Integrins, adhesion and apoptosis

Jere E. Meredith, Jr and Martin A. Schwartz

Integrin-mediated adhesion to extracellular matrix proteins is required for survival of many cell types. This phenomenon appears to be a mechanism of tumour suppression and to participate in embryogenesis. Here, our current understanding of how integrin-dependent signals prevent apoptosis and implications of anchorage-dependent survival for development, physiology and pathology are discussed.

It is now well established that survival of many cell types requires integrin-mediated adhesion to extracellular matrix (ECM) proteins (see Ref. 1 for an earlier review). In the absence of appropriate ECM contacts, cells undergo programmed cell death or apoptosis. Raff has proposed that programmed cell death is a default pathway that cells enter in the absence of extracellular signals instructing them otherwise[2]. Thus, integrins provide one class of signals that prevent entry into this programme.

Integrin-dependent survival was first described by Meredith *et al.*[3] for vascular endothelial and epithelial but not fibroblastic cell types. Frisch and Francis[4] observed suspension-induced cell death for the epithelial MDCK cell line and named the phenomenon 'anoikas', from the Greek word for homelessness. Similar effects have now been reported for many other cell types (see Table 1). It is apparent therefore that anchorage-dependence of survival is widespread.

Functional significance

The physiological significance of the anchorage-dependence of survival may lie in part as a means of tumour suppression. Apoptosis of cells in inappropriate environments would prevent invasion or metastasis, implying that tumorigenesis requires escape from this control mechanism. Indeed, expression of several oncogenes, including v-*ras*, v-*src* or the SV40 large T antigen, prevents apoptosis of detached cells[4–6]. Frisch *et al.*[7] also demonstrated that, in MDCK cells, prevention of apoptosis either by expression of Bcl2 or by expression of a constitutively activated mutant of focal adhesion kinase (FAK) was sufficient to induce tumorigenicity despite the fact that neither protein was mitogenic in adherent cells. These results strongly argue that resistance to apoptosis plays a significant role in tumorigenesis.

Death of cells in the absence of proper cell adhesion is also likely to be involved in development and maintenance of a number of tissues. During embryogenesis, adhesion-dependent survival is involved in the process of cavitation or tube formation[8]. In cavitation, cells in the interior of a cylinder die owing to lack of contact with the basement membrane, leaving a hollow tube. This basic mechanism may play a role in morphogenesis throughout development. In the immune system, adhesion-dependent signals regulate B cell survival and may play a role in the selection process[9]. Adhesion-dependent survival is also likely to be involved in tissue regression, such as occurs in the mammary gland at the end of lactation. In several systems, secretion of matrix-degrading proteases is an early step during tissue regression, suggesting that the widespread apoptosis observed in these systems might be due to loss of ECM. In the mammary gland, blockade of matrix degradation prevents involution[10], whereas the induction of matrix degradation induces apoptosis[11]. ECM degradation as a means to promote regression may have the advantage over hormonal effects in that, unlike hormones that tend to be cell-type specific, all cell types within a tissue will be affected by loss of the ECM, thereby leading to complete tissue destruction. In fact, in some cases, ECM-degrading enzymes appear to mediate the effects of hormones on tissue regression[10].

Cell-type specificity

The initial papers on adhesion and apoptosis suggested that there was a correlation between cell lineage and sensitivity to apoptosis, such that epithelial cell types (including vascular endothelial cells) were highly susceptible, whereas fibroblastic cells were not[3,4]. Examination of additional cell types suggests instead that nearly all cell types are susceptible to apoptosis when deprived of extracellular signals, but that the degree of sensitivity varies. At one extreme are human endothelial cells and MDCK cells[3–5,12], which die after detachment even in the presence of high concentrations of serum or growth factors. At the other extreme are transformed cells such as CHO cells that survive adherent without serum or suspended with serum, but die in suspension in serum-free medium[13]. Many cell types including most primary fibroblasts show an intermediate phenotype such that serum exerts a partial protective effect when cells are in suspension[14,15]. Thus, the sensitivity of cells to apoptosis can be placed along a continuum. A list of cell types and their survival requirements is shown in Table 1.

