Chapter 13

Can Manipulation of Apoptotic Cell Death Benefit Tissue Scarring?

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Abstract

Cell death by apoptosis is an active process of cell removal that is initiated and regulated by activation of specific enzymes and signaling molecules. In contrast to necrotic cell death, apoptotic cell death holds the potential for therapeutic manipulation. Recent studies document important roles for apoptosis in both normal and pathological processes, ranging from embryonic development to tissue scarring. Apoptosis is thought to help in reduce inflammation by the selective removal of inflammatory cells, and help change fibroproliferative mass into an acellular scar tissue, by deleting cellular components. Our understanding on the mechanisms of cell-specific apoptosis during various pathophysiological processes show the opportunity to modulate the rate of apoptosis in a cell type-specific manner, to delay or suppress the progression of such immunoinflammatory diseases as pulmonary fibrosis and formations of pathologic scar tissue. We will briefly present the possible mechanisms of apoptotic cell death, and their impact on the formation of fibrotic mass.

Introduction

Tissue scarring is an active process which is closely involved in end-stage organ failure. Although advanced tissue scarring is believed to be mostly irreversible process, however, identification of important molecules that regulate crucial steps of fibrogenesis has provided an opportunity to delay or reverse the disease process. The nature of initiating events, and subsequent healing responses after an injury partly determine whether the outcome will be controlled wound healing, or uncontrolled tissue scarring. It is the persistent activation of fibrogenic molecules that differentiates uncontrolled tissue scarring from controlled wound healing. Therefore, regulating the healing response by exogenous manipulation of fibrogenic molecules in patients with scarring diseases tantalizing the prospect of modulating uncontrolled tissue scarring to a controlled healing.

Advanced-stage tissue scar is mostly composed of matrix proteins, with no (or very few) cellular components. Recent studies have documented the role of cell death in the pathogenesis of tissue scarring. The presence of dead cells during the early inflammatory phase of scarring diseases triggers the body's defensive responses, such as the release of additional cytokines, chemokines, and growth factors, resulting in a vicious cycle of inflammation, cell death, and scarring. During late-stage of wound healing, a decrease in matrix-producing cells is seen along with an increased rate of apoptosis. This mechanism has been implicated

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in evolution of granulation tissue into an acellular scar tissue.[1] However, when removal of cellular components from granulation tissue is not adequate, keloid and hypertrophic scars form, both of which are characterized by an increased degree of cellular components.[2]

Apoptosis has a determinant role in the clinical outcome of such human scarring diseases as atherosclerosis. Atherosclerotic plaques are delicate tissue, composed of smooth muscle cells, lymphocytes, macrophages, and matrix proteins. Apoptotic deletion of smooth muscle cells from atherosclerotic lesions has been linked to destabilizing plaque, which is then prone to rupture and form emboli, but apoptotic deletion of macrophages has been linked to plaque stabilization, and thus reducing the risk of plaque rupture.[3] In the following chapter, Kuwano and colleagues describe the role of apoptosis in pulmonary fibrosis, and present beneficial effects of modulation of apoptosis by targeting proapoptotic molecules in the experimental models. To provide readers with background information on different modes of cell death, we will review molecular mechanisms of cell death, emphasizing apoptosis.

Apoptosis

Apoptosis is a major form of cell death. This term was coined because the release of apoptotic bodies by dying cells resembled the picture of falling leaves from deciduous trees, called in Greek "apoptosis".[4] During apoptosis, cells induce their own death by chopping their DNA into small fragments after receiving either internal or external death signals. Apoptosis is a multistep, and multifactorial complex process, which occurs in a well-choreographed sequence. These morphological events begin with nuclear and cytoplasmic condensation and blebbing of the plasma membrane. Consequently, the cell breaks up into apoptotic bodies, which are membrane-bound fragments containing structurally intact organelles as well as portions of the nucleus. The apoptotic bodies are rapidly recognized by neighboring cells, then ingested and cleared by degradation.

Mechanisms of Apoptosis

The molecular basis for classical apoptosis is related to the activation of a family of intracellular cysteine proteases, termed caspases. Caspases are present in the cell in a latent state (pro-caspases), but are activated in response to a wide variety of cell death stimuli.[5] Caspases are organized in a cascade which contains the initiator (upstream) caspases (caspase 8 and 9) responsible for activating the effector (downstream) caspases to perform proteolytic cleavage. Once activated, the downstream caspases cleave specific protein substrates to induce apoptosis.[6-8] At present, at least two major pathways of caspase activation have been identified: (i) The mitochondria-mediated apoptosis pathway, in which cytochrome c is released and activates upstream caspase 9 in the presence of cytosolic protein Apaf-1[9] and, (ii) The receptor-mediated apoptosis pathway, in which the stimulation of death receptors activate upstream caspase 8.[10] Both pathways activate a major downstream effector caspase 3.[11] A third, less common, pathway of caspase activation involving granzyme B has also been demonstrated. It bypasses both the mitochondria-mediated and receptor-mediated pathways of caspase activation.[12] Granzyme B is a serine protease synthesized in cytotoxic T lymphocytes and stored in secretory granules. Once activated, the cytotoxic T cells secrete perforin, which induces pores in the membrane of the target cells. These pores will be used by the cytotoxic T cells to inject granzyme B into target cells. The enzymatic activity of granzyme B is crucial to its ability to induce apoptosis through the direct activation of caspases like the downstream caspase 3. In addition, it has been reported that granzyme B can induce apoptosis in a mitochondria-dependent mechanism via Bid-dependent[13] or independent[14] pathways. Bid is a proapoptotic member of the BH3-only family.

