REVIEW

Apoptosis and lung injury

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Abstract. Apoptosis is important in developmental biology and in remodeling of tissues during repair. Apoptosis also plays important roles in the progression of many diseases. The cellular and molecular mechanisms of apoptosis, in general, have been extensively demonstrated. However, the causes and the roles of apoptosis of various cell types in the lung are not well understood. We have determined that adenosine/homocysteine causes lung vascular endothelial cell apoptosis by inhibition of carboxyl methylation of the small GTPase, Ras, through inhibition of isoprenylcysteine carboxyl methyltransferase (ICMT) activity, leading to inactivation of Ras and the subsequent disruption of focal adhesion complexes, resulting in cell-extracellular matrix detachment and anoikis. Apoptosis can either ameliorate or exacerbate lung injury, depending upon the cell type. Although apoptosis of polymorphonuclear leukocytes in the lung prevents inflammation and the development of acute respiratory distress syndrome during acute lung injury, Fas/FasL-mediated alveolar epithelial cell apoptosis promotes acute lung injury and pulmonary fibrosis. Lung epithelial and endothelial cell apoptosis also contributes to the development of emphysema. This article focuses on elucidating the mechanisms of adenosine/homocysteine-induced endothelial cell apoptosis. We also review the current understanding of the role of lung cell apoptosis in acute lung injury, pulmonary fibrosis and emphysema. (Keio J Med 54 (4): 184–189, December 2005)

Key words: apoptosis, adenosine/homocysteine, acute lung injury, pulmonary fibrosis, emphysema

Overview of Apoptosis

The term apoptosis, originally defined as 'falling off of leaves from trees', was first used scientifically to describe energy-dependent cell death by Kerr *et al.* in 1972.[1] Apoptosis or programmed cell death describes a genetically determined elimination of cells. The process is initiated by a death signal that tilts the balance between pro- and anti-apoptotic factors. Apoptotic cells undergo well ordered morphologic and molecular alterations, including cytoskeletal rearrangement, nuclear membrane collapse, chromatin condensation, DNA fragmentation, cell shrinkage, plasma membrane blebbing, and formation of apoptotic bodies.[1] In contrast, cells undergoing necrosis swell and lyse, thereby releasing intracellular contents into the interstitium leading to an inflammatory response.[2] Apoptosis is important in developmental biology, in remodeling of tissues during repair, and in the progression of some diseases.

Cellular and Molecular Basis of Apoptosis

Various factors, such as Fas ligand (FasL), tumor necrosis factor-α (TNF-α), metabolite deprivation, DNA damage, or hypoxia can trigger cell apoptosis. There are two fundamental signaling pathways, the extrinsic and intrinsic pathways, by which apoptosis is mediated, as described in Fig. 1. The extrinsic pathway is activated by external death ligands. The intrinsic pathway is triggered by internal apoptotic signals and involved in mitochondria. The two pathways merge and share mechanisms utilizing the aspartate-specific cysteinyl protease (caspase) cascades.

The caspase cascade is central to the progression of apoptosis. To date, 13 mammalian caspases have been

