Get a ligand, get a life: integrins, signaling and cell survival

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Summary

Programmed cell death is crucial for the development and maintenance of multicellular organisms. The decision to live, or to die, depends, at the cellular level, upon the cell's interaction with extracellular cues that trigger cell signaling pathways promoting survival or death. The extracellular matrix (ECM) influences the execution of the apoptotic program through the actions of adhesion receptors. Among these, integrins initiate a variety of downstream signaling events in response to ECM ligation. Integrins directly activate survival pathways via the PI 3-kinase and MAPK pathways and act as essential cofactors for their stimulation by growth factors. Conversely, elevated integrin expression in the absence of appropriate ligands, or in the presence of natural or synthetic antagonists, can promote apoptosis under otherwise permissive growth conditions. Integrins thus act in a crucial biosensory role, coordinating survival or death responses as a function of ECM composition. This dual function provides an elegant mechanism through which tissue-remodeling events may regulate cell death or survival in a temporal, ECM-governed manner.

Key words: Cell adhesion, Integrin, Apoptosis, Survival, Caspase

Introduction

Complex regulatory systems are required to form and maintain the array of tissue architectures present in multicellular organisms. Among these, programmed cell death is crucial for developmental and physiological processes, including morphogenesis, wound repair and tissue differentiation. Inappropriately triggered cell death can alter tissue structure or function, compromising embryonic viability (Ranger et al., 2001). Conversely, the failure of cells to undergo apoptosis can result in a range of effects from embryonic death to neoplasia (Lee and Bernstein, 1995; Ranger et al., 2001; Reed, 1999) or autoimmune disease (Eguchi, 2001; Moulian and Berrih-Aknin, 1998).

The life and death decision at the cellular level is controlled by environmental cues, including death-receptor (DR) ligands (Ashkenazi and Dixit, 1999) and growth factors (Dvorak et al., 1995; Eliceiri, 2001), as well as physical stimuli such as mechanical stress (Ingber, 1992) or radiation (Wahl and Carr, 2001). This decision is profoundly influenced by the components of the extracellular matrix (ECM), which can change dynamically during differentiation, development and other tissue-remodeling events. Cell adhesion receptors and new ECM proteins, deposited from intracellular stores or synthesized de novo (Brown et al., 1993; Petersen et al., 1998) interact with both pre-existing and proteolytically exposed sites in the assembled ECM (Davis, 1992; Xu et al., 2001). The ongoing remodeling presents a constantly changing environment, contrasting with the static ECM in 'resting' tissues and presents new information to cells that governs their behavior. The principal adhesion receptors that convey this information are the integrins.

Integrins are heterodimeric receptors for cell-surface adhesion molecules and ECM proteins. Different α and β subunits are expressed in limited combinations (Rupp and Little, 2001) and exhibit different ligand specificities (Table1). The integrins that a given cell expresses therefore control the repertoire of ECM components with which the cell can interact. Integrins bind to ligands in a manner that is dependent upon both affinity and avidity and is influenced by ligand conformation and the capacity to anchor and array (multimerize) within the pre-existing ECM. Thus, different ligands, or different forms of a particular ligand, can transmit distinct signals to a cell through the same integrin (Geiger et al., 2001; Koo et al., 2002; Stupack et al., 1999). Because ECM components may be recognized by more than one integrin, competitive or cooperative binding among different integrin heterodimers adds an additional layer of complexity to cellular responses to the ECM.

Anchorage dependence has long been recognized as a requirement for cell viability (reviewed by Frisch and Ruoslahti, 1997). Integrins govern cellular adhesion and shape, which are critical factors in the cellular response to survival factors (Ingber, 1992; Meredith et al., 1993). The integrin requirement for growth factor signaling is partly explained by the physical association of several growth factor receptors with integrins (Borges et al., 2000; Falcioni et al., 1997; Lee and Juliano, 2000; Miyamoto et al., 1996; Eliceiri, 2001). However, integrins also transmit signals directly through ligation-dependent recruitment of non-receptor tyrosine kinases from the focal adhesion kinase (FAK) and Src families, leading to the activation of several major cell signaling pathways (Fig. 1). The consequent downstream signals, especially via the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, are critical for