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Although the molecular basis of classical apoptosis is related to caspase activation, cell death with apoptotic features can also occur independent of caspase[15] through the release of apoptosis-inducing factor (AIF).[16] The two major apoptotic pathways, i.e., the intrinsic (mitochondrial) and the extrinsic (receptor-mediated) are described in the following sections.

The Intrinsic (Mitochondrial) Death Pathway

The mitochondria, which are known collectively as the powerhouse of the cell because it is the place where the oxidative phosphorylation and ATP synthesis take place, play a critical role in apoptosis. Various cell death pathways involve permeabilisation of mitochondrial membranes and release of mitochondrial proteins.[17] Two types of stimuli can induce the mitochondria to release proteins which activate apoptosis. These stimuli are internal, such as DNA damage, or external, e.g., cytotoxic drugs, heat-shock, hypoxia, growth-factor withdrawal, and irradiation. Several chemotherapeutic agents and anticancer drugs also act on mitochondria, although their exact mechanism of action is unclear.[18]

The intrinsic (mitochondrial) apoptotic pathway begins with the release of some mitochondrial proteins to the cytoplasm (Fig. 1). One protein is cytochrome c. The discovery that cytochrome c is released from mitochondria during cell death, as well as its role in triggering apoptosis, has had a dramatic impact on our understanding of the mechanism by which apoptosis is activated and regulated.[19] Cytochrome c is normally located in the space between the outer and inner membranes of mitochondria where it participates in the process of oxidative phosphorylation. Cytochrome c is released from the mitochondria into the cytosol in response to apoptotic stimuli. The released cytochrome c forms a complex with the cytosolic protein Apaf-1 and caspase 9 in the presence of either ATP or ADP. This complex induces autocatalytic activation of caspase 9, the first step in the proteolytic activation of caspase 3 and the other effector caspases 2, 6, 7 and 10, which are instrumental in causing cell death.[20,21] Although it is widely accepted that cytochrome c release is secondary to the loss of the mitochondrial membrane potential, cytochrome c release can also precede this loss.[22]

The detailed mechanisms of the mitochondrial membrane's permeabilization during apoptosis are not clear yet. Many competing models are trying to explain this mechanism.[23] In one model, mitochondrial swelling and rupture is induced by the opening of megachannel permeability transition pores which are formed by the adenine nucleotide translocator (ANT), located in the inner mitochondrial membrane, and the voltage-dependent anion channel (VDAC), located in the outer mitochondrial membrane. These pores span both the inner and the outer mitochondrial membranes where the two membranes are opposed. Bax, the pro-apoptotic Bcl-2 family member, binds to the ATN. This binding induces mitochondrial depolarization and increases the permeability of the inner membrane, which opens the permeability transition pores.[24] Consequently, water and solutes enter into the matrix, causing mitochondrial swelling. Another model proposes that mitochondrial swelling occurs as a consequence of defects in the mitochondrial ATP/ADP exchange induced by the closure of the VDACs, which causes hyperpolarization of the inner mitochondrial membrane and subsequent matrix swelling. On the other hand, at least in some cell types, the decline in membrane potential follows the release of cytochrome c, which contradicts the previous scenario.

Other models predict the formation of pores in the outer mitochondrial membrane, allowing the passage of cytochrome c and other mitochondrial proteins into the cytosol without damaging the membrane. Bax, in addition to its ability to bind to the ANT in the inner mitochondrial membrane, as mentioned above, forms large conducting channels in lipid planar bilayers.[23] Addition of Bax directly to isolated mitochondria induces the release of cytochrome c through a mechanism that is not blocked by permeability transition pore blockers and does not involve mitochondrial swelling. In addition, Bax cooperates with VDAC to form a cytochrome c-conducting channel.[25]

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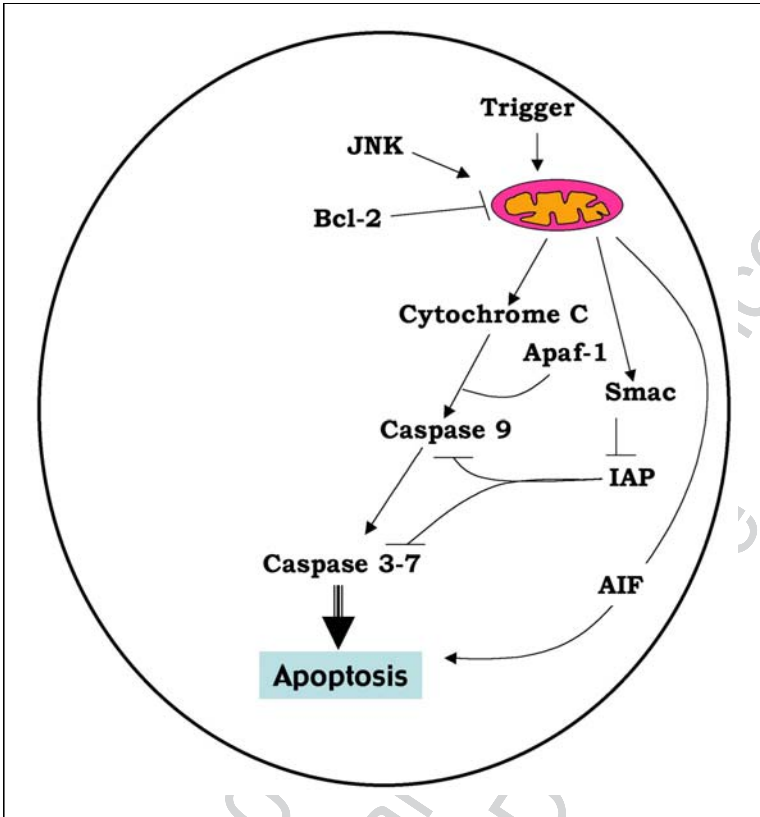

