Evolving Concepts of Apoptosis in Idiopathic Pulmonary Fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic, relentlessly progressive fibrosing disease of the lung of unknown etiology. Significant progress has been made in recent years in elucidating key aspects of the pathobiology of IPF. Insights into disease pathogenesis have come from studies of cell biology, growth factor/cytokine signaling, animal models of pulmonary fibrosis, and human IPF cells and tissue. A consistent finding in the ultrastructural pathology of IPF is alveolar epithelial cell injury and apoptosis. Another consistent finding in the histopathology of human IPF, described as usual interstitial pneumonia, is the accumulation of aggregates of myofibroblasts in fibroblastic foci. The extent or profusion of fibroblastic foci in lung biopsies is strongly correlated with increased mortality in patients with IPF. There is emerging evidence that myofibroblasts in IPF/usual interstitial pneumonia, both in the *in vivo* microenvironment and during the process of differentiation *in vitro*, acquire resistance to apoptosis. Here, we review the current evidence and mechanisms for this apparent "apoptosis paradox" in the pathogenesis of IPF.

Keywords

epithelial cells; fibroblasts; oxidative stress; transforming growth factor β; wound healing

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Orchestrated cell death is critical for maintenance of postnatal adult tissue homeostasis as it eliminates "abnormal" resident cells that may be irreparably damaged, infected, or genetically defective. In addition, during the resolution phase of tissue injury or infection, elimination of both recruited inflammatory cells and mesenchymal cells that participate in tissue repair functions is necessary for restoration of normal cellular homeostasis and the maintenance of tissue architecture and organ function. Thus, apoptosis and subsequent phagocytic clearance of inflammatory cells is required for the resolution of inflammation. Similarly, the orchestrated death and removal of reparative cells, such as myofibroblasts, is expected to be necessary for the normal resolution of tissue repair responses (1). Resistance to apoptosis of inflammatory cells may result in a chronic inflammatory process; impaired apoptosis of myofibroblasts may result in a persistent and dysregulated repair process that culminates in tissue fibrosis. Indeed, myofibroblast accumulation and activation are key features of the pathobiology of usual interstitial pneumonia (UIP), the pathologic correlate of idiopathic pulmonary fibrosis (IPF) (1,2). Exaggerated apoptosis of certain cell types can also result in deranged tissue homeostasis and contribute to disease pathogenesis (e.g., myocyte loss in cardiomyopathies and neuronal cell loss in chronic neurodegenerative diseases). Re-epithelialization is well recognized to be a prerequisite for epithelial regeneration during normal wound repair. Increased apoptosis of alveolar epithelial cells (AECs) may result in ineffective re-epithelialization of a damaged

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alveolar wall, thus promoting a fibrotic tissue-repair response. Interactions with inflammatory cells and endothelial cells may also occur and contribute to this fibrotic response (1); our discussion here is primarily focused on apoptotic phenotypes of alveolar/bronchiolar epithelial cells and fibroblasts/myofibroblasts and their interactions.

Three major types of cell death have been described—apoptosis, autophagy, and necrosis— that may all contribute to disease pathogenesis in different contexts. Apoptosis and autophagy are morphologically distinct forms of programmed cell death (type I and type II, respectively) that occur in the absence of an inflammatory response, typically associated with necrotic cell death. Apoptosis is distinguished from autophagy by the early loss of cytoskeletal elements, relative preservation of intracellular organelles (until late), and a central role for proteolytic enzymes known as caspases. Although much of accumulated data to date on pulmonary fibrosis relate to apoptotic cell death, it must be recognized that there is significant overlap between these different modes of cell death and clear-cut distinctions are not always evident and may not always be possible. Apoptosis regulation is likely to be important in a wide spectrum of injury and repair processes that constitute the idiopathic interstitial pneumonias (IIPs). The focus of this review will be on IPF/UIP, the most common of the IIPs; the extent to which regulation of apoptosis in specific lung cells differentiates IPF/UIP from other IIPs, such as nonspecific interstitial pneumonia (NSIP), is currently not known.

