Scleroderma Fibroblast Survival in Aktion

Nora Sandorfi and Sergio A. Jimenez
Division of Rheumatology, Department of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Basic Mechanisms of Apoptosis and the Role of Akt

Apoptosis is a highly controlled cellular suicide program that plays an important role in a wide range of processes including morphogenesis, immune-cell homeostasis, and immune self-tolerance. Two major apoptotic pathways have been described: the extrinsic pathway that involves activation of a death receptor on the cell surface, and the intrinsic pathway that engages intracellular pro-apoptotic proteins such as Bax, Puma, Noxa, and others (see Fig 1). Both pathways converge on the mitochondria to induce release of cytochrome c and several other proteins from the mitochondrial intermembrane space to the cytosol. These factors promote the activation of caspase-9 and suppress the inhibitor of apoptosis proteins (IAPs) to stimulate downstream executioner caspases that, in turn, carry out apoptosis. In the extrinsic pathway, depending on the cell type, activation of caspase-8 may either directly induce cell death by activating the terminal caspases (type I cells), or recruit the mitochondrial pathway of cytochrome c release and caspase-9 activation (type II cells). In both cases caspase-8 activation is required for the execution of apoptosis. In the extrinsic pathway, activation of the death receptors by natural ligands induces apoptosis in several cell types. Fas receptor is a ubiquitously expressed receptor that belongs to the tumor necrosis factor α (TNF-α) receptor/nerve growth factor receptor family and triggers caspase-8 activation through involvement of Fas-associated protein with death domain. In healthy cells, the effect of the pro-apoptotic factors is balanced by anti-apoptotic proteins such as Bcl-2, Bcl-xL, and Mcl-1, which by acting on the mitochondria inhibit the release of apoptogenic molecules (Martin and Elkon, 2004).

One of the key enzymes in the regulation of apoptosis is Akt (protein kinase B). This 60 kDa serine/threonine kinase is expressed in most cell types and appears to inhibit both spontaneous and stress-induced apoptosis. Akt is activated by numerous factors including insulin-like growth factor-1 (IGF-1) and BDNF using the phosphatidylinositol 3-kinase (PI3K)-generated polyphospho-phosphatidylinositol triphosphates (Datta et al, 1999). During the activation, Akt is phosphorylated (p-Akt), a process achieved at least in part by phosphoinositide-dependent protein kinase-1 (PDK-1) (Brunet et al, 2001). It has been demonstrated that transfection of a variety of cell types with constitutively active Akt alleles blocked apoptosis induced by various apoptotic stimuli including anti-Fas antibody and transforming growth factor-β (TGF-β) (Datta et al, 1999). Akt is postulated to inhibit apoptosis both up- and downstream of the mitochondrial phase (Fig 2). Akt activity may result in the inhibition of pro-apoptotic Bad, Bax, Bik, and caspase-9 by phosphorylation. Akt activation also promotes the upregulation of transcription factors such as cAMP-responsive element-binding protein (CREB) and the nuclear factor-κB (NF-κB) regulator IKK (Datta et al, 1999; Juin et al, 1999; Kennedy et al, 1999).

Indirect immunofluorescence microscopy revealed colocalization of Akt and Bad (Blume-Jensen et al, 1998).Akt phosphorylates Bad to suppress the Bad-dependent cytochrome c release from the mitochondria (Blume-Jensen et al, 1998; Datta et al, 1999). Notably, Akt can also block the release of cytochrome c from mitochondria in cells that lack Bad (Juin et al, 1999), as well as block cell death induced by Bax and Bid (Kennedy et al, 1999). Evidence has recently emerged that Akt also phosphorylates caspase-9 to attenuate the caspase activation downstream of cytochrome c release (Datta et al, 1999). In the presence of survival factors, activated Akt phosphorylates the Forkhead family members (such as FOXO), resulting in their sequestration in the cytoplasm. In the absence of survival factors and Akt activity, the Forkhead family members translocate to the nucleus and initiate expression of various “death” genes (FasL, Fas, TNF-α, TNFR), which, in turn, promote cell death. Growth factors may also be able to prevent cell death by upregulating genes that are capable of promoting cell survival. Akt can associate in vivo with the IKKα and β kinases, which promote the degradation of IκB. Degradation of IκB results in the nuclear translocation of NF-κB and subsequent activation of its target genes. These genes are yet to be identified but may include prosurvival Bcl-2 family member Bfl-1/A1 and certain caspase inhibitors.

Akt also mediates the effect of insulin and IGF-1 on cell metabolism. In this signaling cascade, Akt phosphorylates and inactivates glycogen synthase kinase-3 (GSK-3), a process that affects metabolism and that, in turn, may promote cell survival. However, Akt has several other substrates that have not yet been implicated in growth factor-mediated cell survival. It is also noteworthy that Akt can block Fas-mediated apoptosis by a mechanism that is not explained by the phosphorylation of any of the known substrates.

Abbreviations: BDNF, brain-derived neurotrophic factor; PI3K, phosphatidylinositol 3-kinase; SSc, systemic sclerosis; TGF-β, transforming growth factor-β.
Figure 1
Extrinsic and intrinsic pathways of apoptosis (schematic presentation).

