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Pulmonary Endothelial Cell Apoptosis in Emphysema and Acute Lung Injury

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Abstract

Apoptosis plays an essential role in homeostasis and pathogenesis of a variety of human diseases. Endothelial cells are exposed to various environmental and internal stress and endothelial apoptosis is a pathophysiological consequence of these stimuli. Pulmonary endothelial cell apoptosis initiates or contributes to progression of a number of lung diseases. This chapter will focus on the current understanding of the role of pulmonary endothelial cell apoptosis in the development of emphysema and acute lung injury (ALI) and the factors controlling pulmonary endothelial life and death.

Keywords

Pulmonary; Endothelial cells; Apoptosis; Necrosis; Necroptosis; ER stress; Unfolded protein response; Autophagy; Emphysema; Acute lung injury; ARDS; COPD

4.1. Overview of Cell Death

4.1.1. Apoptosis

Apoptosis is a term first used by Kerr et al. in 1972 to describe a genetically determined energy-dependent active form of programmed cellular suicide. Apoptosis is characterized by well-ordered morphologic and molecular features including: cell surface exposure of phosphatidylserine, plasma membrane blebbing, cell shrinkage, cytoskeletal rearrangement, collapse of nuclear membrane, chromatin condensation, DNA fragmentation, and formation of membrane bound fragments known as “apoptotic bodies” (Kerr et al. 1972). Cell surface-exposed phosphatidylserine acts as a chemoattractant for phagocytes to engulf and clear apoptotic bodies (Henson and Tuder 2008). Apoptosis serves to eliminate unwanted, aged, harmful, injured, or infected cells. Due to limited release of intracellular contents, minimal inflammation occurs (Savill et al. 2002). However, if ingestion of apoptotic bodies by monocytes, macrophages, and dendritic cells (efferocytosis) is impaired, inflammation and autoimmunity may be enhanced (Gaipl et al. 2006). Apoptosis plays an essential role in the maintenance of tissue homeostasis and embryonic development. Further, during embryonic

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development, the timing of apoptosis is genetically determined. Excessive or inadequate apoptosis can, however, contribute to the pathogenesis of a variety of human diseases. Apoptosis is triggered by external stressors (e.g., death ligands, ultraviolet, and γ radiation) and/or internal stimuli (e.g., oxidants, DNA damage, increased Ca^{2+}). Apoptosis is processed by two fundamental signaling pathways: the death receptor-mediated extrinsic pathway and the mitochondria-dependent intrinsic pathway (Olson and Kornbluth 2001; Thorburn 2004). Extrinsic pathway-activated caspase-8 can truncate and activate BID, thus activating the intrinsic pathway (Li et al. 1998). The details on regulation of apoptosis have been reviewed (Harrington et al. 2007; Subramanian and Steer 2010; Ola et al. 2011). Therapies targeting regulators of apoptosis have been used in preclinical and clinical trials for a variety of diseases including the treatment of cancers (Goldar et al. 2015).

4.1.2. Necrosis

Necrosis is a passive and caspase-independent cell death, characterized by cell swelling, mitochondrial degeneration, impaired ATP generation, lysosomal leakage, early rupture of plasma membranes, random fragmentation/degradation of DNA, and leakage of cellular contents into the surrounding environment (Henriquez et al. 2008). Necrosis is usually induced by nonspecific and non-physiological stress. Further, inhibition of caspases leads to necrosis (Henriquez et al. 2008). Due to release of potentially pro-inflammatory and pro-immunogenic cellular contents into surrounding tissues, necrosis often induces inflammation, autoimmune responses, and is often seen concomitant with apoptosis.

4.1.3. Necroptosis

Necroptosis describes a type of active, regulated, and programmed necrosis dependent upon the serine/threonine kinase activity of receptor-interacting protein kinase 1 and 3 (RIPK1/3) (Linkermann and Green 2014). Necroptosis and apoptosis share several upstream signaling elements including death receptors caspase 8 and FLIP. When caspase-8 is inhibited, RIPK1 is activated and forms an intracellular complex with RIPK3 to assemble the necrosome, leading to phosphorylation of mixed lineage kinase domain-like protein (MLKL) and ultimately cell death. Unlike apoptosis, necroptosis promotes harmful innate and adaptive immunologic responses by releasing damage associated molecular patterns (DAMPs). Thus, the reduction of necroptosis might be beneficial by minimizing the release of DAMPs and proinflammatory responses. Necroptosis is, however, a defense mechanism against invading microbes, including viral infections, and promotes the death and removal of virally infected cells. Therefore, blockade of necroptosis may increase susceptibility to viral infections particularly in patients with suppressed immunity. A number of inhibitors of necroptosis, such as necrostatin (specific inhibitor for RIPK1) and necrosulfonamide (specific inhibitor for human MLKL), have been described, providing potential therapeutic tools for treatment. Given the complex role of necroptosis, tissue and cell-specific targeting therapy is needed.