Figure 1. The intrinsic (mitochondrial) apoptotic pathway. Triggering the mitochondria induce the release of certain mitochondrial proteins. Cytochrome C release, with the interaction of Apaf-1, activates caspase 9, which activates caspase 3, among others, inducing apoptosis. Smac, another mitochondrial protein, is released in response to mitochondrial triggering to relieve the inhibitory effect of IAP on caspase activation. AIF release from the mitochondria induces apoptosis in a caspase-independent mechanism. Bcl-2 blocks apoptosis by inhibiting the release of mitochondrial proteins.

In addition to cytochrome c, several proteins are released from mitochondria in cells undergoing apoptosis. The recently identified Smac/Diablo molecule that binds to, and inactivates, the inhibitors of apoptosis proteins (IAPs), is an example of these proteins.[26,27] IAPs inhibit cell death by binding to procaspases and activated caspases, blocking their processing and activity. Smac/Diablo is released from the mitochondria along with cytochrome c during apoptosis and inactivates the IAPs, thus removing inhibition of caspase 9 activation.[28] Because Smac/Diablo is required for the inactivation of IAP by preventing the caspase 8-dependent cleavage and activation of caspase-3,[29] in some cells, Smac/Diablo is more crucial in induction of apoptosis than cytochrome c. Other proteins, in addition to cytochrome c and Smac/Diablo, which regulate caspase activation and apoptosis, are localized between the outer and the inner mitochondrial membrane. Caspase 9, caspase 3, caspase 2, and AIF, which induce caspase-independent apoptosis,[16] are examples of these proteins. These proteins can be released from the mitochondria to the cytosol during the induction of apoptosis.[30]

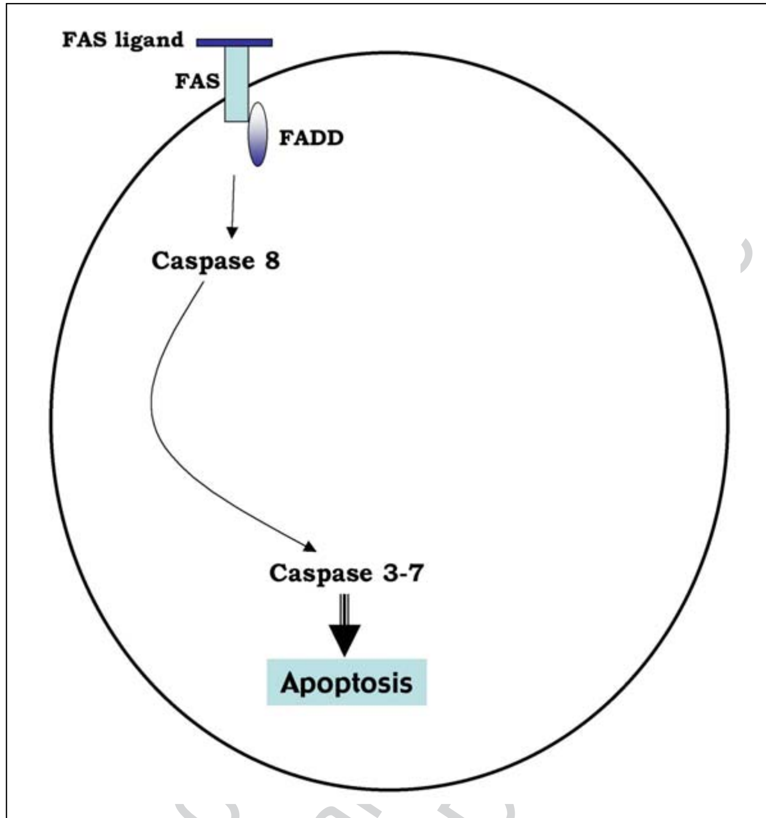

Figure 2. A proposed model for an extrinsic (cell receptor-mediated) apoptotic pathway. Binding of death ligand to a cell membrane receptor activates caspase 8. Activated caspase 8 stimulates downstream caspases like caspase 3, which induces apoptosis.

The Extrinsic (Receptor Mediated) Death Pathway

The other major mechanism of apoptosis induction is the receptor-mediated pathway which involves the activation of death cell receptors. Ligand binding stimulates downstream effectors (Fig. 2). The tumor necrosis factor (TNF) family of cytokine receptors, which differ in their ligand specificity, is an example of the death receptors. Upon ligand binding, the receptor is activated then it recruits and binds the death effector cell protein Fadd/Mort-1.[31] Fadd/Mort-1 binding recruits and cleaves procaspase.8[32] Cleavage of procaspase-8 induces the active caspase 8, which is released to the cytosol, where it cleaves and activates the downstream effector caspase.3[11]