Table 1. Common integrins and ECM ligands bound

Integrin	ECM ligands	Notes
α1β1	Collagens	Binds to fibrillar collagen domains
	Laminins	
α2β1	Collagens	Binds to fibrillar collagen domains
	Laminins	Binding dependent on increased activation state
α3β1	Collagens	Binding to NC1 domains, possibly fibrillar forms
	Laminins	Binds to the Laminin 'Toe', GD6 peptide
	Thrombospondin	Binds to TSP-678 peptide
α5β1	Fibronectin	Binds to RGD site in the cell-binding domain
	Fibrin	Binds to cryptic sites in polymerized fibrin
α6β1	Laminins	May bind several sites in laminin
α6β4	Laminins	May bind several sites in laminin
αvβ3	Vitronectin	Binds to RGD sequence near PEX
	Fibronectin	Binds to the RGD site in tenth FN-III (CBD)
	Laminin	Binds in an activation-dependent manner
	Thrombospondin	Cryptic RGD site
	Tenascin	Binds RGD in a FN-III domain
	Del-1	Binds RGD in an EGF-type domain
	Osteopontin	RGD near a thrombin cleavage site
	Bone Sialoprotein	RGD-dependent and independent binding
	Nonfibrillar collagen	RGD-independent binding to NC1 domains
	Denatured collagen	RGD-dependent binding to a cryptic epitope
	MMP2	RGD-independent binding to PEX domain
	bFGF	Binds to a DGR motif
	von Willebrand's Factor	RGD-dependent binding
	thrombin	RGD-dependent binding
αvβ5	Vitronectin	Binds to RGD and KKQRFRHRNRKG
	Del-1	Binds RGD in an EGF-type domain

regulation of the cyclin-dependent kinases (CDKs) and cell cycle progression (reviewed in Schwartz and Assoian, 2001). Since disruption of CDK signaling can result in cell cycle arrest, leading to apoptosis, integrin-sustained cell cycle signaling presents a basic mechanism by which integrins promote cell survival.

Cells denied adhesion undergo apoptosis far more rapidly than cells denied growth factors. This type of apoptosis (anoikis) results from a variety of events (Frisch and Francis, 1994). Cells in suspension reorganize cytoskeletal architecture (Boudreau and Jones, 1999; Flusberg et al., 2001), alter growth factor receptor and death receptor distribution and activity (Aoudjit and Vuori, 2001; Arora et al., 1995; Finbloom and Wahl, 1989; Schleiffenbaum and Fehr, 1990) and membrane lipid composition (Schulze et al., 2001), elevate cyclic AMP levels, uncouple GTPase signaling (Howe and Juliano, 2000; Lin et al., 1997; Schwartz and Shattil, 2000) and alter nuclear transcription (Sadek and Allen-Hoffmann, 1994; Segaert et al., 1998; Vitale et al., 1999). These distinct events provide a formidable array of apoptotic triggers that insure that unanchored cells remain non-viable. One reasonable explanation for the wide variety of conflicting anoikis data (Frisch and Ruoslahti, 1997; Frisch and Screaton, 2001) is that the 'specific' apoptotic pathway triggered in a given cell type reflects the dominant apoptosis pathway in the cell studied. A precise mechanistic role for integrins in anoikis is therefore elusive, although, clearly, integrin-mediated adhesion prevents these forms of death.

Integrin-mediated 'stress relief'

Beyond preventing apoptosis by maintaining 'normal' cellular function, integrins are also implicated in cellular resistance to

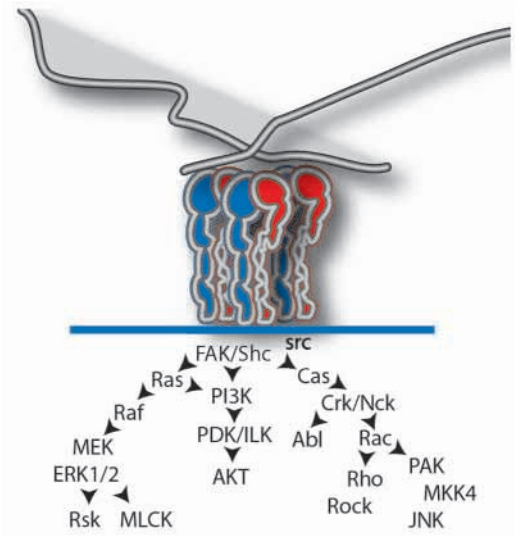

Fig. 1. Common signaling pathways initiated by ECM-ligated integrins. Integrin signaling is dependent upon the non-receptor tyrosine kinase activities of the FAK and src proteins as well as the adaptor protein functions of FAK src and Shc to initiate downstream signaling events. Common kinase signaling cascades are illustrated, but for clarity cooperativity or cross-activation between different cascades has been omitted. One caveat to the interpretation of the role of integrins in mediating survival, as discussed here, is that kinase effects are often studied through the use of constitutively active constructs. It is not clear whether this approach precisely mimics integrin-mediated signaling pathways, as shown here. Also absent are the host of cytoskeletal proteins, including α-actinin, actin, vinculin, talin and paxcillin, that function as adaptors, recruiters and scaffolds on which these signaling events occur.