APOPTOSIS SIGNALING—AN OVERVIEW

Apoptosis is the classical (type I) form of programmed cell death that results in the dismantling of the entire cell within the context of membrane-enclosed vesicles that are recognized and engulfed by phagocytes; this prevents the release of intracellular components from dying cells and the activation of innate immune responses. Apoptosis is executed by the concerted action of a set of cysteine proteases known as caspases. Distinct pathways to apoptosis converge on the activation of effector caspases. Apoptosis can be induced by the stimulation of those members of the tumor necrosis factor–receptor (TNF-R) super family that have a death domain —this is mediated by the *extrinsic* pathway; alternatively, apoptosis may be induced by "internal" cellular stressors such as DNA damage or growth factor withdrawal—this is mediated by the *intrinsic* pathway.

An exhaustive review of the intrinsic and extrinsic pathways to cellular apoptosis is beyond the scope of this discussion (for recent reviews, *see* References 3–5). In brief, the extrinsic pathway is triggered by extracellular signaling "death" ligands such as Fas, which binds to the Fas receptor, CD95, a member of the TNF-R family. Receptor ligation results in multimerization/activation of the receptor that recruits the adaptor protein, Fas-associated death domain. This leads to the assembly of a death-inducing signaling complex that includes an initiator caspase, procaspase-8 or -10. Activated caspase-8 cleaves/activates the effector caspases, caspase-3 and -7, which execute the apoptotic cell death program. The intrinsic pathway is triggered by intracellular "stress" signals, such as DNA damage or starvation that typically activate the BH3-only (proapoptotic) members of the Bcl-2 family proteins. This induces mitochondrial release of cytochrome *c*, which directly activates apoptosis protease-activating factor 1 (Apaf-1) and forms the multimeric complex known as the "apoptosome." The apoptosome mediates the activation of the initiator caspase, caspase-9, which subsequently activates caspase-3 and -7, thus converging with the extrinsic pathway at the level of these effector caspases to execute apoptosis. Activated caspases are subject to negative regulation by the inhibitor of apoptosis (IAP) family of proteins, including the X-linked IAP (XIAP) (6). The antiapoptotic activities of IAP proteins are, in turn, counterregulated by the second mitochondria-derived activator of caspase (Smac/DIABLO), another protein that is released by mitochondria during apoptosis. Finally, convergence and crosstalk between the extrinsic and intrinsic pathways can also occur at an upstream level; this is typically mediated by Bid,

a BH3-only protein that is a physiologic target of activated caspase-8 (of the extrinsic pathway); activation of Bid can then lead to activation of the intrinsic (mitochondrial) pathway.

Loss of cell adhesion or adhesion signaling can lead to a form of apoptosis called anoikis. Anoikis is likely to represent an important apoptotic mechanism in structural cells, including epithelial cells and mesenchymal cells, which are normally adherent to the underlying basement membrane or other extracellular matrix (ECM) substrates. Mechanisms of anoikis have not been fully elucidated but may operate via different pathways in a cell-specific manner. Such mechanisms typically involve loss of "tonic" or constitutive adhesion signals—for example, focal adhesion kinase (FAK) and integrin-linked kinase (ILK). Detachment of cells from the ECM may also induce alterations in cell shape and actin/microtubular cytoskeleton reorganization that allow for translocation and activation of proapoptotic proteins (Bim, Bmf) of the Bcl-2 family (7,8). Thus, in many ways, anoikis is mechanistically akin to an intrinsic form of apoptosis regulation and induction.

SURVIVAL (ANTIAPOPTOTIC) SIGNALING—AN OVERVIEW

Cellular microenvironmental cues may also promote cell survival and the acquisition of an apoptosis-resistant phenotype. Acquisition of an antiapoptotic/prosurvival phenotype is classically mediated by extracellular signaling by soluble trophic factors as well as by signaling from cell–cell or cell–matrix adhesion complexes. Two major pathways for cell survival signaling have emerged: (1) the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (activated primarily by soluble growth factors) and (2) the integrin/FAK pathway (activated primarily by cell adhesion).

The PI3K–Akt Pathway

The PI3Ks constitute a diverse family primarily responsible for conveying growth and survival signals from activated growth factor/cytokine receptors (9,10). PI3K contains an 85-kD regulatory subunit that recruits the 110-kD catalytic subunit of PI3K into close proximity with the plasma membrane. Here, PI3K catalyzes the phosphorylation of inositol phospholipids, producing phosphoinositol (3–5)-triphosphate (PtdIns[3,4,5]P_3) (PIP$_3$). PIP$_3$ is a lipid mediator that recruits proteins that contain a pleckstrinhomology domain, such as Akt, and kinases that activate Akt, the PIP$_3$-dependent kinases, to the plasma membrane. This allows PIP$_3$-dependent kinase to phosphorylate and activate Akt. Phosphorylation of Akt is primarily mediated at two regulatory sites: a threonine (residue 308) and a serine (residue 473). Ser-473 phosphorylation parallels the activation of PI3K and is required for activation of Akt.