4.1.4. Endoplasmic Reticulum Stress-Induced Apoptosis

The endoplasmic reticulum (ER) is the site of posttranslational modifications and folding of secreted and membrane proteins. A variety of insults, such as ER Ca^{2+} chelators, reducing agents, glucose starvation, glycosylation antagonists, and protein mutations, can disrupt ER protein folding and lead to an accumulation of unfolded or misfolded proteins in the ER,

thus initiating ER stress (Schroder and Kaufman 2005). Cells respond to ER stress by the unfolded protein response (UPR). The UPR includes three arms: pancreatic ER kinase (PKR)-like ER kinase (PERK)/eukaryotic initiation factor 2α (eIF2α), transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1) (Schroder and Kaufman 2005). Through the UPR, cells attempt to restore ER homeostasis in order to maintain cell survival by inhibiting global protein synthesis (to reduce the loading of client protein to the ER for folding), enhancing ER protein folding capacity, and promoting ER-associated degradation of misfolded or unfolded proteins (Schroder and Kaufman 2005).

Prolonged ER stress causes cell death due to simultaneous activation of multiple apoptotic pathways by the UPR (Szegezdi et al. 2006). PERK-induced phosphorylation of eIF2α can lead to apoptosis by induction of pro-apoptotic transcription factor, C/EBP homologous protein (CHOP), which suppresses expression of anti-apoptotic protein, Bcl-2. Activated IRE1 activates c-Jun N-terminal kinase (JNK), which causes apoptosis by phosphorylation and thus inactivation of Bcl-2 and by phosphorylation and thus activation of pro-apoptotic protein, Bim. In addition, increased Ca^{2+} in the ER activates the death effector, Bax/Bak in the ER membrane, causing movement of Ca^{2+} from the ER to the mitochondria leading to mitochondrial-dependent apoptosis. ER membrane-localized caspase-12 (rodent) and caspase-4 (human) have also been implicated in ER-stress-induced apoptosis (Szegezdi et al. 2003; Kim et al. 2006). Caspase-12/-4 are cleaved and thus activated by the Ca^{2+}-dependent protease, m-calpain, by ER stress (Groenendyk and Michalak 2005). However, other studies have suggested that ER stress-induced apoptosis depends upon the apoptosome and not caspase-12/-4 (Obeng and Boise 2005; Di Sano et al. 2006).

Cell fate determination is not well understood when both survival (adaptive) and apoptotic pathways are simultaneously activated. It has been proposed that persistent ER stress causes apoptosis due to sustained induction of CHOP and instability of the adaptive pathway (Lin et al. 2007). It has also been suggested that cells survive mild ER stress because of the short half-life of pro-apoptotic proteins, compared to pro-survival proteins (Rutkowski et al. 2006). Robust prolonged ER stress causes apoptosis due to the induction of CHOP excessive to its degradation (Rutkowski et al. 2006).

4.1.5. Autophagy-Associated Cell Death

Autophagy is a dynamic and continuous process by which cells dispose of damaged or unneeded cellular proteins or organelles (mitochondria) by self-digestion to generate intracellular nutrients. During physiological conditions, autophagy is suppressed by mammalian target of rapamycin (mTOR), thus inhibiting the expression of autophagy-related genes (ATGs). Upon external or internal stress: including nutrient starvation, growth factor deprivation, hypoxia, ischemia, or mitochondrial aging, mTOR is inhibited thus initiating autophagy. Autophagy is a multistep sequential process, consisting of the formation of double-membrane vesicles that sequester unwanted cargo (proteins or mitochondria) in autophagosomes, fusion of autophagosomes with endosomes or lysosomes to form amphisomes or autolysosomes, and digestion of cargo by proteases (Hotchkiss et al. 2009; Choi et al. 2013). Autophagy is an evolutionarily conserved housekeeping process that allows recycling of damaged proteins and organelles in order to maintain homeostasis.

Impairment in any step of autophagy causes cellular nutrient deficiency and/or accumulation of damaged proteins and organelles leading to cell death (Hotchkiss et al. 2009). Whether autophagy promotes cell survival or death may depend on cell type and setting (Gustafsson and Gottlieb 2008).

4.1.6. Assessments of Cell Death

Based on the unique characteristics of different types of cell death, a variety of assays have been developed to assess the specific types of cell death in vivo and in vitro. Different types of cell death may share common characteristics at different stages of cell death; therefore, it is often necessary to use multiple assays to confirm cell death. The details on the assessments of cell death have been extensively reviewed (Harrington et al. 2007; Henson and Tuder 2008; Lu and Rounds 2009; Klionsky et al. 2016) and will not be discussed in this review.

4.2. Pulmonary Endothelial Cell Apoptosis

Balance of endothelial cell survival and death is crucial for angiogenesis, vessel regression, and barrier function. Due to the unique position of endothelial cells (EC) at the interface of circulating blood and surrounding tissues, EC may be exposed to various environmental stress including: hypoxia, hyperoxia, oxidants, lipopolysaccharide (LPS), and cigarette smoke (CS), or internal stress including: adenosine, ceramide, tumor necrosis factor (TNF)-α, and angiotensin II. Apoptosis is a pathophysiological consequence of these stimuli. However, a variety of biomechanical and biochemical factors are involved in the anti-apoptotic processes. For example, physiological levels of shear stress and cyclic strain, vascular endothelial growth factor (VEGF), focal adhesion kinase (FAK), activated protein C (APC), and sphingosine 1-phosphate (S1P) protect EC against apoptosis. The pro- and anti-apoptotic effects of these mediators have been reviewed (Harrington et al. 2007; Lu and Rounds 2009); therefore, this review will focus on the current understanding of endothelial pro-survival factors (VEGF and FAK) and apoptosis-inducing stress (adenosine, cigarette smoke, and LPS) in the lungs.