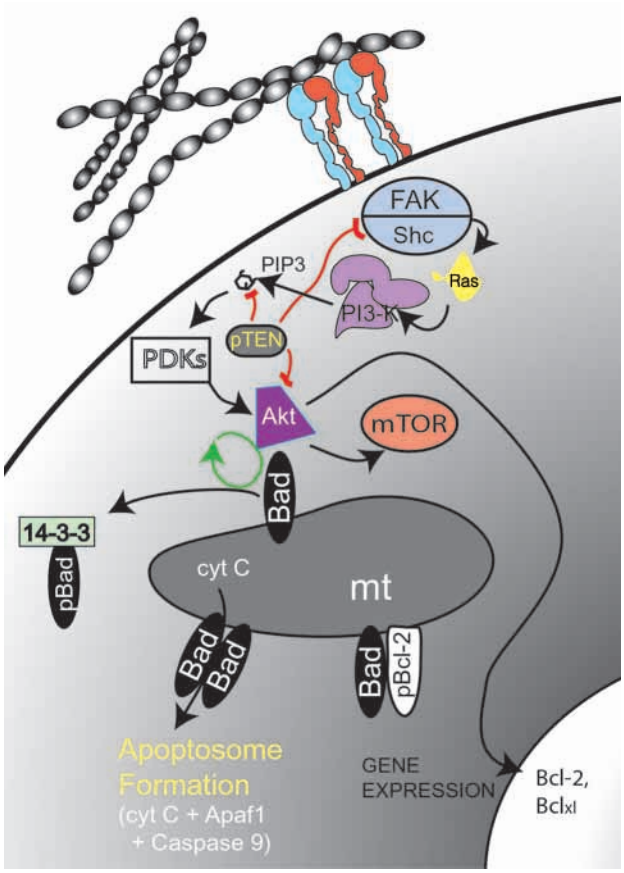

Fig. 2. Integrin-mediated resistance to stress-induced apoptosis via the Ras-PI-3-kinase-Akt pathway. Cell stresses such as serum withdrawal result in the localization of proapoptotic Bcl-2 family proteins, such as Bad (or Bod or Bax), at the mitochondria, which results in cytochrome C leakage and Apaf-1-mediated assembly of the caspase-9-containing apoptosome complex. Ras-mediated activation of PI 3-kinase triggers the phosphorylation of Akt through the functions of phosphoinositide-dependent kinases (PDKs), such as PDK1, PDK2 and ILK. Akt then acts as a central regulator of cell survival, phosphorylating mTOR (and E4BP, allowing iEF-4E activation of CAP-dependent translation; not shown), while also phosphorylating the proapoptotic protein Bad, leading to displacement from the mitochondria and sequestration by chaperones of the 14-3-3 family. Akt also potentiates the transactivating potential of NFkB, leading to the increased expression of NFkB target genes, including Bcl-2 and Bcl-$_{xl}$., Akt signaling is attenuated by the action of phosphatases such as PTEN.