The PI3K–Akt pathway mediates prosurvival/antiapoptotic signaling by several pathways. Phosphorylation of Bad by Akt promotes survival by preventing Bad from binding and inhibiting antiapoptotic Bcl-X$_L$. Akt phosphorylates a member of the forkhead family of transcription factors, forkhead (*Drosophila*) homolog (rhabdomyosarcoma)-like 1 (FKHRL1), promoting its association with 14−3−3 proteins. This retains FKHRL1 in the cytoplasm and prevents the transcriptional activation of genes that promote apoptosis, including Fas ligand. Akt promotes the activation of nuclear factor (NF)-κB by phosphorylation/activation of IκB kinase α. This augments the transcriptional activity of p65 Rel, a subunit of NF-κB, and regulates the expression of genes important in cell survival. Akt also phosphorylates and inhibits the activity of caspase-9, a key initiator in the enzyme cascade that promotes cell death. Akt has recently been shown to phosphorylate XIAP, reducing its ubiquitination/degradation and thereby sustaining higher cellular levels of XIAP (11).

The Integrin–FAK Pathway

The ECM/integrin–FAK pathway may mediate prosurvival signals in addition to its roles in cellular proliferation, migration, and differentiation (12–14). Integrins are the major cell adhesion receptors for ECM ligands that mediate adhesion-dependent signaling in diverse biological processes, including cell proliferation, migration, and survival. Adhesion-dependent integrin activation recruits FAK and activates it at focal adhesions. FAK activation is mediated primarily by autophosphorylation of Tyr-397, which creates a binding site for the Src homology 2 (SH2) domain of Src and PI3K.

The ECM transmits survival signals to adherent cells; the absence of such signals elicits apoptosis or anoikis. Inhibition of FAK can induce apoptosis and overexpression of FAK can prevent anoikis and apoptosis induced in response to other stimuli. The mechanisms by which FAK mediates prosurvival signals are incompletely understood. Because FAK autophosphorylation at Tyr-397 appears to be necessary for protection against anoikis, FAK association with Src or PI3K is thought to be necessary.

APOPTOSIS OF AECS IN IPF

What Is the *In Vivo* Evidence for Apoptosis of AECs in IPF?

AEC injury and death are a consistent finding in human IPF/UIP. Early ultrastructural studies described damage to the epithelium with reactive epithelial cells (15–18). More recently, increased apoptosis of type II AECs has been demonstrated both in areas of IPF/UIP that appear histologically normal, without established fibrosis (19), and in epithelial cells overlying myofibroblasts (20). Increased expression of proapoptotic proteins and decreased expression of antiapoptotic proteins has also been reported (21). Thus, there is increasing evidence that at least certain subpopulations of AECs in IPF/UIP are undergoing apoptosis in association with inadequate or dysfunctional reparative responses of the alveolar and bronchiolar epithelium.

Is Apoptosis of AECs Sufficient to Induce Pulmonary Fibrosis?

Although AEC injury/apoptosis is evident in IPF/UIP, cause–effect relationships have not been clearly established. Data from animal models have provided some insights into whether excessive AEC apoptosis—as the primary event—may lead to lung fibrogenesis. Induction of AEC apoptosis by aerosolized anti-Fas (activating) antibody is associated with the development of pulmonary fibrosis in mice (22). Targeted transgenic overexpression of bioactive transforming growth factor (TGF)-β1 to the murine lung produces a transient wave of epithelial apoptosis that is followed by mononuclear-rich inflammation, tissue fibrosis, myofibroblast hyperplasia, and honeycombing (23). This study also demonstrated that a null mutation of early growth response gene (EGF)-1 or caspase inhibition rescues the fibrotic phenotype, suggesting a role for EGR-1 in TGF-β1–induced epithelial apoptosis. Moreover, the commonly used murine model of bleomycin-induced pulmonary fibrosis is characterized by early induction of reactive oxygen species (ROS)-dependent AEC apoptosis, which is inhibited by caspase inhibitors (24).