4.2.1. Vascular Endothelial Growth Factor

EC express abundant VEGF, which promotes EC survival and maintains normal alveolar structure (Voelkel et al. 2006). Expression of both VEGF and VEGF receptor type 2 (VEGFR2) are decreased in lung tissue of patients with chronic obstructive pulmonary disease (COPD) (Kasahara et al. 2001). This diminished VEGF/VEGFR2 signaling is inversely associated with increased lung EC apoptosis (Kasahara et al. 2001). However, lung-targeted inhibition of VEGF or VEGFR2 causes alveolar septal cell apoptosis in mice (Kasahara et al. 2000; Tang et al. 2004). Our group has also shown that blockade of VEGFR2 causes cultured pulmonary artery EC apoptosis in vitro (Lu 2008). These results indicate that VEGF signaling is essential for lung EC survival.

4.2.2. Focal Adhesion Kinase

EC are linked to the basement membrane through binding of cell surface expressed integrins to extracellular matrix (ECM) proteins at focal adhesion complexes (FAC) (Hynes 1992). As

anchorage-dependent cells, EC undergo detachment-initiated apoptosis, referred to as anoikis, upon loss of adhesion to underlying basement membrane. FAK, a non-receptor tyrosine kinase and an essential component of FAC, is activated upon integrin engagement of ECM (Guan et al. 1991; Guan and Shalloway 1992; Parsons 2003). FAK provides survival signaling for anchorage-dependent cells such as cultured fibroblasts (Hungerford et al. 1996). Similarly, EC isolated from FAK-null embryos undergo apoptosis (Ilic et al. 1995, 2003). Endothelium-specific deletion of FAK (Cre/FAK^{flox}) is embryonic lethal and causes EC apoptosis (Shen et al. 2005; Braren et al. 2006). Guan and colleagues (Guan et al. 1991; Guan and Shalloway 1992) have demonstrated that FAK tyrosine kinase activity is essential for FAK activity. FAK promotes cell survival by recruiting proteins containing SH2 domain including Src and phosphatidylinositol-3-kinase (PI3K) (Schaller et al. 1994). The activated PI3K recruits and activates Akt (Khwaja et al. 1997), which promotes cell survival via phosphorylation and thus inhibition of pro-apoptotic protein, Bad (Kennedy et al. 1997). FAK also promotes survival by activation of NF-κB and ERK signaling pathways (Huang et al. 2007). Additionally, FAK can translocate to the nucleus and inhibit p53 transcriptional activation and enhance p53 degradation, leading to protection against apoptosis (Ilic et al. 1998).

4.2.3. Adenosine

Adenosine is generated from adenosine-5′-triphosphate (ATP) and adenosine-5′-diphosphate (ADP) by extracellular ecto-5′-nucleotidases, CD39 and CD73, and is metabolized by adenosine deaminase (ADA). Extracellular adenosine exists in low concentrations (40–600 nM) under physiological conditions and is increased due to platelet degranulation, cell necrosis, activation of CD39 and/or CD73, or inhibition of ADA (Thompson et al. 2004; Eltzschig et al. 2006; Volmer et al. 2006; Eckle et al. 2007). Increased extracellular adenosine can interact with cell surface G-protein coupled adenosine receptors (ARs) (Feoktistov et al. 2002; Wyatt et al. 2002; Umapathy et al. 2010). Activation of adenosine receptors, specifically A_3-mediated signaling, has been shown to protect against apoptosis and tissue injury (Rivo et al. 2004; Chen et al. 2006; Matot et al. 2006).

However, sustained increased adenosine in ADA-deficient mice enhances alveolar cell apoptosis (Zhou et al. 2009). We have also shown that prolonged exposure to adenosine causes apoptosis of cultured lung EC (Lu et al. 2013). The injurious effect of adenosine is mediated by equilibriative nucleoside transporters. EC predominantly express equilibriative nucleoside transporter 1 (ENT_1) and ENT_2 (Archer et al. 2004). Upon sustained exposure, adenosine may be taken up into cells by ENTs. Further, similar to other G-protein coupled receptors, prolonged engagement of ARs causes receptor desensitization and internalization (Fredholm et al. 2001). This concept is supported by findings that sustained increased adenosine in ADA-deficient mice enhances alveolar cell apoptosis via a mechanism independent of adenosine receptor, $A_{2B}R$ (Zhou et al. 2009). In addition, sustained exposure to adenosine causes endothelial cell apoptosis; this effect is prevented by inhibition of $ENT_{1/2}$ however exacerbated by inhibition of either A_{2A} R or $A_{2B}R$ (Lu et al. 2013). These results are consistent with the concept that $ENT_{1/2}$-facilitated intracellular adenosine uptake and subsequent metabolism mediates adenosine-induced EC apoptosis, whereas AR-mediated signaling limits apoptosis (Simonis et al. 2009).