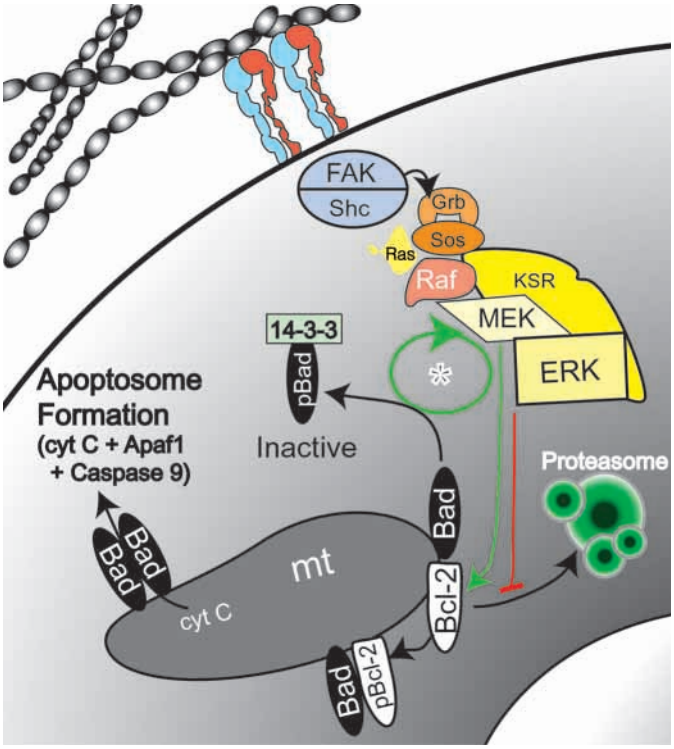

Fig. 3. Roles for ERK activation in resistance to stress-induced apoptosis. Concomitantly with the activation of the Akt pathway (see Fig. 1), integrin ligation leads to the activation of the Ras-Raf-MEK-ERK cascade. Activated Ras recruits Raf, which binds to and activates MEK, and subsequently ERK, which are all bound on a ksr scaffold. The phosphorylation of Bcl-2 by Erk on Thr 56, Thr 74 or Ser 84 prevents its recognition by ubiquitin ligases, whereas phosphorylation at Ser 70 has been reported to both increase and decrease the prosurvival character of Bcl-2. Of the ksr-bound proteins, both Raf and Mek (as well as the Erk-substrate, Rsk, not shown) are capable of phosphorylating the proapoptotic protein Bad (indicated by the green circling arrow, starred), leading to its displacement from the mitochondria and subsequent sequestration by 14-3-3 proteins.

apoptotic stimuli, particularly to signals that activate the intrinsic death pathway (also called the stress pathway or the mitochondrial pathway). Environmental insults, including many cytotoxic drugs, ultraviolet radiation and serum withdrawal (starvation) can lead to mitochondrial release of cytochrome C (Green and Reed, 1998), facilitating the assembly of a proteolytic complex (the apoptosome) containing cytochrome C, caspase 9 and the scaffolding protein Apaf1. The apoptosome processes key cytosolic substrates, including executioner caspases (i.e. caspases 3, 6 and 7) that cleave (and deregulate) kinases, adaptor proteins and nuclear factors, effecting cell death (Carragher et al., 2001; Law et al., 2000; Lesay et al., 2001; Shim et al., 2001; Wolf and Green, 1999).

Integrins preserve cell viability in response to stress at several levels. Signaling by integrins regulates both the expression and activity of several members of the Bcl-2 protein family, affecting the abundance, function and localization of these proteins. The ligation of integrins α5β1 or αvβ3, but not αvβ1, leads to increased expression of Bcl-2 (Matter and Ruoslahti, 2001) and increased resistance to serum withdrawal. Integrin-mediated survival is disrupted by dominant interfering Shc, PI 3-kinase or protein kinase B/Akt constructs (Fig. 2), which suggests a critical role for the PI 3-kinase/Akt pathway. Akt activation induces transcription of the Bcl-2 homolog Bcl$_{xl}$ (Gauthier et al., 2001; Leverrier et al., 1999). This probably occurs because of signaling via the NFκB pathway, since several prosurvival Bcl-2 proteins are regulated by this nuclear transcription factor (Duriez et al., 2000; Grad et al., 2000). NFκB translocation to the nucleus is driven by integrin ligation, although the particular integrin heterodimer that activates NFκB activation appears to be cell type specific (Bearz et al., 1998; de Fougerolles et al., 2000; Lin et al., 1995; Ramarli et al., 1998; Scatena et al., 1998).

At the same time as inducing anti-apoptotic proteins, integrin-mediated signals block the induction of death by proapoptotic Bcl-2 proteins. In addition to triggering the PI 3-kinase/Akt pathway, integrin-mediated Ras activation also results in the activation of the Raf/Mek/ERK pathway (Fig. 3) (Giancotti and Ruoslahti, 1999; Schlaepfer et al., 1999). Both Akt and Raf phosphorylate Bad (at Ser 112 and Ser 136, respectively) (Fang et al., 1999; Hayakawa et al., 2000), promoting its binding and sequestration by members of the 14-3-3 protein family (Petosa et al., 1998). Mechanistically, phosphorylation-based translocation events may be a common means of regulating proapototic Bcl-2 protein function, since Bax also redistributes from the mitochondrial compartment to the cytoplasm as a consequence of integrin-mediated substrate attachment (Gilmore et al., 2000).

Phosphorylation of Bcl-2 by MAP kinases, such as ERK1/2 and JNK, appears to be more complicated. Phosphorylation of Bcl-2 at Thr 56, Thr 74 or Ser 84 (ERK1/2 sites) protects Bcl-2 from ubiquitin-targeted proteolysis, thus effecting increased Bcl-2 accumulation and promoting cell survival (Breitschopf et al., 2000). Bcl-2 phosphorylation by ERK1/2 or JNK at Ser 70 enhances Bcl-2 anti-apoptotic function after growth factor withdrawal (Deng et al., 2001) yet compromises survival in response to the paclitaxel (Srivastava et al., 1999). This conflict in results is mirrored in JNK activation studies, since JNK activation in response to loss of adhesion (Frisch et al., 1996) or chemotherapeutic agents (Avdi et al., 2001; Mandlekar et al., 2000) is proapoptotic but JNK activation downstream of fibronectin-binding integrins suppresses apoptosis after serum withdrawal (Almeida et al., 2000).