What Are the Cellular and Molecular Mechanisms for Injury/Apoptosis to AECs?

Despite the observation that AECs in IPF/UIP appear to be injured with high rates of apoptosis, the causative factors are not known and mechanisms of cell death remain unclear. Both the intrinsic (25) and extrinsic pathways (26–28) have been implicated (Figure 1). Type II AECs express high levels of p53 in association with DNA strand breaks in patients with IPF (21, 29). p53 is a key regulator of the checkpoint response that typically mediates cell cycle arrest, DNA repair, and apoptosis in response to DNA damage. The up-regulation of p53 expression may be further influenced by attenuated conjugation of p53 with Mdm2, which typically triggers p53 degradation (30). Oxidative stress has been proposed as a mechanism for the

alveolar epithelial cell injury in IPF (31,32). Evidence of oxidative DNA damage, specifically by the formation of 8-hydroxy-deoxyguanosine, and the expression of an enzyme that prevents this DNA modification are detected in AECs of patients with IIPs (33). The cellular sources for ROS generation that induce AEC injury/death in IPF have traditionally been thought to represent alveolar inflammatory cells (31,34); however, more recent studies suggest that structural cells of lung, in particular activated myofibroblasts, produce significant concentrations of extracellular ROS that is sufficient to induce injury/apoptosis of adjacent epithelial cells (35).

Epithelial–mesenchymal interactions are important in normal developmental organogenesis as well as in the repair/regeneration of postnatal adult tissues. Dysregulated communication between these cellular compartments may result in fibrotic tissue responses (1). Several pericellular factors may contribute to this dysregulated communication. AEC apoptosis/ necrosis adjacent to underlying α-smooth muscle actin–expressing myofibroblasts has been demonstrated (20); this effect may involve the secretion of angiotensin-II by myofibroblasts (36). In addition, myofibroblast-differentiated cells are potent generators of extracellular ROS, specifically hydrogen peroxide (37). Myofibroblasts isolated from IPF lung tissue, when activated by exogenous TGF-β1, secrete large amounts of hydrogen peroxide that may function in a paracrine manner to induce death of overlying lung epithelial cells (35). Moreover, TGF-β1 itself has been shown to be an enhancer of Fas-mediated AEC apoptosis (38). Thus, myofibroblast-secreted products (angiotensin-II, Fas, and ROS) may function, directly or indirectly, to induce apoptosis of adjacent AECs (Figure 1).

Are there other potential extrinsic or intrinsic factors that may induce AEC apoptosis in susceptible hosts? Environmental triggers such as latent viral infections have been proposed in the initiation or exacerbation of IPF (39). Viral hepatitis B and C have been linked to hepatocyte apoptosis with subsequent liver fibrosis (40). Moreover, viruses may induce or contribute to oxidative stress (41). Intrinsic factors specific to cellular metabolism and function, including protein folding and trafficking, may contribute to cell injury and apoptosis. An inherited mutation in surfactant protein C (SP-C), a type II AEC secreted protein, that predicts a glutamine to leucine residue appears to hinder processing of the precursor protein; this SP-C precursor protein displays aberrant subcellular localization with formation of numerous abnormal lamellar bodies and cellular atypia (42). Interestingly, mutations in the surfactant SP-C gene are associated with familial desquamative and nonspecific interstitial pneumonitis in children and UIP in adults. A similar defect in surfactant processing and secretion appears to result in "giant lamellar body degeneration" of type II AECs observed in the Hermansky-Pudlak syndrome, which is associated with mutations in the Hermansky-Pudlak syndrome-1 gene and interstitial pneumonia (43). The potential role of defects in protein processing and trafficking in sporadic cases of IIPs is unknown; these observations, nonetheless, reinforce the importance of gene–environment interactions in the expression of varied host tissue reactions and phenotypes.

SURVIVAL (APOPTOSIS RESISTANCE) OF MYOFIBROBLASTS IN IPF

What Is the *In Vivo* Evidence for Resistance to Apoptosis of Myofibroblasts in IPF?