Once intracellular, adenosine reacts with homocysteine and generates S-adenosyl-L-homocysteine (SAH) by inhibition of SAH hydrolase (SAHH). SAH induces endothelial cell apoptosis independent of homocysteine (Sipkens et al. 2012). SAH is also a product of carboxyl methylation with S-adenosyl-L-methionine (SAM) as a methyl donor. We have demonstrated that exogenous adenosine causes lung EC apoptosis via increased ratio of intracellular SAH to SAM (Rounds et al. 1998). The increased ratio of SAH to SAM suppresses carboxyl methyltransferase activity. Isoprenylcysteine-O-carboxyl methyltransferase (ICMT) is a major methyltransferase for carboxyl methylation of small GTPase, Ras (Clarke 1992), which is a posttranslational modification essential for membrane localization and activation of Ras (Boivin and Beliveau 1995; Fleming et al. 1996; Kranenburg et al. 1997; Michaelson et al. 2001). We have shown that exogenous adenosine causes lung EC apoptosis in part by ICMT inhibition-mediated inhibition of Ras carboxyl methylation and activation (Kramer et al. 2003).

SAM is a precursor to glutathione (GSH) and is synthesized exclusively in the cytosol (Reytor et al. 2009) and also transported into mitochondria (Agrimi et al. 2004). Exogenous SAM has been shown to elevate GSH levels in vivo and prevent alcohol-induced mitochondrial oxidative stress and dysfunction as well as liver and lung injury in animal models (Holguin et al. 1998; Bailey et al. 2006; Cederbaum 2011). p38 is a redox-sensitive protein (Matsuzawa and Ichijo 2008). Reactive oxygen species (ROS)-mediated p38 activation has been implicated in extracellular ATP-induced macrophage apoptosis (Noguchi et al. 2008) and H_2O_2-induced EC apoptosis (Machino et al. 2003). Activation of p38 has also been implicated in homocysteine-induced apoptosis of endothelial progenitor cells (Bao et al. 2010) and cardiomyocytes (Wang et al. 2011). We have shown that sustained exposure to exogenous adenosine causes mitochondrial defects and endothelial apoptosis via mitochondrial oxidative stress-induced activation of p38 (Lu et al. 2012, 2013). Active p38 causes apoptosis by direct phosphorylation, and thus inhibition of Bcl-2 (De Chiara et al. 2006; Farley et al. 2006) and by increasing mitochondrial translocation of Bax (Capano and Crompton 2006). Future studies are needed to address whether sustained adenosine exposure reduces mitochondrial SAM, thus leading to mitochondrial oxidative stress via increased ratio of SAH to SAM in the cytosol.

In summary, adenosine displays seemingly paradoxical effects on lung EC life and death. Acute exposure protects EC against apoptosis via AR-mediated signaling, whereas prolonged exposure causes EC apoptosis via $ENT_{1/2}$-mediated intracellular adenosine uptake and subsequent metabolism and mitochondrial oxidative stress.

4.2.4. Cigarette Smoke

Lung EC apoptosis is significantly elevated in human smokers with emphysema (Kasahara et al. 2001) and mice with mild emphysema caused by CS exposure (Sakhatskyy et al. 2014). We (Sakhatskyy et al. 2014) and others (Tuder et al. 2000; Damico et al. 2011) have shown that CS extract (CSE) causes cultured lung macro- and microvascular EC apoptosis in vitro. The mechanisms underlying CS-induced lung EC apoptosis are rather complicated and involve FAK, p53, UPR, and autophagy.

FAK is a survival signal for anchorage-dependent cells (Hungerford et al. 1996). Tyrosine 397 phosphorylation of FAK is essential for its activation (Schaller et al. 1994). CSE decreases FAK phosphorylation at tyrosine-397 in an oxidative stress-dependent manner (Lu et al. 2011)—essential in CSE-induced EC apoptosis (Sakhatskyy et al. 2014). FAK also promotes cell survival via suppression of p53 (Ilic et al. 1998). Further, activation of p53 has contributed to CSE-induced pulmonary EC apoptosis (Damico et al. 2011). Thus, we speculate that CSE causes lung EC apoptosis via oxidative stress-mediated inhibition of FAK and subsequent activation of p53.

The UPR is an important mechanism of the elimination of ER stress and enhanced cell survival (Schroder and Kaufman 2005). The UPR is activated in lung tissue of smokers who do not have emphysema (Kelsen et al. 2008). The UPR is also activated by CSE in cultured human bronchial epithelial cells and 3T3 fibroblasts (Hengstermann and Müller 2008; Jorgensen et al. 2008) and cultured pulmonary EC (Sakhatskyy et al. 2014). Using mouse models of CS exposure, we have demonstrated a strong link between impairment of eIF2α signaling with lung EC apoptosis (Sakhatskyy et al. 2014). Future studies are necessary to determine if impaired eIF2α signaling contributes to lung EC apoptosis.

Autophagy is increased in response to deficiencies in extracellular and intracellular nutrients. Enhanced autophagy is observed in the lung tissue of smokers with emphysema (Chen et al. 2008). Autophagy is also activated by CSE exposure in lung epithelial cells and fibroblasts (Kim et al. 2008) as well as lung EC (Sakhatskyy et al. 2014). Increased autophagy has contributed to CS-induced alveolar epithelial cell apoptosis in mice (Chen et al. 2010). In contrast, increased autophagy has also been shown to protect against pulmonary endothelial cell apoptosis induced by cadmium, a component of cigarette smoke (Surolia et al. 2015). We have reported that autophagy was not altered in the lung tissue of a mouse strain susceptible to CS-induced lung EC apoptosis and emphysema (Sakhatskyy et al. 2014). The role of autophagy in CS-induced apoptosis may be dependent on cell types and stimuli.