This view is consistent with our current understanding of signal transduction by integrins and growth factors. There are now several instances where signals from growth factor receptors are modulated by cell adhesion, such that events downstream of growth factor binding are inhibited in suspended cells or cells anchored to inappropriate substrata. Examples include hydrolysis of inositol lipids[16], associations of the insulin receptor substrate-1 (Ref. 17) and activation of the epidermal growth factor (EGF) receptor[18]. There are also instances where signals from integrins require priming from growth factors[19]. These results suggest that differential sensitivity to adhesion and serum growth factor deprivation may be primarily a

The authors are in the Dept of Vascular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd, La Jolla, CA 92037, USA. E-mail: meredith@scripps.edu

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quantitative matter. Survival of cells that are more susceptible to apoptosis may require occupancy of both integrins and growth factor receptors to reach a critical threshold of an intracellular mediator. Cells with lower requirements may obtain a sufficient level of stimulation with one or the other alone, and other cell types may lie in between.

In addition to effects of growth factors and adhesion on survival, certain cells may also be sensitive to the presence of cell–cell adhesions, although both positive and negative effects have been reported. Pullan *et al.*[20] found that cell–cell adhesion is required for protection by the basement membrane in differentiated mammary epithelial cells. Wary *et al.*[21] found that vascular endothelial cells died when plated on laminin at low density but survived at high density. Cells also survived at low density on vitronectin or fibronectin. By contrast, Frisch and Francis found that, when sparse MDCK cells were placed in suspension, they showed markedly less cell death than those detached from confluent monolayers[4]. One possible explanation for these disparate results is that cell–cell adhesion does not modulate survival directly but does so via effects on differentiation or the cell cycle.

TABLE 1 – CELL TYPES DEPENDENT ON ADHESION FOR SURVIVAL

Cell type	Specific adhesive requirements[a]	Protection by GF/serum[b]	Refs
Epithelial/endothelial			
HUVECs	FN or VN not Lam	–	3,5,21
MDCK		–	4,12
HaCat (keratinocytes)		?	4
Rat intestine epithelial (IEC-18)		–	6
Colon carcinoma (LIM 1863)	Cell–cell	–	39
M21 melanoma		+	25
HeLa		+	14
Mammary epithelial			
CID-9	Basement-membrane	+	11
Primary		?	20
PK-15		+	14
Myoblastic			
RD, C2C12	Merosin (Lam 2 or 4) not Lam 1	?	22
Fibroblastic			
CHO		+	13
Rat I		+	14
Primary fibroblasts			
CEF		+/–	15
MEF, sciatic nerve		+	14
p53 $^{-/-}$ MEF		+	14
Haematopoietic			
B cells	ICAM/VCAM	?	9
P3V1 mouse myeloma		+	14
HL60		?	14

[a]In some cases, cells will survive only when adherent to a specific ligand.
[b]All cell types listed above have been found to undergo programmed cell death in the absence of adhesion; some cells, however, will not die in the absence of adhesion when growth factors (GF) or serum are present ('+').
Abbreviations: CEF, chicken embryo fibroblasts; CHO, Chinese hamster ovary cells; FN, fibronectin; GF, growth factors; HUVECs, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule; Lam, laminin; MDCK, Madin–Darby canine kidney epithelial cells; MEF, mouse embryo fibroblasts; RD, rhabdomyosarcoma; VCAM, vascular cell-adhesion molecule; VN, vitronectin.

Integrin specificity

There is now ample evidence that integrins differ in their ability to promote cell survival. An effect of this type was first observed by Zhang *et al.*[13] in CHO cells, where integrin α5β1 binding to fibronectin was especially potent at preventing the death of cells in serum-free medium. In that system, the α5 cytoplasmic domain was found to be crucial. In skeletal myoblast cell lines, isoforms of laminin have different effects on myogenesis. Merosin (laminin 2 and 4) will prevent apoptosis and promote myogenesis, whereas laminin 1 will not[22].

