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Involvement of integrins in cell survival

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Summary

Apoptosis is a regulated process of cell death by which cells actively participate in their own destruction. In multicellular organisms, the balance between cell proliferation and apoptosis provides homeostatic control, and a regulatory failure of either event can contribute to oncogenesis. The extracellular matrix (ECM) is known to play a regulatory role in cellular growth and differentiation, but only more recently has it been recognized as a regulator of apoptosis. In these processes the major transmitters of ECM-derived signals to the cell are members of the integrin family, although the mechanical process of cell spreading also plays a role. Both *in vivo* and *in vitro* the loss of adhesion to specific components of the ECM can lead to cell death, and such apoptosis can be induced experimentally by blocking integrin binding. Heterotypic and homotypic cell-cell adhesion can also protect from adhesion-dependent apoptosis and there is evidence to suggest that this too is integrin mediated. In addition, some integrin mediated signaling appears to promote apoptosis. The downstream mechanisms of integrin signaling causing cell death have not been greatly explored, but there is evidence from two different systems that the induction of ICE transcription and nuclear translocation of p53 are candidate processes. Alterations in integrin expression or signaling therefore are likely to contribute to tumor development by enabling escape from apoptosis. Also, the recognition of the importance of cell-cell adhesion in tumor cell survival offers the potential of developing improved drug regimes for the treatment of malignancy.

Involvement of integrins in cell survival

All integrins are transmembrane glycoproteins which exist as heterodimeric complexes of an α and β subunit in non-covalent association [reviewed 1, 2]. The ligand binding domain is formed by sequences in the extracellular amino terminals of each contributing subunit, which allows for the differences in ligand specificity resulting from different α/β combinations. Because of their direct association with the cytoskeleton, the cytoplasmic domains of integrins, and in particular the β subunits, may be the targets of signals which alter integrin binding affinity ('inside-out' signaling) [3–5]. Also, the various α subunits have very different cytoplasmic sequences and different receptors for a given ligand can differ in their apparent association with the cytoskeleton [1]. For example, α3β1 and α5β1 both recognize fibronectin, although only α5β1 localizes to focal contacts [6]. Individual embryonic fibroblasts have been shown to co-express at least three αv-defined integrins, all of which can bind vitronectin [7]. It has been suggested that the expression of separate receptors for a single matrix glycoprotein, while apparently redundant, could allow different signals to be transduced in response to the same matrix component ('outside-in' signaling), presumably through variations in cytoskeletal associations [7].

Apoptosis and anoikis

Integrin-mediated cellular contact with the extracellular matrix (ECM) has long been known to regulate normal cell growth and differentiation. Although the phenotypic changes that result from such interactions have been well documented, the actual mechanisms of intracellular signaling have yet to be elucidated. Past studies have concentrated on the cellular alterations that occur when various integrins are specifically activated through contact with their corresponding matrix proteins. As a result, it is recognized that aspects of gene expression, differentiation, growth control and cytoskeletal architecture can be regulated by integrin engagement [8–10]. Only recently, however, have researchers begun to focus on what changes occur in the *absence* of such interactions. In 1968, Stoker *et al.* [11] speculated that by restricting proliferation to those cells that were attached to the ECM, the body maintained a natural mechanism for preventing dysplasia. There is now increasing evidence that not only proliferation, but actual cell survival may be ultimately dependent on these interactions. Recent studies have shown that normally adherent cells that lose contact with the ECM activate a suicide pathway known as apoptosis [12–14]. Such suspension-induced cell death has been termed 'anoikis', the Greek word for 'homelessness', by Frisch and Francis [13]. This phenomenom can be used to explain the naturally occurring processes of cell death that occur in tissue such as the skin [15].

Apoptosis, a morphological series of events described over twenty years ago by Kerr *et al.* [16], is a process of active cellular self destruction seen under a wide variety of physiological and pathological conditions. Because it is a gene-directed process, apoptosis can be considered, along with more familiar processes such as proliferation and differentiation, as an option available to cells in response to external and internal stimuli. The cytological characteristics of such 'programmed' cell death and its discrimination from necrotic (trauma-induced) cell death have been extensively characterized [for reviews see 17, 18]. The terms apoptosis and programmed cell death (PCD) have been used interchangeably in the literature and, since the precise meaning of these terms is different (although overlapping), this has lead to some confusion [18, 19]. In developmental biology, PCD refers to the gene-regulated death of cells in response to a defined set of developmental stimuli. In contrast, other biologists, particularly in immunology, use the term to describe any form of cell death that requires a genetic programme of self destruction, irrespective of the trigger. For the purpose of this review, we will use the term apoptosis to describe the gene-directed process of active self-destruction, even when this is induced by loss of cell contact.

In many cases of apoptosis, there is a requirement for new gene expression for both the morphological changes and death itself to occur [reviewed in 17] – indeed, this was originally considered to be the case for all forms of apoptotic cell death. Inhibitors of transcription or protein synthesis could in many cases prevent cell death. Cohen [17], who has characterized the different forms of apoptosis, refers to this form of apoptotic activation as an induction mechanism. Conversely, there are examples in which inhibitors of macromolecular synthesis can actually induce apoptosis within a cell. This implies that the suicide programme may be constitutively expressed, but is continuously inhibited by the presence of factors with short half-lives. This mechanism Cohen termed release. However, in other forms of apoptosis, such as the activation of apoptosis in target cells by the action of cytotoxic T lymphocytes, inhibitors have no effect and the time course is such that it appears that these cells constitutively express all of the neccessary molecules required for apoptotic induction, and require only a specific signal for its promotion. This transduction mechanism has stimulated the search for presumptive 'death genes'.