There is currently no conclusive evidence that resistance to apoptosis is responsible for the persistence of myofibroblasts in fibroblastic foci of IPF/UIP. However, studies of human IPF/ UIP tissues demonstrating high rates of apoptosis and increased levels of apoptosis-related markers in AECs generally show relatively little evidence of apoptosis in adjacent fibroblasts/ myofibroblasts (20,21,44). Moreover, myofibroblasts in fibroblastic foci of IPF/UIP have been reported to undergo less apoptotic activity in comparison to myofibroblasts in the fibromyxoid lesions of bronchiolitis obliterans organizing pneumonia (BOOP) (45). Few studies have explored altered apoptosis/survival signaling in fibroblasts/myofibroblasts of human IPF. In

patients with persistent/nonresolving acute lung injury, a disorder that is often associated with fibroproliferative responses, constitutive activation of prosurvival signaling pathways and an antiapoptotic phenotype in alveolar mesenchymal cells have been demonstrated (46). This supports the concept that the acquisition of an apoptosis-resistant phenotype of mesenchymal cells, which participate in the lung injury/repair process, may contribute to persistent or progressive fibrosis.

Are Alterations in Mesenchymal Cells Sufficient to Induce Fibrogenic Tissue Responses?

A serendipitous finding in a transgenic animal model does suggest that genetic alterations in mesenchymal cells, at least in principle, might be sufficient to initiate and promote a fibrogenic tissue response. Denton and colleagues expressed a kinase-deficient type II TGF-β receptor (TβRIIΔk) in fibroblasts of transgenic mice, using a potent fibroblast-specific transcriptional enhancer within the far upstream region of the mouse pro-α2(I) collagen gene (47). This led to paradoxical ligand-dependent activation of downstream TGF-β signaling and the development of spontaneous skin and lung fibrosis (48). Subsequent analysis of the fibroblasts derived from these transgenic mice suggested activation of an autocrine TGF-β1 loop with low-level transgene expression, whereas a dominant-negative effect was observed only with high-level expression of the transgene (49). The "autoactivated" fibroblast phenotype was associated with heightened type I TGF-β receptor (ALK5) activity, myofibroblast differentiation and altered production of matrix metalloproteinases. These studies provide proof-of-concept that a mesenchymal cell–specific genetic alteration—in this case, sustained activation of TGF-βsignaling in myofibroblasts—is sufficient to promote, perpetuate, or even initiate the fibrotic tissue phenotype.

What Are the Cellular and Molecular Mechanisms for Apoptosis Resistance in Mesenchymal Cells?

Elimination of myofibroblasts by apoptosis is essential during normal cutaneous wound healing (50), a process that may be hindered in fibrotic disorders. The combined effects of reduced growth factor expression, increased extracellular matrix turnover, and nitric oxide generation were proposed as the mechanism for fibroblast apoptosis seen during remodeling of skin-flap-induced regression of granulation tissue (51). Moroever, strong correlation between declining levels of TGF-β1 expression, reduced myofibroblast presence, and histopathological improvement in fibrosis is observed later in the course of bleomycin-induced pulmonary fibrosis in rodents (52,53). Thus, altered or dysregulated cellular microenvironmental cues may permit the persistent survival of myofibroblasts in progressive fibrotic disorders.

The alveolar microenvironment in IPF/UIP is characterized by the persistent and high concentrations of fibrogenic cytokines/growth factors, ROS, and other reactive biomolecules in association with damaged basement membranes and altered extracellular matrices. Why mesenchymal cells survive while epithelial cells succumb to apoptosis within the same alveolar microenvironment is not entirely clear. Inherent differences between these cell types exist. Human lung fibroblasts have been shown to be inherently resistant to Fas-mediated apoptosis, an effect that is associated with increased expression of inhibitors of the apoptotic pathway, specifically XIAP and Fas-like interleukin (IL)-1β–converting enzyme-inhibitory protein (FLIP$_L$) (54) (Figure 2). In addition, differences between the senescent phenotypes of fibroblasts versus those of other cell types may help to explain this "paradox": human diploid fibroblasts enter a stable growth arrest phenotype at the end of their life span and are resistant to apoptosis, whereas senescent endothelial cells acquire a proapoptotic phenotype (55).