In angiogenic endothelial cells *in vivo*, integrin αvβ3 appears to be of particular importance in maintaining cell survival. Blocking αvβ3 with antibodies or peptides will inhibit angiogenesis by specifically inducing apoptosis of the migrating endothelial cells[23]. Antagonists of other integrins are without effect. This specificity is dependent upon the nature of the angiogenic stimulus, however. Whereas basic fibroblast growth factor (bFGF)-stimulated angiogenesis works through αvβ3, in vascular endothelial growth factor (VEGF)-stimulated angiogenesis, αvβ5 is crucial to angiogenesis and presumably survival[24]. Integrin αvβ3 will promote survival of melanoma cells in collagen gels in serum-free medium, whereas integrin α2β1 will not[25]. As discussed below, *in vitro* studies of endothelial cells[21] also showed that integrins differ in their abilities to promote survival, such that integrins α5β1 and αvβ3 were more effective than α2β1 in promoting cell survival. Finally, one integrin, α6β4, has even been shown to promote cell death when overexpressed in rectal carcinoma cells[26]. Thus, specific integrins clearly differ in their abilities to regulate programmed cell death – this may reflect differences in the signalling capacities of specific receptors and also differences in the ability of a given receptor to signal in different contexts. A list of integrins that promote cell survival is given in Table 2.

Proximal integrin signals involved in survival

Integrin-mediated adhesion regulates a variety of intracellular events (reviewed in Ref. 27). These include induction of cell spreading and cytoskeletal organization; activation of phosphoinositide synthesis and, in some cases, hydrolysis; activation of serine/threonine protein kinases such as protein kinase C and MAP kinases; activation of tyrosine protein kinases such as focal adhesion kinase (FAK), c-Src and c-Abl; and elevation of intracellular pH and Ca^{2+} concentrations. Thus, it has been possible to investigate which of these signals is involved in maintaining survival.

The role of integrin-mediated cell spreading in the regulation of cell survival is still unclear. Re *et al.*[5]

TABLE 2 – SPECIFIC INTEGRINS ABLE TO PROMOTE CELL SURVIVAL

Integrin	Cell type	Ref.
β1	HUVECs,	3
	Columnar epithelial,	8
	CID-9 mammary epithelial,	11
	Primary mammary epithelial	20
α2β1	MDCK epithelial	12
α4β1	B cells	9
α5β1	CHO	13
	HT29 colon carcinoma	40
	HUVECs	21
αLβ2	B cells	9
αV	LIM 1863 colon carcinoma	39
	Columnar epithelial	8
αVβ3	Endothelial	23

Abbreviations: CHO, Chinese hamster ovary cells; HUVECs, human umbilical vein endothelial cells; MDCK, Madin–Darby canine kidney epithelial cells.

attempted to determine whether cell spreading was required to prevent cell death by treating suspended cells with RGD-coated 4.5 μm beads. These beads bind and activate integrin-specific signals but fail to induce spreading because of their small size[28]. The beads did not protect endothelial cells from death in suspension, suggesting that spreading is necessary. However, particles of this size can be internalized by endothelial cells with a half-time of ~30 min (Ref. 28). Thus, signals from beads are likely to be transient and may not be sufficient to protect cells from apoptosis at later times. Other investigators have found that cell spreading does not correlate with protection from apoptosis. Zhang *et al.*[13] and Wary *et al.*[21] both observed that certain integrins (αvβ1, α2β1) mediated cell spreading but did not prevent cell death under conditions where other integrins (αvβ3, α5β1) did.