Due to open structure and limited repair capacity, mitochondrial DNA is 50 times more sensitive to oxidative damage than nuclear DNA (Yakes and Van Houten 1997). Oxidative stress-induced mitochondrial DNA damage triggers mitochondrial dysfunction and apoptosis of lung EC (Ruchko et al. 2005). The role of mitochondrial DNA damage in CS-induced lung EC apoptosis remains to be studied.

4.2.5. Lipopolysaccharide

LPS, also known as lipoglycans or endotoxin, is a component of the outer envelope of gram-negative bacteria and elicits pro-inflammatory responses. It is well established that LPS-induced EC activation, dysfunction, and apoptosis play an important role in bacterial sepsis and endotoxemia. In the blood circulation, LPS binds to soluble CD14 via LPS-binding protein (LBP), followed by engagement of toll-like receptor (TLR)-4. This engagement results in the recruitment of adaptor, myeloid differentiation factor 88 (MyD88), and subsequent activation of interleukin (IL)-1 receptor associated kinase (IRAK)-1, TNF receptor associated (TRAF)-6, NF-kB, and MAPK pathways (Desch et al. 1989; Wang et al. 2001; Bannerman and Goldblum 2003).

NF-kB has been shown to transcriptionally upregulate anti-apoptotic genes such as IAP-1, IAP-2, and FLIP (LaCasse et al. 1998; Bannerman et al. 2004). However, suppression of NF-kB has minimal effect on LPS-induced EC apoptosis (Zen et al. 1999). This is due to FADD/MyD88-dependent negative regulation of LPS-induced NF-kB activation (Martin et al. 2005; Zhande et al. 2007); Fas is no longer able to activate MyD88, thus stimulating LPS/TLR4/NF-kB signaling (Martin et al. 2005). LPS also stimulates MyD88-independent signaling of endothelial apoptosis (Dauphinee and Karsan 2006). Heterotrimeric Gi/Go proteins play a role in LPS-induced TLR signaling independent of the MyD88-dependent pathway, leading to MAPK, Akt, and IFN activation of endothelial cells (Dauphinee et al. 2011). Whether LPS-induced stimulation of heterotrimeric G coupled proteins plays a role in EC apoptosis is unknown. LPS can activate the BID-dependent intrinsic pathway of apoptosis in lung EC (Wang et al. 2007). Conversely, LPS has been shown to upregulate mRNA of anti-apoptotic molecules, thus preventing EC apoptosis (Hu et al. 1998). LPS-induced intrinsic apoptosis and cytoprotection in disease states are not well understood and require further study.

4.3. Pulmonary EC Apoptosis in Lung Diseases

Apoptosis has been shown to ameliorate or exacerbate lung injury. Pulmonary EC apoptosis plays an important role in physiological processes including vasculogenesis and angiogenesis during lung development. Pulmonary EC apoptosis may also initiate or contribute to the progression of a number of lung diseases, as reviewed elsewhere (Harrington et al. 2007; Lu and Rounds 2009). In this review, we will focus on the role of pulmonary EC apoptosis in development of emphysema and Acute Lung Injury (ALI).

4.3.1. Emphysema

Chronic obstructive pulmonary disease (COPD), a progressive respiratory condition consisting of emphysema and chronic bronchitis, is the fourth leading cause of death worldwide and may become the third leading cause of death by 2030 based on prediction by the World Health Organization (Khaltaev 2005). The prevalence of COPD in the United States in 2013 was estimated to be 6.4% (15.7 million adults) (Wheaton et al. 2015). COPD is also an important contributor of mortality and disability in the United States (Murray et al. 2013). Further, COPD-related medical costs were estimated at $32 billion in the USA in 2010 with an additional $4 billion in costs due to absence from work (Ford et al. 2015). α1-antitrypsin (AAT) deficiency and other genetic predispositions contribute to the development of COPD (Sandford et al. 1997). However, tobacco smoke remains the leading cause of this devastating disease. Indoor air pollution (such as biomass fuel used for cooking and heating), outdoor air pollution, and occupational dusts and chemicals also increase the risk of COPD (Diette et al. 2012). Although the pathology of COPD has been well defined, the pathogenesis of the disease initiation and progression is not understood. Currently, there is no specific treatment available to reverse COPD.

Emphysema, a common and debilitating manifestation of COPD, is characterized by alveolar airspace enlargement, loss of alveolar capillary septa, and resultant impaired gas exchange. Several hypotheses have been proposed to explain alveolar wall damage in

emphysema. Protease/anti-protease imbalance has been accepted as a major mechanism for emphysematous lung destruction (Shapiro 1995, 1999; Shapiro et al. 2003; Taraseviciene-Stewart and Voelkel 2008). It is believed that neutrophil elastase and macrophage matrix metalloproteinases enzymatically degrade elastin in alveolar septa, leading to emphysema (Taraseviciene-Stewart and Voelkel 2008). This notion is supported by findings that patients with genetic deficiency of the anti-protease, AAT, develop emphysema (No Authors 1997).