Ligation of integrin αvβ3 may also suppress the expression of the proapoptotic protein Bax, possibly via downregulation of the transcriptional activity of p53 (Stromblad et al., 1996). Accordingly, glioma, melanoma and endothelial cells that express αvβ3 integrins (in the context of an appropriate ECM), have elevated Bcl-2 to Bax ratios and display increased survival in response to stress both in vivo and in vitro (Petitclerc et al., 1999; Uhm et al., 1999).

Integrin signaling is coordinated with other cellular responses to stress

Integrin-mediated signaling intersects and is coordinated with other cellular responses to stress. For example, phosphorylation mediated by Akt or Raf facilitates the binding of Bad or Bax to 14-3-3 proteins, which sequesters them during the anti-apoptotic response. However, 14-3-3 proteins bind to a variety of other cellular proteins, including p53 (increasing its transcriptional activity), Raf-1 (increasing its kinase activity) transcription factors (which has varied effects) and cell-surface receptors, including cytokine receptors (van Hemert et al., 2001) and integrins (Han et al., 2001). Since changing the equilibrium of targets available for the relatively promiscuous 14-3-3 chaperones can alter cell fate (Maslyar et al., 2001), integrins may mediate part of their survival effects via indirect ‘cascades’ that promote signaling through intersecting pathways.

There are now several examples of integrin signaling interacting with chaperone functions in the cell. Integrin-mediated actin remodeling promotes phosphorylation and dephosphorylation of the heat shock protein Hsp27 (Polanowska-Grabowska and Gear, 2000), an anti-apoptotic chaperone that stabilizes actin dynamics but also prevents mitochondrial release of cytochrome C and binds and inactivates caspase 3 (Paul et al., 2002). Integrin ligation also influences binding of Hsp90 (Gear et al., 1997), an important cofactor for activity of the serine/threonine kinases Raf-1 and Akt, to its targets (Hostein et al., 2001). The precise roles of other heat shock proteins, such as hsp60, which appears to activate integrin α3β1 (Barazi et al., 2002) and Hsp70, which binds Hsp90, caspase 3 and BAG (Bcl-2 athanogene) proteins, are less well characterized.