Caspase 8 is not the only link between death receptors and induction of apoptosis. Activation of Fas can induce apoptosis by alternative pathways. For example, Fas receptors, via adapter proteins, activate the mitogen-activated protein 3 (MAP3) kinase, ASK1. When triggered, the proapoptotic ASK1 activates a phosphorylation cascade that culminates in the stimulation of c-Jun N terminal kinase (JNK).[31] Activated JNK phosphorylates c-Jun and p53, among other substrates, and induces apoptosis through modifying and transcriptionally regulating proteins in the Bcl-2 family. Activation of Fas can induce apoptosis through multiple pathways.[33]

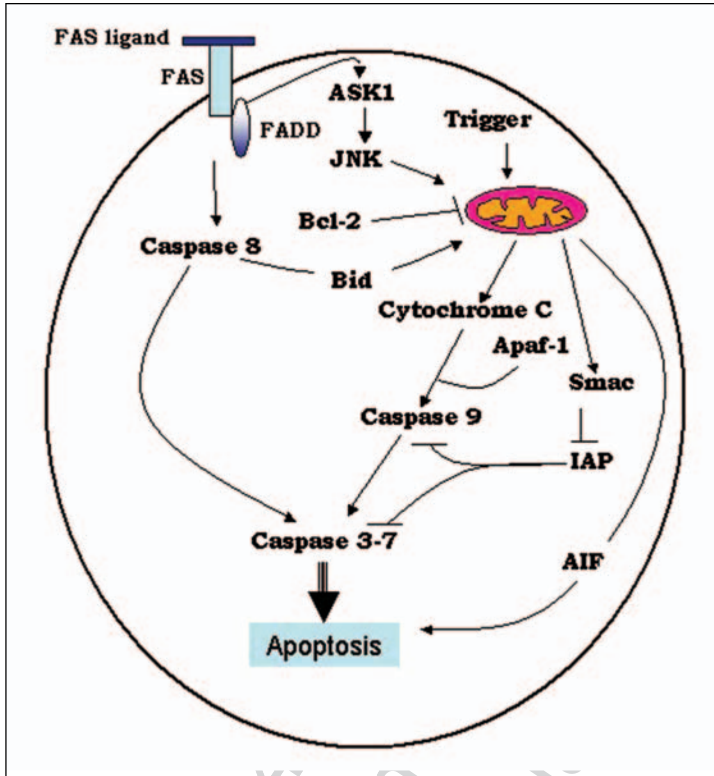

Figure 3. The cross talk between the intrinsic (mitochondrial) and the extrinsic (cell receptor-mediated) apoptotic pathways. One level of cross talk occurs at the level of Bid, where Bid cleavage by caspase 8 triggers the mitochondria to induce apoptosis. Another level of cross talk occurs when receptor mediated apoptotic pathway activates ASK 1. Active ASK 1 activates JNK, which stimulates the mitochondria to induce apoptosis. This figures also shows a different mechanism of inducing apoptosis, by the ability of granzyme B to directly activate caspase 3.

Cross Talk between Pathways

Despite the fact that mitochondrial-mediated and receptor-mediated pathways are separate, cross talk between them occurs (Fig. 3). Bid is an example of the cross talk between the two pathways. Bid is cleaved and activated by caspase 8. When cleaved, Bid activates the mitochondria-mediated apoptotic pathways via the interaction with Bax as well as ANT.[34] In addition, cleaved Bid forms ion channels in liposomes in vitro suggesting that it can directly disrupt the mitochondria and induce cytochrome c release.[35]

The mechanisms by which Bid rapidly and selectively targets the mitochondria are not known. Granzyme B, the cytotoxic T-cell specific serine protease, cleaves Bid and activates the mitochondrial pathway.[12] Granzyme B also affects mitochondria in a Bid-independent way resulting in mitochondrial depolarization and cell death, even though these mitochondria fail to release cytochrome c.[14]

Another level of cross talk is BAR, which inhibits apoptosis in both pathways. BAR contains a protein-interacting domain that binds and inhibits procaspase 8 and thereby blocks the receptor-mediated pathway. On the other hand, BAR inhibits the mitochondrial-mediated

pathway through an unknown mechanism that involves interactions with Bcl-2 and Bax.[36] ASK1 is another example of the cross talk between the two pathways. As mentioned before, stimulation of death receptors can induce activation of ASK1, which phosphorylates and activates the JNK pathway. JNK activation induces mitochondria-mediated cell death in some cell lines.

Bcl-2 Proteins and Apoptosis

The Bcl-2 gene family includes genes that either suppress or promote apoptosis.[37,38] The anti-apoptotic members Bcl-2 and Bcl-x_L reside in the outer mitochondrial membrane, where they suppress apoptosis either by blocking cytochrome c release or binding to Apaf-1 to prevent activation of caspase 9. On the other hand, the mammalian pro-apoptotic Bcl-2 family members, such as Bak, Bax, and Bik, may promote apoptosis by displacing Apaf-1 from Bcl-2 and Bcl-x_L.