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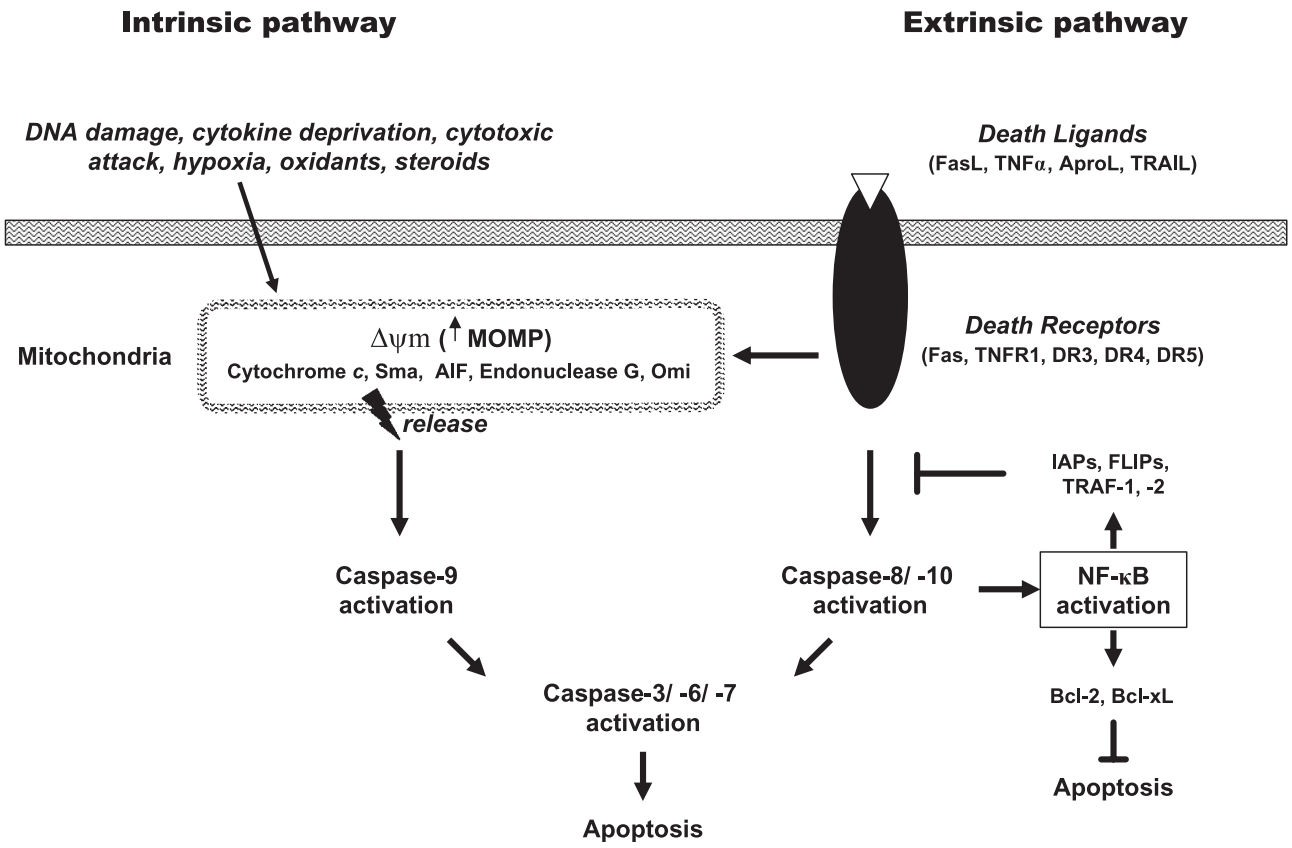

Fig. 1 Schematic representation of the cellular and molecular basis of apoptosis.

identified, which are classified into initiator caspases and effector caspases. Initiator caspases, caspase-8, -9, and -10, are activated by autoproteolysis in response to death signals and initiate apoptosis.[3,4] Effector caspases, caspase-3, -6, and -7, are activated by initiator caspases and cleave substrate proteins, culminating in cell death.[4] Over 100 caspase substrates have been identified; including inhibitor of caspase-activated DNase (ICAD), poly ADP-ribose polymerase (PARP), Bcl-2, lamin, and several cytoskeleton binding proteins. Cleavage of these proteins causes DNA fragmentation,[4] inhibition of DNA synthesis and repair,[5] nuclear membrane disruption and chromatin condensation,[6] and cytoskeleton collapse.[7–11]

The extrinsic pathway

Binding of specific ligand to cell surface or soluble receptor initiates the extrinsic pathway and apoptosis. The best characterized ligands and their corresponding death receptors include FasL/Fas, TNF-α/TNF receptor 1 (TNFR1), AproL/death receptor 3 (DR3) and Apo2L (TRAIL)/death receptor 4 and 5 (DR4, DR5).[12,13] Upon ligand binding, adapter proteins are recruited, resulting in association and activation of initiator caspases, which subsequently cleaves and activates effector caspases or activates Bid-dependent mitochondrial/intrinsic pathway[14,15] and apoptosis ensues.

The intrinsic pathway

Caspase activity is not necessary for apoptosis.[16] Mitochondria also play important roles in executing apoptosis. Exposure to stresses, such as cytotoxic drugs, oxidants, radiation, and growth factor deprivation, promotes mitochondrial outer membrane permeabilization (MOMP), resulting in mitochondrial release of several apoptogenic proteins to the cytosol; including cytochrome *c*, Smac, apoptosis-inducing factor (AIF), endonuclease G, and a serine protease called Omi.[17] The released cytochrome *c* rapidly binds to Apaf-1, leading to activation of caspase-9, with subsequent activation of caspase-3, -6, or -7, and culminating in apoptosis.[18,19] Released Smac and Omi activate effector caspases by removal of inhibitor of apoptosis proteins (IAPs).[20,21] AIF translocates to the nucleus and initiates chromatin condensation,[22] and endonuclease G contributes to DNA fragmentation by cleavage of genomic DNA.[23]