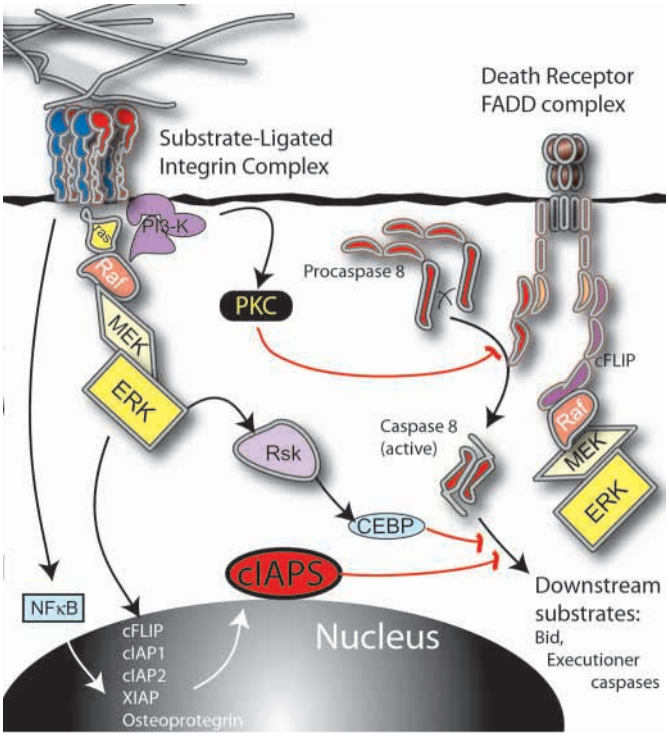

Fig. 4. Mechanisms for integrins to influence DR-mediated apoptosis. The induction of apoptosis by DRs is triggered by ligand-induced clustering, leading to the recruitment of FADD and subsequently initiator caspases such as caspase 8 or 10 via their DED domains (banana shape) to form a DISC (death-inducing signal complex). Caspases autoactivate and form dimers, leaving the DED domains at the DISC, and go on to cleave downstream targets, such as Bid (which will activate the intrinsic apoptosis pathway) and the executioner caspases. The formation of the DISC is influenced by both the presence of DED-containing inhibitory proteins, such as FLIPs, and protein kinase C activity. Activated PKC blocks recruitment and/or activation of caspase 8 at the DISC and is permissive for the assembly of signaling downstream of cFLIP via the Raf-MEK-ERK pathway or through Traf to JNK (not shown). Integrin ligation leads to signaling via the ERK pathway (as described in Fig. 3) as well as to NFκB translocation to the nucleus, which together promote the transcription of anti-apoptotic proteins such as the IAPs and cFLIP. Erk activation of Rsk also leads to phosphorylation of the transcription factor CEBP, creating an XEXD motif that blocks caspase 8 activity.

Life after cytochrome C release

After the release of cytochrome C from mitochondria, cell

viability may be sustained if caspase activity is blocked; thus the mobilization of the caspase-binding heat shock proteins mentioned above may play an important role in maintaining cell survival. However, integrin-mediated signaling via FAK leads to increased expression of the inhibitors of apoptosis (IAPs), specifically cIAP1, cIAP2 and XIAP (Sonoda et al., 2000). Like some of the heat shock proteins, IAPs bind to and inactivate executioner caspases but also serve other functions in the cell, including the regulation of cell division and cytokinesis, as well as signaling from TGFβ receptors to SMAD, NFkB and JNK pathways (reviewed in Salvesen and Duckett, 2002).

Activated Akt directly phosphorylates and inactivates human caspase 9 at S196; however, this site is not conserved in other species and it does not appear that this is a general mechanism by which Akt promotes survival (Fujita et al., 1999). Akt also phosphorylates both nuclear factors (including FKHRL) (Brunet et al., 1999) and the apoptosome scaffold protein Apaf-1 (Zhou et al., 2000), which may influence proapoptotic factor expression and/or apoptosome formation. Integrin-mediated activation of Akt proceeds via several mechanisms downstream of PI 3-kinase, including via integrin-linked kinase (ILK) (Persad et al., 2001) and the phosphoinositide-dependent kinases (PDKs) (Parise et al., 2000). Akt is negatively regulated by the action of the phosphatase PTEN on itself as well as ILK, PtdIns(1,4,5)P_3, FAK and Shc (Yamada and Araki, 2001). Notably, lack of PTEN activity leads to increased cellular resistance to apoptosis and a decreased requirement for integrin-mediated adhesion to maintain cell viability, whereas, conversely, overexpression of PTEN sensitizes cells to apoptosis and increases the integrin-adhesion requirement (Lu et al., 1999; Tamura et al., 1999).

Akt also phosphorylates and activates mTOR and its target p70S6 kinase, critical regulators of CAP-dependent translation in the cell (Scott et al., 1998). Activated mTOR phosphorylates 4E-BPI (Gingras et al., 1998) in cooperation with MEK (Herbert et al., 2002), preventing it from sequestering the CAP-dependent translational initiator eIF4E.

Blockage of CAP-dependent translation by inhibition of Akt or mTOR mimics growth factor deprivation and presents a mechanism by which cells sense this stress. Conversely, overexpression of eIF4E prevents apoptosis and allows growth-factor-independent cell growth (Polunovsky et al., 1996). Importantly, antagonism of integrin αvβ3 (and possibly other integrins) results in inhibition of both MEK activity in vivo (Eliceiri et al., 1998) and mTOR activity and CAP-dependent translation in vitro (Maeshima et al., 2002).