Bcl-2, the prototype of the family, exerts an anti-apoptotic action. Bcl-2 over-expression in some cell lines protects them from apoptosis.[39,40] Conversely, inhibition of Bcl-2 expression increases apoptosis and sensitivity to chemotherapeutic agents.[41]

The final cell death decision either towards or against apoptosis could be a result of protein-protein interactions between Bcl-2 and other members of the Bcl-2 family. Heterodimerization of Bcl-2 and the proapoptotic Bax prevent Bax-mediated apoptosis.[42] Similar results have been reported between Bcl-2 and Bak, another member of the same family.[43] Bcl-2 phosphorylation at serine-70 and serine-87 results in inhibition of Bcl-2 dimidiation with Bax. Many microtubule-damaging agents can trigger this phosphorylation, which releases Bax from the inhibitory effect of Bcl-2.[44,45]

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Signal Transduction (MAP Kinase) Pathways and Apoptosis

During the last decade, considerable research has been focused on the role of signaling transduction pathways in cell proliferation, differentiation, and survival. mitogen-activated protein kinase (MAPK) represents one of the most extensively studied pathways (Fig. 4).[46]

The MAPK family comprises c-Jun N-terminal kinase (JNK), p38 MAPK, and extracellular signal-regulated kinase (ERK). The ERK pathway is activated by such stimuli as growth factors,[47] lipopolysaccharide,[48] and chemotherapeutic agents.[49] ERK activation exerts in most cases an antiapoptotic,[50,51] and less commonly proapoptotic[49,52] influence, depending upon the cellular context and by yet unclarified regulatory mechanisms. The JNK and p38 MAPK signaling pathways are also activated by various and overlapping stimuli, such as heat or osmotic shock, radiation, and growth factors.[47,53] The JNK and p38 MAPK signaling pathways are associated, with few exceptions, with enhanced activation of the apoptotic pathway.[54]

The JNK family was first described as stress kinases, and their response to cellular stress has been studied extensively.[55] The JNK family consists of JNK1 and JNK2, which are widely distributed, and of JNK3, which is primarily expressed in the heart, brain and testis. Differential splicing yields a total of 10 different isoforms.[56] After activation, JNKs phosphorylate various substrates in the nucleus, e.g., c-Jun or activating transcription factor-2 (ATF-2) and in the cytoplasm, e.g., Bcl-2.[57]

PI3K/AKT Pathway and Apoptosis

An important pathway downstream of the receptor tyrosine kinase (RTK), in many cases via RAS, involves the phosphatidylinositol 3-kinase (PI3K) cascade. This pathway is a major regulator of mammalian cell proliferation and survival became apparent when it was described that PI3Ks activity was physically and functionally associated with the transforming activity of viral oncogenes.[58]

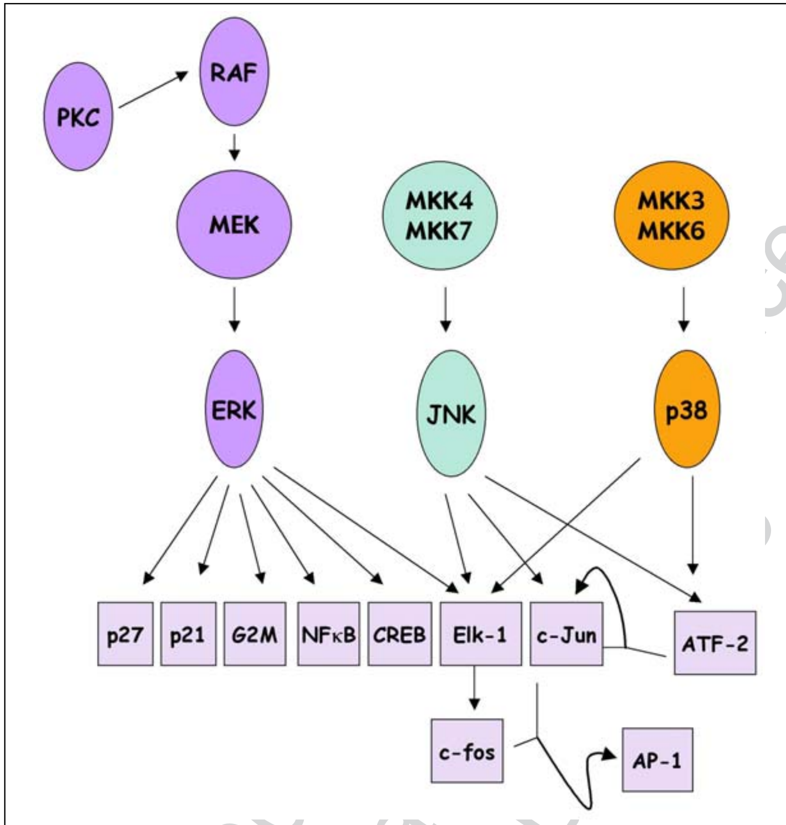

Figure 4. Different MAP kinases pathways: ERK, JNK, and p38 pathways, with the possible cross talk between them at the level of downstream targets.

In addition to their roles in signaling pathways, PI3Ks also control many important cell responses.[59,60] Members of the PI3K family can also be considered oncogenes because they control cell cycle progression, differentiation, survival, invasion and metastasis, and angiogenesis.[61] Several downstream targets for PI3Ks have been detected, and many biological effects of PI3Ks are mediated through the activation of the downstream target AKT/protein kinase B (PKB).[62]

At present, three members of the AKT family have been identified and are termed AKT1, AKT2, and AKT3. They are closely related, exhibiting more than 80% sequence homology, although they are products of different genes.[63] After exposure to hormones, growth factors, or cytokines, inactive (cytosolic) AKT is translocated to the plasma membrane by the products of PI3K, PIP_2, and PIP_3, where it is phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1).