Regulation of apoptosis

Development and tissue homeostasis require a proper balance between survival and apoptotic signals. Anti-apoptotic Bcl-2 family members, such as Bcl-2, Bcl-x_L and Mcl-1 heterodimerize nonspecifically with pro-apoptotic Bcl-2 family members, such as Bax, Bak, Bim, and Bid, and sequester them in an inactive state in cytoplasm. This sequestration protects against apoptosis by preventing MOMP. Fas-associated death domain (FADD)-like inhibitory proteins (FLIPs) block apoptosis by competing with procaspase-8 for binding with adapter protein FADD.[24] In endothelial cells, down-regulation of c-FLIP is implicated in extracellular matrix detachment-induced anoikis.[25] IAPs prevent apoptosis by binding to effector caspases, rendering them inactive.[26] Death signaling-induced NF-κB activation promotes expression of various anti-apoptotic proteins, including Bcl-2, Bcl-x_L, FLIPs, IAP-1 and 2, TNFR-associated factor 1 and 2 to protect against apoptosis.[27–29]

Endothelial Cell Apoptosis

Endothelial cells form a monolayer lining the vasculature. Due to the positioning of the endothelium at the interface between the blood and surrounding tissue, the endothelium is exposed to multiple biochemical and biomechanical stresses, such as lipopolysaccharide (LPS), endotoxin, TNF-α, and oxidative stresses. One pathological consequence of stresses in the blood vessel is the induction of endothelial cell apoptosis. In this article, we review endothelial cell apoptosis caused by adenosine/homocysteine.

Increased levels of adenosine triphosphate (ATP) or adenosine may occur in blood vessels upon exocytotic release of nucleotides from stimulated platelet granules, during cytolytic release from necrotic cells, or from endothelial cell membrane transporters.[30] We have demonstrated that elevated levels of ATP or adenosine promote endothelial cell apoptosis *in vitro*.[31,32] Ectonucleotidase-mediated hydrolysis of ATP and the subsequent uptake of adenosine were necessary for the induction of endothelial cell apoptosis.[31] We have also shown that adenosine-induced endothelial cell apoptosis was exacerbated by homocysteine and mimicked by inhibitors of S-adenosyl-L-homocysteine hydrolase.[32] In addition, adenosine- and adenosine/homocysteine-induced apoptosis correlated with protein tyrosine phosphatase-dependent inhibition of p38 mitogen-activated protein kinase (p38 MAPK)[33] and degradation of focal adhesion kinase (FAK), paxillin, and $p130^{CAS}$ proteins, with subsequent disruption of focal adhesion complexes.[8] Importantly, overexpression of wild type FAK blunted adenosine/homocysteine-induced endothelial cell apoptosis, while over-expression of mutants of FAK, neither FAT nor FRNK, which lack the central catalytic domain, didn't protect against adenosine- or adenosine/homocysteine-induced apoptosis.[34] Mutation of the autophosphorylation site of FAK also failed to protect against apoptosis,[34] suggesting that kinase activity is required for FAK protection.

The small GTPases are a superfamily of monomeric regulatory GTP-binding proteins. The Ras protein was the first characterized and now is known to consist of 5 major classes – Ras, Rho, Rab, Arf, and RAN. Ras GTPases are molecular switches, in that they are activated when bound to GTP, and inactivated when bound to GDP. This process is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide dissociation inhibitors (GDIs). GEFs accelerate the exchange rate of bound GDP for GTP. GAPs enhance hydrolysis of bound GTP to GDP, whereas GDIs inhibit the exchange of bound GDP for GTP, inhibit GTP hydrolysis, and prevent membrane association.[35] Our studies have demonstrated that increased adenosine/homocysteine decreased Ras GTPase activity by inhibiting carboxyl methylation of this small GTPase through inhibition of isoprenylcysteine carboxyl methyltransferase (ICMT) activity.[36] Transient overexpression of wild type or dominant active H-Ras blunted adenosine/homocysteine-induced endothelial cell apoptosis,[36] suggesting that Ras GTPase methylation and subsequent activation play an important role in adenosine/homocysteine-induced endothelial apoptosis.

Thus, it is speculated that increased levels of adenosine and homocysteine promote endothelial cell apoptosis by multiple signaling pathways, resulting in disruption of cell-extracellular matrix interactions and anoikis, as described in Fig. 2.