Integrins and the extrinsic death pathway

In contrast to the intrinsic (stress) pathway, the extrinsic apoptosis pathway is triggered by stimuli at the cell surface, typically death receptor ligands such as Fas or TRAIL, that initiate the clustering of death receptors (DRs) at the cell surface. Depending upon the cell type and environmental conditions, DR signaling can be productive, and, in common with integrins, DRs can activate the ERK, JNK and and NFκB pathways (Fig. 4) (Strasser et al., 2000). However, DR ligation more typically results in the recruitment of adaptor proteins such as FADD. During apoptosis, the N-terminal death domains (DDs) of these adaptors interact with the DDs of the clustered DRs. The DR-bound FADD can recruit initiator caspases via homotypic interactions of death effector domains (DEDs) found on both FADD and on caspase 8 and caspase 10. This accumulation of proteins, termed the death-inducing signaling complex (DISC), leads to initiator caspase autoactivation (the 'induced proximity model') (Muzio et al., 1998). Caspase 8 activation may result in direct activation of executioner caspases (the extrinsic pathway, type I) or may cleave the cytosolic protein Bid, subsequently triggering the mitochondrial release of cytochrome C and activation of the caspase 9 pathway discussed above (extrinsic pathway, type II). The proportion of caspase 8 that needs to be activated to result in apoptosis is both cell-type and cell-environment specific and is influenced by cellular expression of IAPs, heat shock proteins and other caspase inhibitors, as discussed above. Signaling events, such as activation of Raf-1 or Akt, that exert control over the caspase 9 can thus impact the 'amplification' step that occurs at the mitochondria in the type II extrinsic apoptosis cascade. Since caspase 8 activation appears to be dependent upon recruitment to the DISC, the abundance of DED-containing proteins that can compete with caspase 8 for binding to the DISC will also influence susceptibility to apoptosis. In this respect, integrin signaling via the ERK, Akt and NFkB pathways influences the relative abundance of the DED-containing proteins c-FLIP and PEA-15.

Integrin-mediated remodeling of the actin cytoskeleton is also likely to play a major role in regulating the extrinsic apoptosis pathway. The type I extrinsic pathway is modulated by the actin cytoskeleton (Algeciras-Schimnich et al., 2002). Dysregulation of actin with cytochalasin B results in lateral clustering of DR1 (Fas) and its association with disrupted actin filaments (Kulms et al., 2002). Similarly, simultaneous ligation of DRs and integrins potentiates apoptosis, possibly because the coordinated actin remodeling facilitates DISC formation (Aoki et al., 2001; Krzyzowska et al., 2001; Moreno-Manzano et al., 2000). This result may provide a context for the observation that the DR ligands TNFα and FAS physically associate with ECM proteins such as fibronectin. Disruption of integrin adhesion, which results in actin remodeling, may similarly contribute to the FADD-dependent caspase 8 activation observed during anoikis (Frisch, 1999).

By contrast, stable integrin adhesion that sustains cytoskeletal integrity appears to block the extrinsic apoptosis pathway. In this case, integrin signaling, probably via ERK1/2, upregulates the caspase 8 inhibitor c-FLIP and decreases the expression and activity of both Fas and Fas ligand on endothelial cells (Aoudjit and Vuori, 2000). Thus, integrins may either promote or block apoptosis triggered by the extrinsic pathway in a manner that is almost certainly dependent upon the cells' current cytoskeletal status.

In addition to influencing susceptibility to DR-mediated apoptosis, integrins may also promote a form of cell death similar to the type I extrinsic pathway. Non-ligation or antagonism of αvβ3 or β1 integrins can lead to caspase-8-dependent apoptosis among attached cells in vivo (Brooks et al., 1994; Storgard et al., 1999) and in vitro (Kozlova et al., 2001; Stupack et al., 2001; Bonfoco et al., 2000; Brassard et al., 1999; Kuzuya et al., 1999). This form of apoptosis, called integrin-mediated death (IMD), appears to result from the clustering of the integrins themselves rather than DRs. Although actin, integrin and caspase 8 colocalize in complexes

on dying cells, DRs and the adaptor FADD are not observed (Stupack et al., 2001) (D.G.S. and D.A.C., unpublished). Moreover, dominant interfering forms of DD/DED adaptors, such as FADD, catalytically inactive caspase 8 and PEA-15, do not block IMD as they do other forms of the extrinsic apoptosis pathway. However, in common with DRs, integrins can cluster in a cytoskeleton-regulated manner, independently of ligation or focal adhesion formation and are available to bind to ligands or antagonists (Byzova et al., 2000; Grabovsky et al., 2000; van Kooyk and Figdor, 2000).

Whether an integrin-bound protein acts as a ligand or an antagonist is dependent upon the context of the binding event. The transmission of integrin-mediated signals is strongly dependent upon a mechanical element or physical resistance factor (Schwartz, 2001; Vogel et al., 2001). Soluble ligands, in general, provide little mechanical resistance and can be endocytosed through integrin-applied forces (Nemerow and Cheresh, 2002). With few exceptions, these signals are incomplete, failing to recruit the full complement of signaling proteins found in substrate-immobilized integrin contacts (Miyamoto et al., 1996). Although sufficient to mediate internalization of integrin-binding viruses (Li et al., 2000), the abortive nature of signaling by soluble ligands/antagonists ultimately conveys 'negative' or unproductive signals to the cell regarding its environment (Fenczik et al., 1997; Klinowska et al., 1999). This may be one reason that plasma-borne ECM proteins (including fibronectin, fibrinogen and vitronectin) do not serve as integrin ligands in their native soluble state but rather require conformational changes associated with deposition or denaturation to reveal integrin-binding sites (Narasimhan and Lai, 1989; Tomasini and Mosher, 1988; Zamarron et al., 1990).