Figure 2
Phosphatidylinositol 3-kinase (PI3K/Akt)-related substrates and their role in cell survival (schematic presentation).
It has been postulated that TGF-β may activate PI3K in epithelial cells and fetal rat hepatocytes to promote cell survival via Akt-dependent regulation (Valdes et al., 2004). It has also been demonstrated that in kidney mesangial cells TGF-β1 is able to activate PDK-1 and Akt. Blockage of the PI3K or Akt abrogated the TGF-β1-stimulated COL1A2 gene transcription. The inhibition of the PI3K pathway resulted in diminished Smad3 activity, indicating a possible cross-talk between Smad and PI3K/Akt pathways in the regulation of type I collagen expression in response to TGF-β (Runyan et al., 2004).

Role of Akt in Rheumatic Diseases

Although the role of apoptotic pathways in the pathogenesis of rheumatic/autoimmune diseases has been extensively investigated, the role of Akt has previously only been studied in rheumatoid arthritis. Evidence has emerged to indicate that Akt may play a role in the pathogenesis of rheumatoid arthritis. In macrophages isolated from rheumatoid arthritic-affected joints, apoptosis was induced following inhibition of the PI3K/Akt pathway (Perman et al., 2001). In rheumatoid arthritis synovial fibroblasts, higher levels of activated Akt were detected and treatment with TNFα-activated Akt activation. Inhibition of PI3K allowed TNFα to induce synovial fibroblast apoptosis (Zhang et al., 2001), and more recent studies revealed the PI3K/Akt pathway involvement in the TGF-β-mediated growth and anti-apoptotic effects in synovial rheumatoid arthritis fibroblasts (Kim et al., 2002).

Significance of Apoptosis in Systemic Sclerosis (SSc) Fibroblasts

The most remarkable pathologic alterations in SSc are caused by the exaggerated deposition of interstitial collagen (mainly type I), and other extracellular matrix components in affected tissues. Inflammatory elements including mononuclear cells such as macrophages and T cells also play a role. These inflammatory cells produce numerous factors among which TGF-β appears to play a pivotal role in the pathogenesis of SSc (Jimenez and Derk, 2004). The TGF-β-activated fibroblasts induce further production of TGF-β by an autocrine signaling mechanism and also express increased levels of TGF-β receptors. The binding of activated TGF-β by phosphorylation of its receptor forms an active receptor complex on the target cell. Subsequent signaling to the nucleus occurs through the Smad family of proteins.

Although it has been recognized that the pathology of SSc primarily involves activation of fibroblasts, it has only recently been discovered that SSc fibroblasts are resistant to certain forms of programmed cell death. Two recent studies have shown that SSc fibroblasts are more resistant to Fas-mediated apoptosis compared with control fibroblasts (Jelaska and Korn, 2000; Santiago et al., 2001). Following sustained exposure to TGF-β, SSc fibroblasts became resistant to anti-Fas antibody-induced apoptosis and also displayed increased proliferation. Defective apoptosis was not linked to a ubiquitous element of the apoptotic pathways because staurosporine, a non-specific protein kinase inhibitor, induced cell death similarly in SSc and normal fibroblasts. It is noteworthy that the downregulation of Bcl-2 expression caused increased susceptibility to TGF-β-induced apoptosis in SSc fibroblasts, suggesting a role of Bcl-2 in the development of apoptosis resistance (Santiago et al., 2001).

The current issue of Journal of Investigative Dermatology features a study by Jun et al., examining the potential role of Akt in the resistance of SSc fibroblasts to cell death. In vitro experiments found a relatively high level of p-Akt in SSc fibroblasts and showed an elevated activity of this anti-apoptotic factor in GSK-3 phosphorylation. When the number of fibroblasts expressing p-Akt was quantitated in skin biopsies, more cells stained positive for p-Akt in SSc than in control skin, further supporting an elevation of p-Akt in SSc fibroblasts. The authors were able to demonstrate the expression of both p-Akt and α smooth muscle actin (SMA-α) in one of three samples studied. The presence of SMA-α indicates fibroblast–myofibroblast trans-differentiation, possibly induced by TGF-β as it has been described in other studies. The relationship between such a transdifferentiation process and the occurrence of p-Akt expression is a very important and relevant suggestion that requires further confirmatory studies. Although in the study of Jun et al., TGF-β-induced Akt activation was shown in SSc fibroblasts, this finding did not reach a statistically significant difference when compared with normal fibroblasts.

In this regard, it may be important to examine whether blocking TGF-β activity either with specific antibodies or by blockade of its receptor can abrogate the difference in the level of p-Akt observed between SSc and normal fibroblasts. It is also possible that the Akt activation in SSc fibroblasts involves not only TGF-β also other factors (cytokines, ligands, etc.) that have also been identified to play a role in the pathophysiology of SSc. Further studies should address the potential role of these factors in the upregulation of p-Akt. The other emerging question is whether the presence of activated Akt indeed brings about prolonged SSc fibroblast survival. Alteration in SSc fibroblast survival needs to be determined in the presence and absence of activated Akt. In case that the occurrence of elevated p-Akt is indeed associated with attenuated apoptosis of SSc fibroblasts, downstream elements of the apoptotic pathway related to Akt should be examined to identify the factors that mediate the Akt activity. Jun et al.'s study is an important step in the investigation of the pathophysiology of SSc fibroblast resistance to apoptosis. This study raises several provocative questions and also reinforces the potential role of altered cell death in this severe autoimmune disease. Every step that brings us closer to understand the complex pathophysiology of SSc is therefore extremely welcome and undoubtedly will lead us closer to the development of effective therapy for this devastating and, to date, incurable disease.

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References