Apoptosis and Lung Diseases

The lung is a complex organ which includes many different types of cells; including endothelial cells, epithelial cells, fibroblasts, and inflammatory cells. Apoptosis can either ameliorate or exacerbate lung injury, depending upon the cell type. This review article focuses on the effects of apoptosis on acute lung injury, pulmonary fibrosis, and emphysema.

Apoptosis and acute lung injury

PMN may infiltrate lung tissue in acute lung injury

Polymorphonuclear leukocytes (PMN) undergo apoptosis *in vitro*. Macrophages phagocytose apoptotic PMN. It has been suggested that enhanced PMN apop-

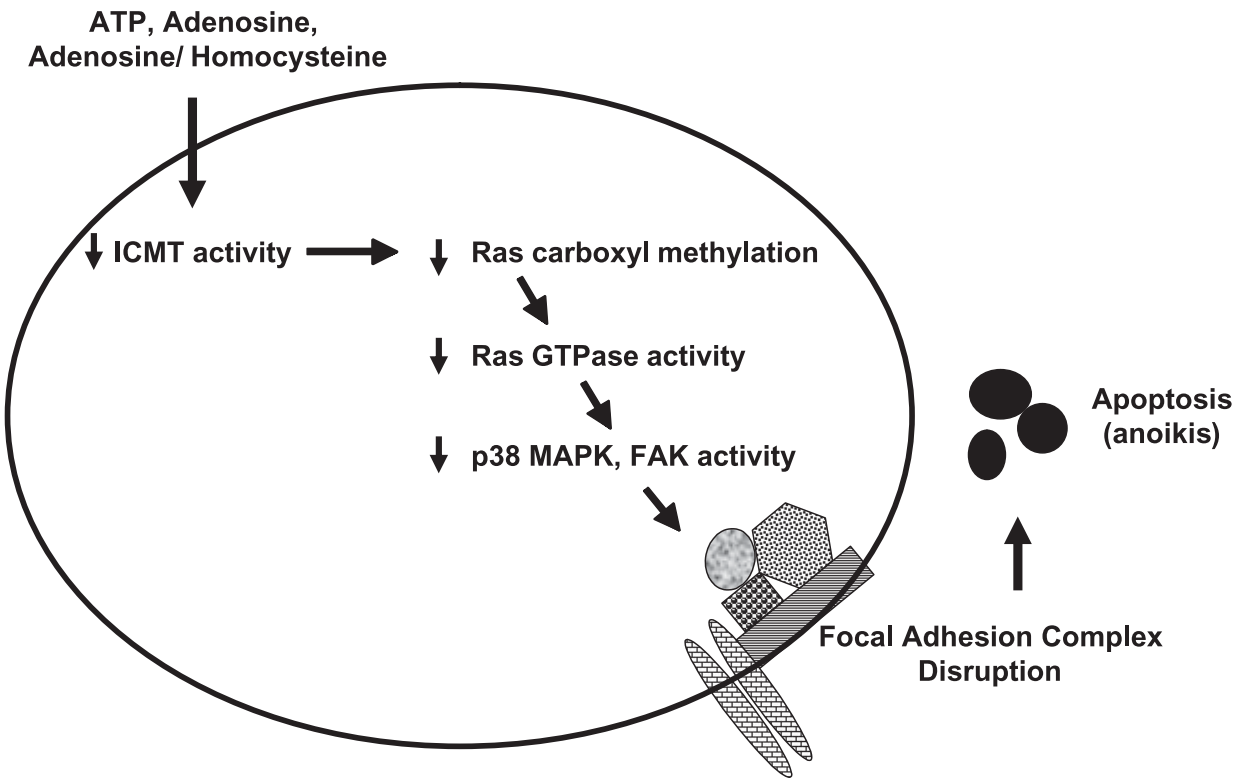

Fig. 2 Schematic representation of the signal mechanism of adenosine/homocysteine-induced endothelial apoptosis.

tosis may blunt inflammation.[37] The work of Matute-Bello and colleagues has extended this observation to acute lung injury. They found that there are fewer apoptotic PMN in bronchoalveolar lavage (BAL) of patients with acute respiratory distress syndrome (ARDS) or at risk of ARDS. Furthermore, BAL from ARDS patients decreases apoptosis and prolongs survival *in vitro* of normal human PMN, due to the presence of anti-apoptotic factors, such as GM-CSF.[38] These observations suggest that lack of PMN apoptosis may prolong inflammatory responses and predispose the patients to ARDS after acute lung injury.

