receptor deficient mice, the degree of emphysematous changes and lung cell apoptosis are decreased after intratracheal instillation of elastase (117). Therefore, inflammation and protease activation accelerate epithelial, endothelial cell apoptosis and emphysema.

Cigarette smoke induces epithelial cell apoptosis, activated caspases, protease activation, and chemokines *via* IL-18R α -dependent manner (118). Cigarette smoke induces mitochondrial dysfunction by blocking mitochondrial respiratory chain, loss of ATP generation, which leads to cellular necrosis rather than apoptosis (119). HO-1 mRNA expression was elevated in the lungs of mice chronically exposed to cigarette smoke. The mitochondrial localization of HO-1 in bronchiolar epithelial cells was confirmed using electron microscopy. Overexpression of HO-1 inhibited cigarette smoke extract-induced cell death (120). As well as cigarette smoke, air pollution contain high levels of nitrogen oxygen species and reactive oxygen species, which activate different sphingomyelinases to induce apoptosis in airway epithelial cells (121).

Chronic treatment of rats with the vascular endothelial growth factor (VEGF) receptor blocker induces alveolar cell apoptosis, which subsequently leads to enlargement of the alveolar spaces without inflammatory cell infiltration or fibrosis (122). VEGF and VEGF receptor type II expressions are also decreased in lung tissues from patients with pulmonary emphysema compared with controls (123). Rats

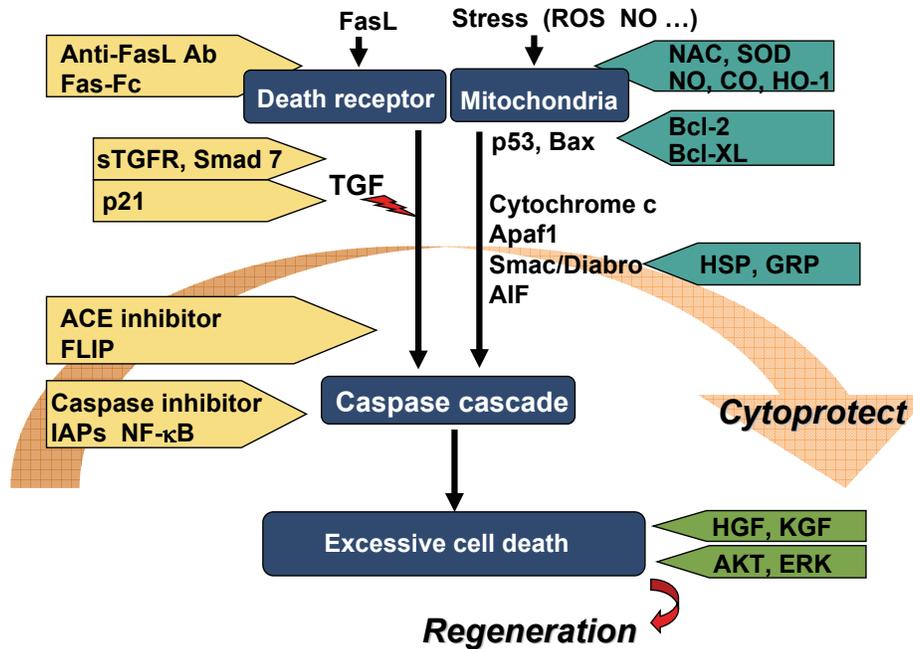


Figure 3. Cytoprotective strategies against epithelial cell death. Cytoprotective strategies against apoptosis-signaling molecules may be a promising strategy against lung diseases accompanied with remodeling.

treated with the VEGF receptor blocker show increased alveolar enlargement, alveolar septal cell apoptosis, and expression of oxidative cell markers (124). These results suggest that the defect of alveolar wall maintenance factors may lead to alveolar cell apoptosis.

While neutrophils are the predominant cells in the lung parenchyma of smokers without emphysema, CD8⁺ and CD8⁺ cells are predominant cells in the alveolar wall in smokers with emphysema. Furthermore, apoptosis in smokers is correlated with the amount of smoke (125). CD8⁺ T cells express perforin and granzyme B, which induce apoptosis. T-cells expressing perforin and granzyme B are increased in blood and BAL fluid from patients with COPD and smokers. There is a significant correlation between granzyme B expression and the number of apoptosis of bronchiolar epithelial cells (126). These results suggest that apoptosis mediated by T lymphocytes induced by smoking may be one of factors which induce alveolar wall destruction. Apoptotic cells should be removed rapidly by phagocytosis for the resolution of inflammation without damaging the tissues. Defective removal of apoptotic cells as well as more apoptosis is thought to be important as a contributor to COPD (127). Surfactant protein (SP)-D deficient mice accumulate apoptotic macrophages in the lung, and exogenous SP-D binds to apoptotic macrophages (128). These results suggest that SP-D may have an important role in the clearance of apoptotic cells, and have preventive effects on the development of emphysema.

In lung tissues from patients with emphysema, alveolar cell apoptosis and expression of PCNA in epithelium are increased, which suggests the activation of regenerative

processes (129). However, cigarette smoke extract induces oxidative stress and apoptosis not only epithelium but also lung fibroblast, and impairs repair processes (130). Apoptosis is involved in organogenesis. *Hoxa5* is required for embryonic respiratory tract morphogenesis. *Hoxa5*^{-/-} lungs present an emphysema-like morphology because of impaired alveologenesis, in which goblet cell mataplasia and elastic fiber abnormalities were observed (131). Since apoptosis is likely to be involved in not only the destructive phase but also remodeling process, regulating apoptosis may become an effective treatment against COPD.

Conclusion

Death receptors/ligands, death signals such as reactive oxygen species, nitrogen species, proinflammatory cytokines, and signaling molecules associated with mitochondria-mediated cell death are involved in remodeling process after lung injury. Promotion of inflammatory cell apoptosis and protection of parenchymal cells from cell death may be an effective therapeutic strategy against inflammatory lung diseases accompanied with fibrosis or destruction (Figure 3). Once parenchymal cells are damaged, accelerating the repair and regeneration in damaged tissues could also be an effective treatment. However, when parenchymal cells are severely damaged, rescue of these cells may be not sufficient to normal repair or lead to carcinogenesis. To avoid this problem, inhibiting apoptosis at early stage may be an effective strategy against devastating lung diseases accompanied with remodeling.

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