Fibroblasts/myofibroblasts isolated from lungs of patients with fibrotic lung disease show significant variability in proliferative and apoptotic properties when cultured in *ex vivo* conditions (56–59). It is difficult to draw definitive conclusions from these studies because the

in vivo microenvironment, composed of a complex three-dimensional matrix and soluble factors, cannot be precisely reproduced *ex vivo*.TGF-β1, a profibrotic growth factor that is expressed at high levels in IPF tissues, has been shown to protect myofibroblasts from IL-1 (60) and serum-deprivation–induced apoptosis (46) when cells are grown on rigid, two-dimensional substrates. In this context, the acquisition of an apoptosis-resistant phenotype of myofibroblasts induced by TGF-β1 is, at least in part, due to the autocrine secretion of a soluble growth factor that activates the PI3K–Akt pathway (46). Interestingly, a recent study by Kobayashi and colleagues showed that TGF-β1 stimulates apoptosis of fibroblasts cultured in a contractile three-dimensional collagen gel, whereas no induction of apoptosis was noted in gels that were constrained from contraction (61). TGF-β1–induced myofibroblast differentiation is also dependent on the resistance of the substrate to deformation (62), suggesting that biomechanical signaling is a key regulator of this phenotype. On rigid substrates, TGF-β1 is capable of inducing the activation of cell adhesion/integrin-dependent FAK, a pathway that is required for the differentiation of fibroblasts to myofibroblasts (14); thus, this TGF-β1–activated FAK pathway may integrate biochemical and biomechanical signals for induction of myofibroblast differentiation and survival (Figure 2). Cumulatively, these studies suggest that the cellular context, particularly the nature of ECM, may dictate divergent signaling/physiologic responses to the soluble growth factor/morphogen TGF-β1.

There is an urgent need to understand alterations in the biochemical and biomechanical properties of the ECM in progressive fibrotic diseases such as IPF/UIP. Few studies have addressed this question *in vivo*. In a model of myofibroblast differentiation *in vitro*, biochemical cross-linking reactions in the matrix overlying myofibroblasts are induced in the context of oxidative stress (63). Further studies are required to determine if such matrix modifications occurs *in vivo* and how they might alter biomechanical properties of and signaling from the ECM. A better understanding of how such signals are transduced to generate apoptotic and survival signals is also required. Tian and colleagues showed that fibroblasts transfected with constitutively active PI3K display increased Akt phosphorylation and are protected from anoikis and collagen gel contraction-induced apoptosis (64). Furthermore, integrin β1 may activate FAK signaling upstream of PI3K-Akt to transduce viability signals to fibroblasts within collagen matrices (65). Thus, there appears to be significant crosstalk between the FAK and Akt signaling pathways that promote fibroblast/myofibroblast survival.

Strategies that induce fibroblast/myofibroblast apoptosis may prove to be an effective approach to the treatment of fibrotic lung diseases (66). Induction of fibroblast apoptosis may be achieved by blockade of cell surface–associated CD44 activity (67). Recent studies from our laboratory demonstrate that activation of FAK and Akt in fibrotic foci correlates with the emergence and persistence of myofibroblasts after bleomycin-induced lung injury in mice; systemic administration of protein kinase inhibitor that inhibits the activity of FAK and Akt reduces the accumulation of myofibroblasts and abrogates fibrotic responses to lung injury (68). This study provides proof-of-concept that the *in vivo* modulation of prosurvival protein kinase signaling pathways, highly activated in apoptosis-resistant myofibroblasts, may represent an effective antifibrotic therapeutic strategy.

CONCLUSIONS

Accumulating evidence supports the emergence of an "apoptosis paradox" in IPF—apoptosis susceptibility in epithelial cells and apoptosis resistance in myofibroblasts. Although progress has been made, we have much to learn of the underlying mechanisms for this paradox. For example, the profibrotic cytokine/morphogen TGF-β1 mediates primarily proapoptotic effects on epithelial cells and antiapoptotic effects on myofibroblasts—despite ligand activation of presumably the same cell surface TGF-β receptor complex. Postreceptor signaling mechanisms in these different cell types need to be elucidated. Another contrasting feature is the

susceptibility of epithelial cells to ROS-induced injury and apoptosis whereas myofibroblasts appear to be relatively resistant to similar levels of oxidative stress. Epithelial cells that escape apoptosis appear to do so by adopting a mesenchymal cell fate—a process referred to as epithelial–mesenchymal transition (69); this likely further amplifies the fibrotic response and impedes alveolar re-epithelialization. Finally, epithelial–mesenchymal interactions may induce certain genetic or epigenetic alterations that permit the emergence of a fibroblast phenotype that is resistant to apoptosis (70). Deciphering the biochemical and molecular bases for differential apoptosis susceptibilities, cellular fate decisions, epigenetic changes, and the acquisition of apoptosis-resistant mesenchymal phenotypes will, ultimately, lead to more specific and targeted therapies for IPF.