Recent studies have implicated FAK in cell survival. FAK is a tyrosine kinase that is autophosphorylated and activated in response to integrin-mediated signals. Hungerford *et al.*[15] found that injecting chicken embryo fibroblasts with antagonists of FAK induced apoptosis. Xu *et al.*[29] found that antisense oligonucleotides to FAK induced apoptosis of tumour cells. Conversely, Frisch *et al.*[7] found that expression of a constitutively activated variant of FAK blocked apoptosis of suspended MDCK cells. Taken together, these results provide good evidence that FAK is an important mediator of integrin-dependent survival signals. FAK has been implicated as a potential activator of the mitogen-activated protein (MAP) kinase through the Ras–Raf pathway, at least under some conditions[30], and MAP kinase activation is reported to suppress programmed cell death[31]. Thus, FAK may inhibit cell death via this pathway, although other FAK effectors may also be involved (see below).

FAK may not mediate survival in all cases. While Xu *et al.*[29] demonstrated increased apoptosis of FAK antisense-treated tumour cells, they found that normal fibroblasts were not affected. Furthermore, microinjection of adherent endothelial cells with C3 exoenzyme, which inactivates Rho and thereby inhibits phosphorylation of FAK (Ref. 32), does not trigger apoptosis (M. A. Schwartz, unpublished). Finally, when endothelial or CHO cells were plated on different substrata, cell survival did not correlate with FAK phosphorylation[13,21] since the cells died under conditions where FAK was highly phosphorylated. Thus, the ability of FAK to regulate cell survival may depend on the cell type, the integrin(s) involved and the culture conditions.

Wary *et al.*[21] have identified a novel pathway linking integrins to the activation of MAP kinase through the Ras–Raf pathway and reported that use of this pathway correlates with survival of endothelial cells plated on different ECM proteins. In their system, certain integrin α subunits, notably α1, α5 and αv, physically associate with the adaptor protein Shc, possibly via a bridging interaction with caveolin. They find that this association with Shc and the subsequent activation of MAP kinase both correlate with cell survival. MAP kinase, however, is not the only downstream effector of Ras, and recent findings from the Downward laboratory suggest that the phosphoinositide (PI) 3-kinase may mediate cell survival

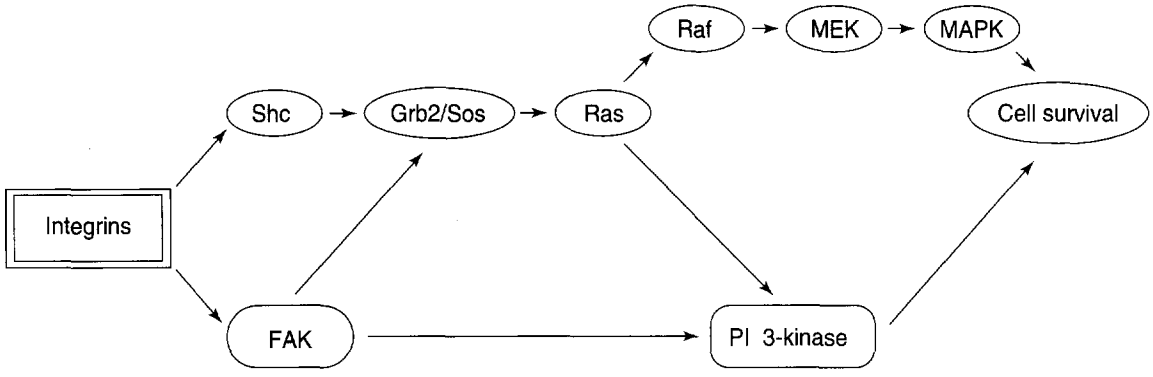

FIGURE 1

Integrin-activated survival signals. Integrin-mediated survival signals may be regulated by PI 3-kinase- or MAP kinase-dependent pathways. There are several potential routes whereby integrins may activate these mediators: mitogen-activated protein (MAP) kinase could be activated via Shc and Ras or via focal adhesion kinase (FAK), Grb2 and Ras. Phosphoinositide (PI) 3-kinase could be activated via binding to FAK or via activation of Ras. Other integrin-mediated signals may also be involved. How these proximal signals contribute to cell survival is not known.