Many AKT substrates have been identified such as BAD, CREB, procaspase 9, $p21^{WAF1}$, members of the forkhead family of transcriptions factor, Iκ-B kinase, GSK-3- kinase, and mTOR/FRAP.[64] The large number of downstream targets of AKT explains why this kinase is rapidly emerging as a key mediator of cell proliferation, differentiation, and survival, and an inhibitor of apoptosis (Fig. 5). AKT stimulates many anti-apoptotic proteins like NFκB, and

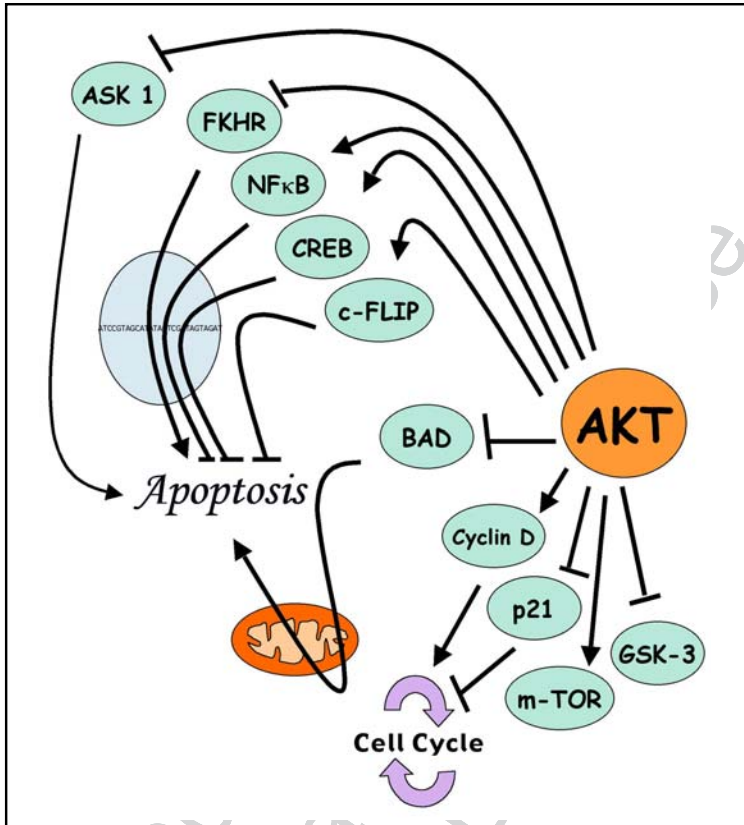

Figure 5. The schematic illustration of the large number of downstream targets of AKT and the roles that they play in cell proliferation, differentiation, and survival. AKT stimulates many anti-apoptotic proteins like NFκB, and c-FLIP. In addition, it inhibits many pro-apoptotic proteins such as BAD and ASKI. AKT also stimulates cyclin D, which is needed for the cell cycle and inhibits p21, the cyclin-dependent kinase inhibitor that blocks the cell cycle. The overall effects of AKT are to antagonize apoptosis and stimulate cell survival.

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Other Modes of Cell Death

Necrosis

Necrosis represents another form of cell death, which occurs as a consequence of a cell exposure to toxic stimuli such as hyperthermia, hypoxia, ischemia, complement attack, metabolic poisons, and direct cell trauma. Necrosis is characterized by irreversible swelling of the cytoplasm

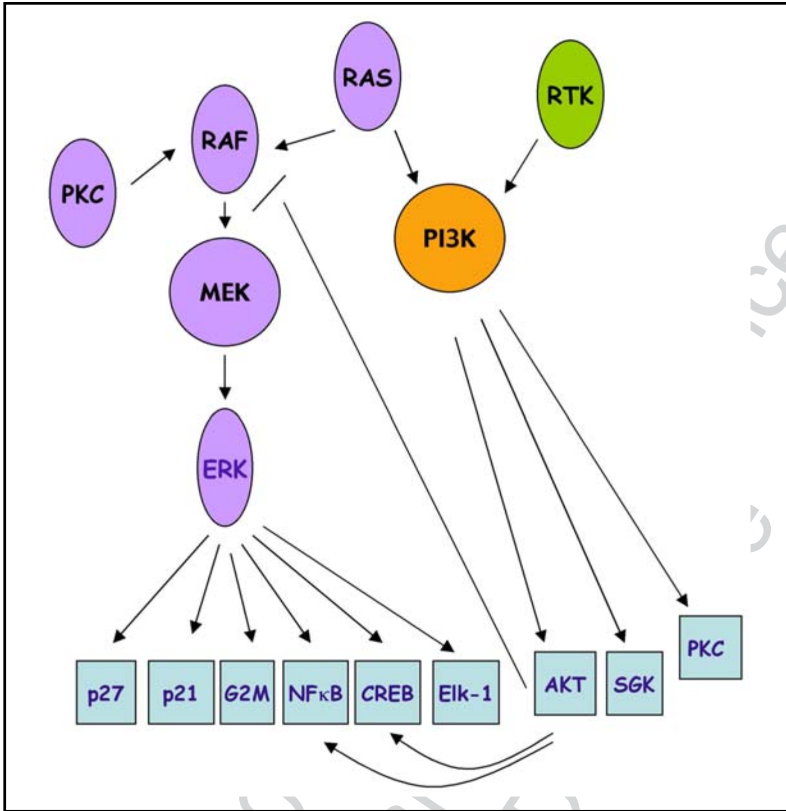

Figure 6. The possible cross talk between the MAPK (ERK) pathway and the PI3/AKT pathway at different levels. RAS, the upstream activator of the RAF/MEK/ERK pathway, activates PI3K, which stimulates AKT. AKT can inhibit RAF activity. CREB and NFκB are downstream targets for both pathways.