Additionally, intra-tracheal instillation of proteases causes an emphysema phenotype in rats (Pastor et al. 2006). However, less than 5% of emphysema patients have AAT deficiency. Inflammatory cell infiltration is also seen in human emphysema. However, lung inflammation in pneumonia or acute lung injury does not usually result in emphysema. This suggests that inflammation may not be sufficient by itself for the development of emphysema. Oxidant stress and immunological injury also play a role in the pathogenesis of emphysema (Taraseviciene-Stewart and Voelkel 2008). Emerging evidence has highlighted a role of apoptosis, particularly EC apoptosis, in the initiation and progression of emphysema (Kasahara et al. 2000, 2001; Giordano et al. 2008).

Lung tissue from patients with emphysema displays increased apoptosis of both epithelial and endothelial cells in the alveolar septa (Kasahara et al. 2001; Imai et al. 2005). Bcl-2 single-nucleotide polymorphisms have been associated with severity of human emphysema (Sata et al. 2007). We have shown that lung EC apoptosis is elevated in a mouse model of emphysema induced by CS exposure (Sakhatskyy et al. 2014). Interestingly, induction of alveolar cell apoptosis by intratracheal instillation of the active caspase-3 causes emphysema in rats (Aoshiba et al. 2003). Additionally, inhibition of VEGF signaling causes alveolar septal cell apoptosis and emphysema in mice (Kasahara et al. 2000; Tang et al. 2004). Similarly, intra-tracheal instillation of C_{12} ceramide triggers alveolar endothelial and epithelial cell apoptosis and emphysema-like changes in mice (Petrache et al. 2005). Further, lung EC-targeted induction of apoptosis led to emphysema and enhanced oxidative stress and lung inflammation (Giordano et al. 2008). More importantly, inhibition of apoptosis using pan-caspase inhibitors prevented the emphysematous changes induced by either ceramide (Petrache et al. 2005) or blockage of VEGF signaling (Kasahara et al. 2000; Tang et al. 2004). These results support a central role of lung EC apoptosis in the development of emphysema. Anti-protease, AAT, inhibits CSE-induced pulmonary EC apoptosis in vitro by direct interaction with caspase-3 (Aldonyte et al. 2008). Overexpression of AAT also inhibits lung endothelial apoptosis and attenuates emphysema caused by either active caspase-3 or blockade of VEGF signaling (Petrache et al. 2006). These studies suggest that lung EC apoptosis is a critical step in the pathogenesis of emphysema.

Inhibition of FAK causes emphysema-like change in rat lungs (Mizuno et al. 2012). We have shown that CS exposure for 3 weeks enhanced pulmonary EC apoptosis and decreased FAK activity in mice susceptible to CS-induced emphysema (Sakhatskyy et al. 2014). Further studies are necessary to address whether reduced FAK activity contributes to CS-induced lung EC apoptosis and emphysema in humans in vivo. We have shown that CS exposure increases lung tissue adenosine levels in mice, an effect associated with lung EC apoptosis and early emphysema (Lu et al. 2013). Sustained increased adenosine in ADA-deficient mice also enhances alveolar cell apoptosis and causes emphysema in mice (Zhou et al.

2009). ADA expression and activity are reduced in the lung of smokers with COPD (Zhou et al. 2010). Whether chronically elevated adenosine contributes to CS-induced lung endothelial cell apoptosis and development of emphysema remains to be investigated.

Ceramide is upregulated in emphysematous lungs of patients and animal models, as well as in cultured pulmonary EC exposed to CSE (Petrache et al. 2005). This increase in ceramide is associated with enhanced alveolar cell apoptosis (Petrache et al. 2005). Interestingly, intratracheal instillation of C_{12} ceramide triggers airspace enlargement and apoptosis of alveolar EC and type II epithelial cells (Petrache et al. 2005). Further, inhibition of de novo ceramide synthesis significantly attenuated lung cell apoptosis and emphysema induced by VEGFR2 blockade (Petrache et al. 2005). These results suggest that ceramide is also an important mediator of alveolar cell apoptosis and emphysema (Petrache et al. 2005).

Only 10–15% of smokers develop emphysema. The mechanism underlying increased susceptibility to emphysema remains unclear. The UPR is elevated in the lungs of smokers without evidence of emphysema (Kelsen et al. 2008). Nrf2, a redox-sensitive, antioxidant transcription factor, is activated by eIF2α, a branch of UPR (Digaleh et al. 2013). Nrf2 knockout mice demonstrate enhanced susceptibility to cigarette smoke-induced emphysema in comparison to wild-type mice (Iizuka et al. 2005). We have shown that active eIF2α was significantly reduced in the lungs of AKR mice with mild emphysema induced by CS (Sakhatskyy et al. 2014). Future studies are needed to address whether Nrf2 is reduced in the lungs and whether inadequate induction of Nrf2 contributes to development of emphysema.

Autophagy is significantly increased in lung tissue of patients with COPD; the degree of autophagy positively correlates with the clinical severity of disease (Chen et al. 2008). Increased autophagy has contributed to CS-induced alveolar epithelial cell apoptosis and emphysema in mice (Chen et al. 2010; Mizumura et al. 2014). In contrast, increased autophagy protects against pulmonary endothelial cell apoptosis and emphysema induced by cadmium, a component of cigarette smoke (Surolia et al. 2015). We have reported that autophagy was not altered in lung tissue of a mouse strain with increased lung EC apoptosis and mild emphysema induced by CS (Sakhatskyy et al. 2014). Thus, the role of autophagy in regulating lung EC apoptosis and early onset of CS-induced emphysema needs further study.