Fas/FasL-mediated apoptosis causes acute lung injury

The Fas/FasL pathway is an important apoptosis-signaling system. Apoptosis mediated by Fas/FasL interaction has been implicated in acute lung injury (ALI) and ARDS. Fas is a 45-kDa type I membrane protein, while FasL is a 37-kDa type II protein. In the lung, Fas has been found on the surface of alveolar and bronchial epithelial cells, Clara cells, alveolar macrophages, and myofibroblasts, while FasL is expressed in neutrophils and lymphocytes. Alveolar epithelial injury in humans with ALI or ARDS is associated with local upregulation of the Fas/FasL system and activation of the apoptotic cascade in alveolar epithelial cells.[39] Recent studies show that Fas or FasL deficient mice have lesser degrees of acute lung injury compared with wild type mice when challenged with intrapulmonary deposition of IgG immune complexes.[40] Inhibition of caspase activity blunts PMN-induced acute lung injury in wild type mice.[40] These results suggest that Fas/FasL-induced apoptosis contributes to acute lung injury.

Fas ligand is cleaved by metalloproteinases to form a soluble form, referred to as sFasL. sFasL accumulates at sites of tissue inflammation and can initiate apoptosis of leukocytes, epithelial cells, and other lung cells. sFasL is increased in BAL and pulmonary tissue of patients with multisystem organ failure (MSOF)[39] and in BAL of patients with ARDS.[41] Importantly, BAL from patients with ARDS caused apoptosis of cultured lung epithelial cells, and this effect was inhibited by blocking the Fas/FasL system,[41] suggesting that sFasL can be released as a biologically active, death-inducing mediator capable of inducing epithelial apoptosis by interaction with Fas during acute lung injury. Mechanical ventilation with high tidal volumes of rabbits with ARDS causes renal and intestinal epithelial cell apoptosis and renal dysfunction, which correlated with increased plasma sFasL.[42] These studies suggest that sFasL also contributes to failure of other organs in MSOF.

Apoptosis and pulmonary fibrosis

Pulmonary fibrosis begins with alveolitis, which progresses to excess collagen deposition and destruction of normal lung architecture. Epithelial apoptosis and necrosis are increased in lungs of patients with idiopathic pulmonary fibrosis (IPF).[43] Apoptosis has been thought to be a non-inflammatory means of removing injurious cells thus facilitating lung repair. However, there is increasing evidence that Fas/FasL-mediated lung epithelial apoptosis induces release of proinflammatory cytokines (such as TNF-α and transforming growth factor-β1), leading to inflammation and progression from ARDS to fibrosis.[44] FasL was upregulated on inflammatory cells in BAL from patients with IPF.[45] Fas expression was also increased on alveolar and bronchiolar epithelial cells from patients with IPF.[45] sFasL was significantly enhanced in both serum and BAL from IPF patients.[46] Thus, we speculate that Fas/FasL-induced epithelial cell apoptosis may stimulate lung fibrosis.

Apoptosis and emphysema

Emphysema is characterized by loss of alveolar capillary septal tissue, resulting in impairment of gas exchange. The imbalance of proteases and anti-proteases caused by cigarette smoke is important in the pathogenesis of emphysema.[47] However, recent work suggests that apoptosis may also be important in the pathogenesis of emphysema. Emphysema lungs displayed apoptosis of both epithelial and endothelial cells and decreased expression of lung vascular endothelial growth factor (VEGF) and its receptor 2 (VEGF R2).[48] Overexpression of active caspase-3 by intratracheal instillation causes alveolar epithelial cell apoptosis and emphysema in rats.[49] These studies provide direct evidence that epithelial and/or endothelial apoptosis is important in the pathogenesis of emphysema. VEGF is abundantly expressed in normal lung and promotes endothelial cell proliferation. VEGF level is decreased in emphysema,[48] suggesting that lack of VEGF may result in apoptosis. Indeed, blockade of VEGF receptor causes apoptosis of alveolar epithelial and endothelial cells and emphysema *in vitro* and *in vivo*.[50–52] These studies have expanded the clinical concept of emphysema as a disease of protease-antiprotease imbalance to include the idea that programmed cell death could also be playing a role.

Summary

There are a variety of stimuli that may cause apoptosis via either the extrinsic or intrinsic pathways. Epithelial and/or endothelial apoptosis contributes to acute lung injury, pulmonary fibrosis, and emphysema. However, the mechanisms of endothelial apoptosis in human disease are not well understood and further study is needed. Inhibition of apoptosis in a cell-specific and vascular bed-specific manner may be potentially therapeutic for some diseases.

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