and organelles, including the mitochondria, followed by loss of membrane integrity, resulting in cell lysing and release of noxious cellular constituents.[65] In contrast to apoptosis that can occur in a single cell surrounded by a group of viable cells, necrosis simultaneously involves a group of cells in which the necrotic cell ruptures and releases chemical mediators that induce inflammatory reactions. However, under some pathological conditions, both necrosis and apoptosis may occur concurrently. For example, ischemic injury is frequently characterized by damaged necrotic cells surrounded by cells that undergo delayed apoptotic death.[66] The severity and nature of the stimulus may determine if the cell will die by necrosis or apoptosis.[67]

Senescense

Senescense is another mechanism of cell death. Normal human cells in culture, after dividing for about 60 populations, undergo growth arrest known as replicative senescence in which the cells remain metabolically active, but lose permanently their proliferating activities.[68] Senescense is characterized by a cell enlargement and elevated expression of a pH-dependent galactosidase activity, the cyclin-dependent kinases $p16^{Ink4a}$, $p21^{Cip1/Waf1}$, and hypophosphorylated Rb.[69] The exact underlying mechanisms of senescence are not known yet. Progressive telomere shortening, which occurs at every cell division, is a proposed mechanism

for the induction of replicative senescence. When telomeres reach the critical length of less than 5 kb, the Rb and p53 pathways become activated by a mechanism that is not yet well understood. This activation triggers irreversible growth arrest.[70]

Sublethal stress may induce a state that closely resembles replicative senescence.[71] Stress-induced senescence may occur following H_2O_2 or hyperoxia-induced oxidative stress,[72,73] UV- or irradiation-induced DNA damage,[71,74] and treatment with histone deacetylase inhibitors.[75] The mechanisms of stress-induced senescence are very similar to replicative senescence,[76] but neither is fully understood. In chronic hyperoxia, telomere shortening occurs 5 to 10 times faster.[77,78] In addition, it has been reported that induction of stress-induced senescence is triggered when the p53-dependent cell cycle is arrested via generation of nonspecific as well as telomerespecific DNA damage.[71]

It has been suggested that senescence represents a barrier against tumorgenesis,[79] and that an essential step in the malignant transformation of normal cells, comes when they acquire ability to proliferate an unlimited number of times (immortalization).

Paraptosis

Paraptosis, another mechanism of cell death, is a nonapoptotic programmed cell death with somewhat different cellular features, characterized by cytoplasmic vacuolation without nuclear fragmentation. Similar to apoptosis, it appears to be an active process requiring transcription and protein synthesis.[80]

Apoptosis and Tissue Scarring

Although scar formation is part of the normal wound-healing process, excessive scarring is the hallmark of fibrotic disease. Extensive scarring from chronic injury severely compromises the function of the affected tissues or organs, and eventually becomes irreversible. Irreversible fibrotic diseases are major causes of morbidity and mortality in clinical practice. For instance, cirrhosis of the liver is the seventh leading cause of death among young and middle-aged adults in the United States. Approximately 10,000 to 24,000 deaths from cirrhosis may be attributable to alcohol consumption each year. This number goes higher when other causes of liver cirrhosis, such as hepatitis, are taken into consideration. Alcohol-induced cell death and inflammation can initiate the scarring process that distorts structural integrity of the liver and impairs its functional abilities.

Similar to other fibrotic diseases, the complex interaction of certain cytokines, chemokines, and growth factors with resident hepatic cells (i.e., stellate cells) could initiate and propagate the disease process, which eventually lead to advanced stages of cirrhosis. Upon activation by cytokines and other factors, stellate cells start to proliferate, and begin to produce excessive amounts of matrix proteins, which are the main components of scar tissue. In addition to hepatic stellate cell activation, proliferation, differentiation and matrix synthesis,[81] increased rates of apoptotic cell deletion have been documented during hepatic fibrogenesis.[82,83] Hepatic stellate cell-secreted TGFβ1 in alcoholic liver disease might have a dual impact on the hepatic injury, possibly by promoting fibrogenesis and eliminating hepatocytes by apoptosis.[84]

In a similar study, experimental ligation of the common bile duct initiated inflammatory events, proliferation of ductal epithelial cells and periportal fibrosis but release of such obstruction resulted in reversal of the fibrotic process, presumably due to apoptotic deletion of matrix-producing cells and matrix degradation.[85] Theoretically, the inhibition or inactivation of hepatic stellate cells in the early fibroproliferative phase could serve as a basis for developing new therapeutic strategies to control the progression of fibrotic liver diseases; such a therapeutic approach might have clinical impact in the general treatment of fibrotic diseases. Although this hypothetical concept is provocative, it still needs to be proven.