Acknowledgment

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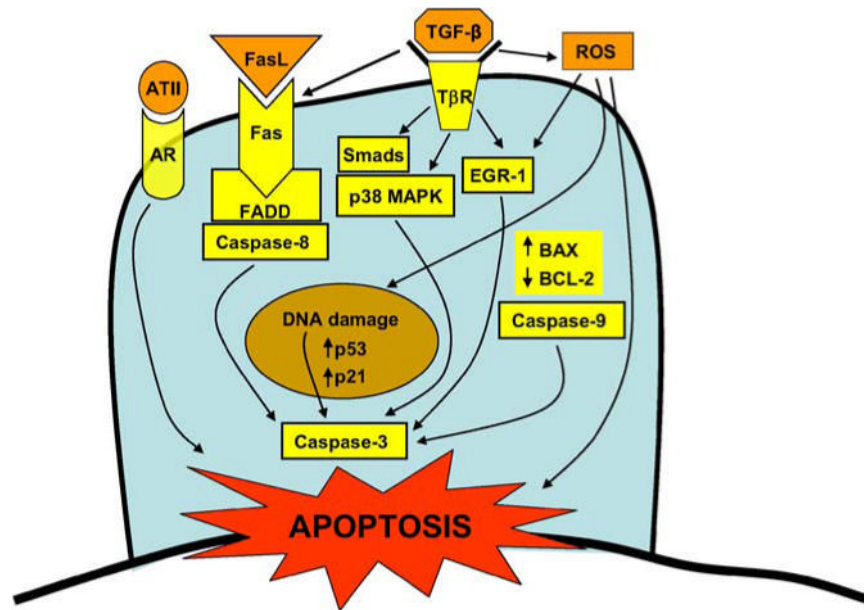
Figure 1.
Proposed proapoptotic mediators and pathways activated in alveolar epithelial cells of idiopathic pulmonary fibrosis (IPF). Additional proapoptotic mechanisms in epithelial cells that may need to be considered include latent viral infections and acquired defects in protein processing and trafficking (not depicted—*see* text for details). AR = angiotensin receptor (subtype I); ATII = angiotensin II; BAX = BCL-2–associated protein; BCL-2 = B-cell lymphoma 2; EGR-1 = early growth response protein-1; FADD = Fas-associated death domain; Fas = apoptosis-mediating surface antigen (aka Apo-1 and CD95); FasL = Fas ligand; p38 MAPK = p38 mitogen-activated protein kinase; ROS = reactive oxygen species; Smads = mothers against decapentaplegia in *Drosophila* (*Mad*) gene and the related *Sma* gene in *Caenorlabditis elegans*.
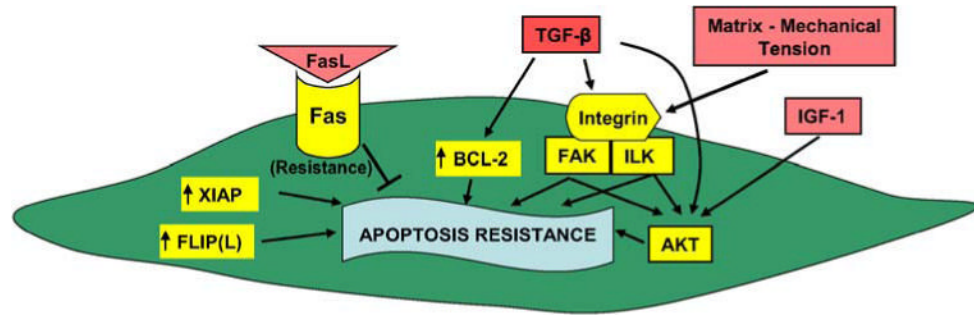
Figure 2.
Proposed antiapoptotic (survival) mediators and pathways activated in fibroblasts/ myofibroblasts of IPF. Akt = protein kinase B; FAK = focal adhesion kinase; FLIP(L) = Fas-like interleukin-1β–converting enzyme-inhibitory protein; IGF-1 = insulin growth factor-1; ILK = integrin-linked kinase; XIAP = X-linked inhibitor of apoptosis.