(J. Downward, pers. commun.). They found that Ras effector mutants that activate PI 3-kinase but not the MAP kinase pathway prevent death of suspended MDCK cells, whereas mutants that activate the MAP kinase pathway but not PI 3-kinase do not. Moreover, they find that activated PI 3-kinase but not activated Raf blocks the death of cells in suspension. These results suggest that PI 3-kinase is a critical transducer of integrin-mediated survival signals.

Interestingly, the ability of FAK to regulate cell survival may also be related to regulation of PI 3-kinase. Phosphorylated FAK can bind directly to the p85 subunit of PI 3-kinase and contribute to its activation[33]. In addition, phosphorylated FAK can activate the Ras pathway[30], which could lead to the activation of PI 3-kinase, as mentioned above. Thus, integrin-mediated cell survival may be linked to the activation of multiple overlapping pathways (Fig. 1). However, it seems likely that, in addition to these pathways, other integrin-mediated signals are also involved.

Integrins and the death programme

The cellular pathways that mediate apoptosis have been subjected to intense study over the past few years, and several components of these pathways have been identified. A family of proteases related to interleukin-1β-converting enzyme (ICE) has been implicated in apoptosis, and recent work has identified a cascade of ICE-like enzymes (caspases) that link Fas and the tumour necrosis factor (TNF) receptor to apoptosis (reviewed in Ref. 34).

A second set of regulatory proteins is the Bcl-2 family. This protein was originally identified because its overexpression leads to lymphoid tumours. These tumours arise not because of growth stimulation but because Bcl-2 inhibits cell death, thereby leading to net accumulation of cells. Bcl-2 interacts with a protein named Bax, which promotes apoptosis; the Bcl-2 : Bax ratio rather than the absolute amount of either is thought to be the crucial determinant of cell survival (reviewed in Ref. 35).

The tumour-suppressor protein p53 also plays a role in apoptosis (reviewed in Ref. 36). p53 is required for apoptosis in certain instances, for example following exposure to DNA-damaging reagents. p53-mediated apoptosis is important for the suppression of transformation and the loss of p53 is a frequent event in tumour progression. p53 function may be upregulated by signals that induce cell death. p53 may regulate apoptosis by its ability to induce transcription of Bax and the cyclin/cyclin-dependent kinase (CDK) inhibitor p21, and by its ability to inhibit transcription of Bcl-2. However, p53 may also induce cell death by transcription-independent mechanisms[36].

Integrins have been found to regulate a number of the events described above (Fig. 2). In mammary epithelium, loss of the basement membrane induces expression of ICE prior to cell death, and inhibitors of ICE block apoptosis[11]. In MDCK cells, both ICE and the *jun* N-terminal kinase (JNK) are activated in the absence of adhesion, while inhibition of either ICE or JNK alone will block activation of the other protein and will suppress apoptosis[37]. These results suggest that activation of ICE or an ICE-like protease and