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Recent studies have documented that selective induction of an increased rate of apoptosis of lung cells, by activating the proapoptotic system in mice, could develop pulmonary fibrosis.[86] Suppressing such apoptosis by caspase inhibitors attenuated the fibrotic process in the lung,[87] suggesting that selective modulation of apoptosis has therapeutic implications in fibrotic diseases. Similarly, a recent study with a rat glomerulonephritis model showed that inducing apoptosis of the mesangial cells could attenuate the matrix accumulation in the glomeruli.[88]

The pathogenesis of fibrotic disorders is mostly similar regardless of the tissues or organs involved.[89-95] Early phases of fibrosis show inflammatory changes such as inflammatory infiltrates and proliferation of matrix-producing cells. In later stages, most of these infiltrating and proliferating cells are cleared (possibly by apoptosis), leaving mostly an acellular fibrotic mass. The microenvironment influences the intracellular signaling cascade. The altered microenvironment during fibrosis might facilitate the apoptotic removal of cellular components, by either influencing apoptotic stimuli or by immobilizing the survival state of the surrounding and neighboring cells.[96] Such cytokines and growth factors as platelet-derived growth factor (PDGF), transforming growth factor (TGF-β) and fibroblast growth factor (FGF) have both profibrogenic and proapoptotic effects.[97-99] PDGF, TGF-β_1 and bFGF not only have proliferating and migrating effects on both fibroblasts and myofibroblasts, but also induce excessive matrix production.[100-103]

For instance, PDGF is a potent mitogen for various types of cells including vascular smooth muscle cells. Interestingly, PDGF-BB has the ability to induce apoptosis in the vascular smooth muscle cells, while PDGF-AA could prevent such apoptosis.[103] Similarly, a cyclic peptide analog of PDGF-BB could induce apoptotic cell death in growing cardiac fibroblasts.[104] In contrast to the vascular smooth muscle cells, normal rat kidney fibroblasts undergo apoptosis, when stimulated by either PDGF-AA or PDGF-BB homodimers.[105] Similarly, bFGF-induced apoptosis was much higher in fibroblasts isolated from the rat palatal scar tissue than normal palatal fibroblasts.[106] TGF-β1 is able to induce apoptosis in a wide range of cells, such as microglia.[107]

Although knowledge about the contribution of apoptosis to fibrotic diseases is expanding, the exact pathways leading to the development of fibrosis remain to be elucidated. The slow and progressive nature of the disease process in humans may explain why the direct role of apoptosis and its initiating events during fibrogenesis is not always conclusive. In some cases, it takes years to form a fibroproliferative mass into an acellular scar tissue. In experimental models, where fibrotic lesions can be induced in relatively short period of time, it has been demonstrated that an increased rate of apoptosis is closely associated with fibrotic changes in the affected organs. For instance, in an experimental model of Thy 1.1 nephritis, glomerular hypercellularity and expansion of mesangial matrix is followed by resolution of glomerular hypercellularity, which is mediated by apoptotic deletion of mesangial cells.[108]

Although, apoptotic removal of proliferating mesangial cells and infiltrating inflammatory cells has a beneficial effect on the resolution of glomerular hypercellularity in anti-Thy1.1 nephritis, an injurious role for apoptosis has been documented in several human and experimental renal diseases. In nephrotoxic nephritis, an increased rate of apoptosis is related to tubular atrophy and subsequent interstitial fibrosis.[109] The degree of apoptosis was not only associated with renal scarring, but also positively correlated with levels of serum creatinine and proteinuria in the remnant kidney model.[110,111] The types of cells undergoing apoptosis during renal scarring varied with different phases of the disease process. For instance, apoptosis of such infiltrating inflammatory cells as neutrophils and macrophages contributed to the resolution of relatively early inflammatory events,[112 113] but apoptotic deletion of resident and transforming cells was noted in the later stages of the disease process, and led to interstitial fibrosis.

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Using a nephrotoxic nephritis model, El Nahas and colleagues have shown two distinct peaks of apoptosis on day 7 and at 4 to 6 weeks.[109] The first peak of apoptosis was mostly inflammatory infiltrates of glomeruli, while the second peak was due to removal of tubulointerstitial cells, resulting in tubular atrophy and interstitial fibrosis. Association between apoptotic cell death and accumulation of matrix proteins was found in age-associated renal scarring in Fischer 344 rats.[114,115] Similar induction of apoptosis in the kidney has been documented in hypertensive nephrosclerosis of Dahl rat, which was associated with augmented expression of the pro-apoptotic molecules Fas, Bax, and Bcl-X_S.[116]

Conclusions

Fibrosis is a complex pathological process that involves prolonged inflammation, proliferation of matrix-producing cells, increased deposition of matrix proteins, and eventual tissue remodeling.[117-119] As the fibrotic tissue matures, a striking decrease in cellularity occurs, due to apoptotic removal of cellular components, forming mostly an acellular fibrotic mass.[120-122] Given the dire consequences of scarring diseases, to find more effective therapies to reverse or prevent the damage related to widespread tissue scarring is an active field of study. The search continues to develop novel treatments that can modulate the scarring response to control healing, but the complexity of the scarring process makes it difficult to develop such magical therapy. Will the advances in molecular biology open doors to new magical therapies? The molecular knowledge of nonmammalian systems allows us to selectively transfect human cells to target a specific cell population to modulate their undesirable functions. Targeted removal of the unwanted cells, or rescuing essential cells from dying, might move us closer to develop site- and phase-specific therapeutic strategies to treat mostly irreversible scarring diseases. Finally, further research is needed to understand how normal cells may switch to fibrotic phenotypes in response to stimuli received from the extracellular environment. For example diverse stimuli that induce inflammatory and fibrotic responses generate such complex intracellular molecular events as synthesis of second messengers, initiation of phosphorylation cascades, and protein-protein interactions. Understanding the precise nature of such intracellular events is pivotal for the development of effective strategies of artificial manipulation to alter, amplify, or prevent cellular responses, so as to control the progression of fibrosis.

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