Do integrins communicate with the DED?

Integrin and DR crosstalk appears to be centered on the actin cytoskeleton. Although most DED-containing proteins such as cFLIP, procaspase 8 and 10, FADD and PEA-15 exhibit a cytosolic distribution, it is clear that a fraction of these molecules also associates with cytoskeletal components such as microfilaments or microtubules (Shain et al., 2002; Stupack et al., 2001). Since the local accumulation of caspases is sufficient to trigger apoptosis, the cytoskeleton may concentrate threshold quantities of caspase 8. It is not clear how this might occur, although newly described propoptotic 'pseudo-DED'-containing proteins that contain domains homologous to the cytoskeletal proteins talin and myosin (Hip and Hippi) may offer clues (Gervais et al., 2002). Nevertheless, caspase 8 does not appear to be present in established focal adhesions, nor does caspase 8 activation occur during integrin ligation events during cell spreading. However, caspase 8 is not always activated by DR ligation and membrane recruitment – in fact, the activation of signaling molecules such as Akt (Abreu et al., 2001; Li et al., 2002; Thakkar et al., 2001) or PKC (Gomez-Angelats and Cidlowski, 2001; Miranti et al., 1999; Zhuang et al., 2001; Meng et al., 2002) suppresses activation of caspase 8 and may be involved in DR-mediated signaling to the NFκB, JNK or ERK1/2 pathways (Fig. 4). It is possible that the activation of these or other signaling molecules at the focal adhesion similarly prevent activation of the caspase cascade. Alternatively, targets phosphorylated downstream of the ERK pathway may directly inhibit caspase 8 (Buck et al., 2001).

A second link between integrin-mediated signaling pathways and DR-mediated signaling pathways may be the DED-containing protein PEA-15. Identified in astrocytes as a PKC substrate (Araujo et al., 1993), PEA-15 acts both as a modulator of apoptosis (downstream of PKC and/or calcium/calmodulin-dependent kinase II) (Condorelli et al., 1998) and as a regulator of integrin function (Ramos et al., 1998). Overexpression of active Raf-1 or H-Ras suppresses the integrin conformational change necessary for efficient ligand binding (Ramos et al., 1998). However, overexpression of PEA-15 relieves the H-Ras/Raf-1-induced blockage but activates and sequesters ERK1/2 in the cytosol (Formstecher et al., 2001). Different domains of PEA-15 appear to be involved in integrin regulation and apoptosis – the DED is sufficient to inhibit the extrinsic apoptosis pathway but is insufficient to restore integrin function. However, PEA-15 expression also increases PKC activity (Condorelli et al., 2001), which could affect caspase 8 activation and integrin signaling (Keely et al., 1999) independently.

Conclusions

As the major receptors for ECM expressed on most cell types, integrins are perfectly poised to transmit information to the cell regarding its immediate environment and thus to influence the decision to live or die. Integrin signaling can impact upon both the intrinsic (stress) and the extrinsic (death receptor) apoptotic cascades, thus providing a means to regulate cell survival positively and negatively.

Why should integrins preserve cell viability under conditions that might otherwise kill the cells? Strong integrin interactions between the local ECM and the cell might indicate to the cell that it is well suited to its immediate environment, regardless of the presence of proapoptotic insults. Thus, if local adverse conditions should be overcome, the exogenous stress is relieved, or is transient in nature, the cells present in the stressed tissue can be rescued rather than lost and/or replaced. Conversely, cells lacking the expression of integrins appropriate to the local ECM are more susceptible to stress and succumb more rapidly to apoptosis. Excessive trauma to the tissue, resulting in extensive ECM denaturation or elimination of structural integrity, would similarly lead to elimination of integrin-ECM interactions and cell death. Thus, integrins perform a biosensory role through the cell's interactions with the surrounding ECM.

This signaling may be particularly important during invasive processes, such as inflammation, angiogenesis, tumorigenesis, metastasis and tissue differentiation or remodeling. During these processes cells often break contact with neighbors and rely upon ECM interactions to sense their surroundings. Clearly, different combinations of extracellular cues are present in these processes and resistance to extrinsic or intrinsic apoptosis pathways may play different roles in the progression of these events. As the mechanistic details of the integrin-mediated and apoptotic signaling pathways become clearer, it should become possible to develop logical combinations of drugs that optimize (or minimize) the susceptibility of selected target cell populations to apoptosis during therapeutic interventions.

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