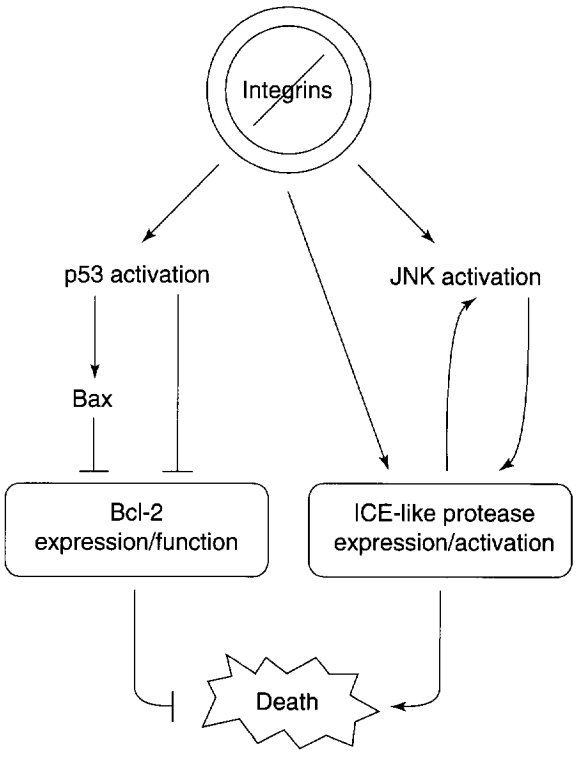

FIGURE 2

Death signals activated in the absence of integrin-mediated adhesion. In the absence of integrin-mediated survival signals, Bcl-2 expression and/or function is downregulated while expression and/or activation of ICE-like proteases is induced. Bcl-2 may be regulated by the p53-dependent transcription of the Bcl-2 inhibitor Bax or by the p53-dependent suppression of Bcl-2 expression. The role of Bcl-2 in the death programme is not known but may involve the regulation of signals upstream or downstream of ICE-related enzymes. In the absence of integrin-mediated signals, ICE-like protein expression may be induced by an unknown pathway or ICE-like protein activity may be induced by a positive feedback loop involving *jun* N-terminal kinase (JNK). In addition to their effects on Bcl-2 and ICE, p53 and JNK may also regulate other aspects of the cell death programme. ICE, interleukin-1β-converting enzyme.

JNK may be linked by a positive feedback loop that is sensitive to adhesion[37].

Integrin-mediated signals induce Bcl-2 expression in CHO cells[13] and are required for expression of Bcl-2 triggered by growth factors in endothelial cells[38]; the specific integrin-mediated signals involved in regulating Bcl-2 expression are not known. Conversely, expression of Bax is increased in the absence of integrin-mediated signals in mammary epithelial and endothelial cells, suggesting that integrins suppress the expression of this protein[20,38]. Thus, integrin-mediated signals can regulate the Bcl-2 : Bax ratio.

The expression of Bax in the absence of adhesion may be linked to the activation of p53. p53 DNA-binding activity was found to increase in endothelial cells in which integrins were blocked[38], and p53 was observed to translocate from the cytoplasm to the nucleus in a colon epithelial cell line upon detachment[39]. Conversely, SV40 large T antigen, which binds to p53 and inhibits its function, prevents the death of endothelial cells upon detachment[5]. However, not all cells are dependent on p53 function for the

activation of apoptosis: p53-null mouse fibroblasts still undergo apoptosis when deprived of extracellular signals[14]. Thus, the role for p53 in integrin-dependent survival may be cell-type specific.

Summary and conclusion

That cell adhesion is required for cell survival should have come as no surprise. It makes good physiological sense that the same molecules that control cell location should also regulate survival. In that way, cells that accidentally localize to improper environments will be deleted and pose no threat to the organism. The death of cells lacking proper adhesive contacts also appears to contribute to normal morphogenesis and organ regression. Furthermore, our current understanding of signalling by growth factors and integrins indicates that critical endpoints often depend upon signals from both; in many instances, signalling pathways from growth factor receptors and integrins converge at relatively early points. Thus, the ability of growth factors to promote cell survival might be expected to depend on adhesion. Control of cell survival by growth factors and adhesion may therefore be interdependent and involve similar mechanisms.

Our current understanding of integrins and cell survival includes preliminary identification of signalling intermediates such as FAK, MAP kinase and PI 3-kinase that may mediate effects of integrins on survival, and of mediators of programmed cell death such as p53 and Bcl-2 that appear to participate. There is also intriguing evidence that integrins differ in their abilities to maintain cell survival. However, much remains to be learned about the details of these pathways. In particular, almost nothing is known about how early events such as PI 3-kinase activation influence the later events in the apoptosis pathways. Finally, the roles of anchorage-dependent survival in physiology, cancer and embryogenesis have yet to be determined.

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