4.3.2. Acute Lung Injury

ALI and its more severe form, acute respiratory distress syndrome (ARDS), are life-threatening disorders clinically characterized by severe hypoxemia and pulmonary bilateral infiltrates. In the United States, ARDS affects approximately 190,000 patients annually (Rubenfeld et al. 2005). ARDS accounts for 3.6 million associated hospital days (Rubenfeld et al. 2005; Adhikari et al. 2010). The global impact of ARDS has been difficult to assess due to varying definitions of the broad clinical phenotypes and limited data. Thus, ARDS remains an underreported disease of treated incidence, as opposed to actual incidence, in the undeveloped world (Buregeya et al. 2014). Although the mortality rate of ARDS has decreased to around 30–40% due to lung protective ventilation strategies (Amato et al. 1998; Villar et al. 2006), ARDS remains a deadly syndrome without a specific cure. Currently, there are no pharmacological interventions available to reduce the mortality of ARDS.

Sepsis, bacterial and viral pneumonia, and trauma remain the leading risk factors for the development of ARDS. Emerging evidence from epidemiologic studies, animal models, and cultured cell models have suggested that both active and passive cigarette smoke exposure modifies the susceptibility for development of ALI and ARDS (Iribarren et al. 2000; Calfee et al. 2011; Lu et al. 2011, 2013; Hsieh et al. 2014; Borgas et al. 2016).

The pathophysiology of ARDS is characterized by increased permeability of the alveolar-capillary barrier, influx of protein and inflammatory cell-rich fluid into the alveolar space, attenuated gas exchange between alveolar-capillary barrier, and dysregulated inflammation. Increased permeability of the microvascular endothelium and alveolar epithelium promotes edema formation, and this concept has been accepted as an important mechanism for the initiation of ARDS (Matthay et al. 2012). It is well established that polymorphonuclear cells (PMN) and immunological injury also play a significant role in the pathogenesis of ARDS (Perl et al. 2011). PMN accumulation is observed in the broncheoalveolar lavage fluid (BALF) (Pittet et al. 1997) and lung biopsies of early ARDS patients (Bachofen and Weibel 1977, 1982). Further, neutrophilia has been correlated with exacerbation of sepsis-induced ALI (Steinberg et al. 1994). However, ARDS may also develop in neutropenic patients, and neutrophil activation and migration may be observed in human lungs without injury (Martin et al. 1989; Downey et al. 1999). This suggests that inflammation may not be sufficient by itself for the development of ARDS.

Emerging evidence has suggested a role of pulmonary cell apoptosis in the initiation and progression of ARDS. The death receptor, Fas, and its ligand, FasL system, is an important death receptor-mediated extrinsic pathway of apoptosis. FasL is expressed and released by inflammatory cells, including neutrophils and lymphocytes, whereas Fas is expressed on the surface of lung EC, alveolar and bronchial epithelial cells, Clara cells, and alveolar macrophages. Fas and FasL are increased in pulmonary edema fluid and in lung tissue of patients with ARDS (Albertine et al. 2002). Silencing of Fas/FasL reduces lung cell apoptosis and ALI in a mouse model of sepsis (Perl et al. 2005, 2007). Soluble FasL (sFasL) is a cleaved form of FasL by metalloproteinases and is increased in BAL fluid of patients with ARDS (Matute-Bello et al. 1999). sFasL released from inflammatory cells is capable of inducing lung epithelial cell apoptosis (Matute-Bello et al. 1999). The role of Fas/FasL in lung EC apoptosis is not yet clear. Robust pulmonary endothelial cell apoptosis has been observed in patients with severe ARDS (Abadie et al. 2005) and in mice with ALI induced by LPS (Fujita et al. 1998). Sepsis-induced ARDS in mice indicates evidence for pulmonary microvascular endothelial cell death as a cause of barrier dysfunction and edema (Gill et al. 2014, 2015). Inhibition of apoptosis using a broad-spectrum caspase inhibitor prolonged survival of mice exposed to LPS (Kawasaki et al. 2000). Since apoptosis of alveolar endothelial, epithelial, and interstitial inflammatory cells occurs during ALI, future studies are needed to address the role of apoptosis of specific cells in initiation of ALI/ARDS.

Apoptosis has been thought of to be a non-inflammatory means of removing injurious cells, thus facilitating lung repair. However, there is increasing evidence indicating that Fas/FasL-mediated lung epithelial apoptosis results in release of pro-inflammatory cytokines (such as TNF-α and TGF-β1), leading to inflammation and progression from ARDS to fibrosis

(Chapman 1999). Whether pulmonary endothelial cell apoptosis occurs during initiation or progression of pulmonary fibrosis is unknown.

The role of necroptosis in development of ARDS is yet to be determined. Of interest, a recent study of blood transfusion-related acute lung injury indicates that banked red blood cell (RBC) transfusion enhances susceptibility to lung inflammation and ARDS in critically ill transfused patients and mice through necroptosis of lung EC and subsequent release of DAMPs (Qing et al. 2014).

4.4. Conclusions and Perspectives

Cell life and death are tightly regulated by survival signaling and death inducing programs. Pulmonary EC apoptosis significantly contributes to the development of emphysema and ALI/ARDS, as depicted in Fig. 4.1. Pan-caspase inhibitors have been used to inhibit lung cell apoptosis and prevent emphysema and ALI in animal models. However, use of such drugs to treat apoptosis-associated lung diseases may be problematic due to breakdown of tissue homeostasis and activation of necroptosis (Linkermann and Green 2014). The therapeutic potential of drugs that modulate cell death is dependent upon cell type-specific, tissue-specific, and vascular bed-specific actions. Thus, drugs acting locally and with cell type specificity are needed. Areas where research is needed include: (1) apoptosis susceptibility of different EC (conduit artery versus microvascular versus progenitor); (2) role of apoptosis of specific lung cells in initiation and/or progression of lung diseases; (3) role of necrosis and necroptosis in development of lung diseases, such as emphysema and ALI.

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List of Abbreviations

AAT	Alpha1-anti-trypsin
ADA	Adenosine deaminase
ADP	Adenosine-5′-diphosphate
APC	Activated protein C
ARDS	Acute respiratory distress syndrome
Ars	Adenosine receptors
ATF6	Transcription factor 6
ATGs	Autophagy-related genes

ATP	Adenosine-5′-triphosphate
BALF	Broncheoalveolar lavage fluid
CD39	ecto-5′-nucleotidase
CD73	ecto-5′-nucleotidase
CHOP	C/EBP homologous protein
COPD	Chronic obstructive pulmonary disease
CS	Cigarette smoke
CSE	Cigarette smoke extract
DAMPs	Damage associated molecular patterns
EC	Endothelial cells
ECM	Extracellular matrix
eIF2α	Eukaryotic initiation factor 2α
ENT1/2	Equilibrative nucleoside transporter 1/2
ER	Endoplasmic reticulum
FAC	Focal adhesion complexes
FAK	Focal adhesion kinase
GSH	Glutathione
ICMT	Isoprenylcysteine-O-carboxyl methyltransferase
IRAK-1	Interleukin (IL)-1 receptor associated kinase
IRE1	Inositol-requiring enzyme 1
JNK	c-Jun N-terminal kinase
LPS	Lipopolysaccharide
MLKL	Mixed lineage kinase domain-like protein
mTOR	Mammalian target of rapamycin
MyD88	Myeloid differentiation factor 88
PERK	Pancreatic ER kinase like ER kinase
RBC	Red blood cells
RIPK1/3	Receptor-interacting protein kinase 1 and 3
ROS	Reactive oxygen species

S1P	Sphingosine 1-phosphate
SAH	S-adenosyl-L-homocysteine
SAHH	S-adenosyl-L-homocysteine hydrolase
SAM	S-Adenosyl-L-Methionine
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-alpha
TRAF-6	TNF receptor associated factor-6
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
VEGFR2	VEGF receptor type 2

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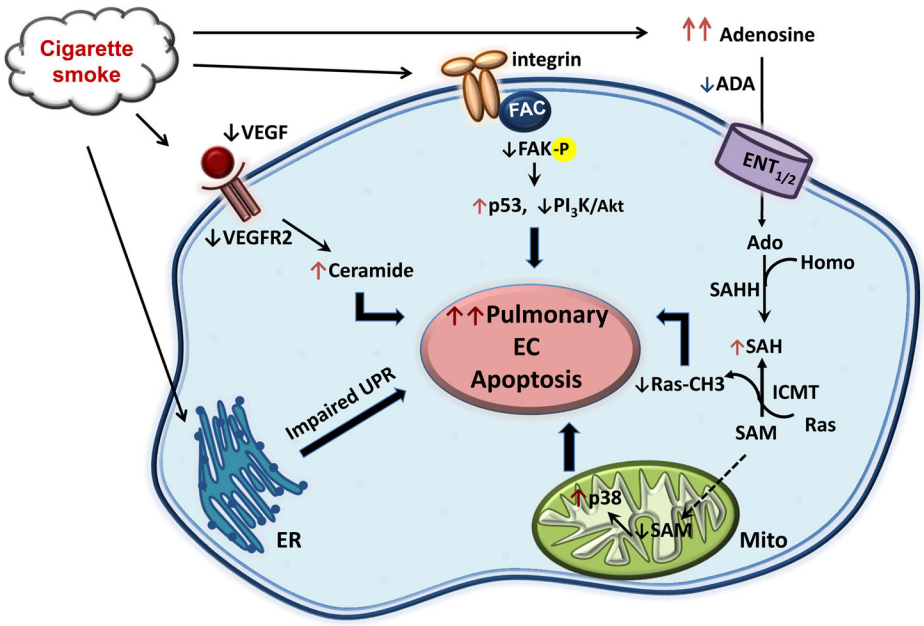

Fig. 4.1. Signaling pathways to CS-induced pulmonary endothelial cell apoptosis

Multiple signaling pathways are involved in CS-induced pulmonary endothelial cell apoptosis. (1) CS reduces VEGF/VEGFR2 signaling, leading to induction of ceramide and consequent apoptosis; (2) CS reduces FAK activation, leading to activation of p53 and inhibition of PI3K/Akt signaling, which results in apoptosis; (3) CS causes mitochondrial oxidative stress and mitochondrial dysfunction, leading to apoptosis; (4) CS elevates adenosine levels, leading to inactivation of Ras and mitochondrial oxidative stress, resulting in apoptosis; (5) CS impairs unfolded protein response, leading to apoptosis