Within an organism, the dynamic balance between regulated cell growth and apoptosis provides a means of maintaining correct cell numbers; pertubation of this balance has the potential of leading to malignant disease [20, 21]. Because apoptosis is dependent on active cell participation, and can therefore potentially be suppressed – aberrant cell survival resulting from such inhibition would be expected to contribute to oncogenesis [21]. In support of this, recent studies have shown that oncogene

transformed endothelial or epithelial cells are more resistant to suspension-induced apoptosis than their normal counterparts [13, 14]. The mechanisms responsible for escape from apoptotic control by tumor cells are likely to prove almost as numerous as those enabling escape from growth control, and, for example, oncogene products such as Bcl-2 have been shown to function by blocking the apoptotic pathway [22, 23]. However, the close association between cell attachment and survival point to a pivotal role for cell adhesion molecules in this process. In particular, the diversity and signaling role of the integrin family of receptors suggest that these molecules act as primary regulators of apoptotic control, with alterations of the integrin phenotype of signaling function contributing to the development of malignancy.

Integrins and protection from apoptosis

The extracellular matrix as a survival factor

During the process of mammary gland involution *in vivo*, following lactation when the milk producing glands are no longer required, the cells secrete metalloproteinases which degrade the basement membrane and initiate their own self destruction [24]. Boudreau *et al.* [25] have characterized this process in the CID-9 mammary epithelial cell line. These cells will undergo apoptosis if plated on plastic, type I collagen, or fibronectin, but will survive to differentiate and express milk proteins when grown on exogenous ECM. The apoptotic response can be induced by disruption of cell-ECM interactions achieved by addition of either anti-β1 integrin antibodies, or by proteolytic degradation of the existing basement membrane by stromelysin-1. Presumably the laminin-rich basement required for milk production [reviewed in 26] can also suppress the apoptotic response through β1-mediated interactions. Upon degradation of the ECM during involution, these contacts are lost, and the apoptotic response is enabled to proceed.

Other studies of the relationship between integrin-matrix engagement and cell survival are not as well served by *in vivo* correlates, but a causitive relationship is now well established from *in vitro* experiments and several distinctive features of the process are emerging. Not surprisingly, dependence on ECM interactions differ between naturally motile cells, such as mesothelial cells and fibroblasts [12], and ECM-dependent endothelial, epithelial and neuronal cells [27]. However, this is not a static phenomenom. Frisch and Francis [13] demonstrated that apoptosis could be induced by disruption of the interactions between normal epithelial cells and the extracellular matrix. When two epithelial cell lines were plated out under conditions in which matrix attachment was prevented, the cells in suspension subsequently underwent apoptosis. This could be abrogated by overexpression of Bcl-2 protein. The same authors [13] also demonstrated that the MDCK cells used in their experiments resisted apoptosis when they were treated with scatter factor, which promotes motility. Thus it might be considered that the dependence upon ECM attachment for survival of the epithelial cells would stabilize tissue architecture within an organism, by preventing inappropriate cell attachment and proliferation by cells which detach from their tissue localization. During times of tissue proliferation, and in the presence of the appropriate growth factors, the cells are transiently released from the ECM-determined survival constraints.

But how does the extracellular matrix signal to the cells to allow them to survive? It is now becoming clear that there are two components of cellular response to ECM interactions: one physical, involving shape changes and cytoskeletal organization; and the other biochemical, involving integrin clustering and increased protein tyrosine phosphorylation [10, 28]. Thus, Meredith *et al.* [12] showed that in the absence of ECM interactions, human endothelial cells rapidly underwent apoptosis. However, attachment *per se* was not enough to circumvent the apoptotic response – plating cells onto immobilized antibodies against VCAM-1 or class 1 histocompatability antigen (HLA) had no significant effect in preventing cell death. Apoptosis could be blocked by attachment to anti-β1 integrin antibody, suggesting that integrin-mediated signals were required for maintaining viability. In the MDCK epithelial cells described above the addition of RGD-containing

peptides, resulting in a functional blocking of integrin-mediated adhesion, also induced apoptosis [13]. These observations have been taken further by Re *et al.* [14] who showed, again in endothelial cells, that occupancy and clustering of integrin receptors by the use of soluble vitronectin or Gly-Asp-Gly-Asp-Ser-coated microbeads – conditions which did not allow spreading – did not prevent the apoptosis of the rounded cells. In these and other cases where the cells remained round in shape, the apoptotic pathway was activated. Only when the cells were plated onto high substrate concentrations on which they are able to adhere and spread were they rescued from cell death. Furthermore, shape changes induced by the binding of either αvβ3 (on a vitronectin matrix) or α5β1 (on fibronectin) were able to prevent apoptosis of the endothelial cells, suggesting that the effects are not specific to any one integrin, but instead rely on the cytoskeletal organization that resulted from integrin clustering together with cell spreading.

Studies with intrinsically motile cells have yielded more complex findings for the role of integrins in promoting cell survival. The integrin αvβ3 (the classical 'vitronectin receptor') has been shown to correlate with the invasive capacity of melanoma cells by Albelda *et al.* [29], who noted that this complex was restricted exclusively to cells within the vertical growth phase and to metastatic melanomas as compared to radial growth phase melanoma cells. It was suggested that this integrin may therefore constitute a useful marker of melanoma cells entering a more aggressive phase of the malignant process. Further, Seftor *et al.* [30] explored the relationship between expression and function of αvβ3 on melanoma cells and found that invasiveness could be stimulated by ligation of this receptor with either antibodies or the ligand vitronectin itself. In addition, there appeared to be a corresponding increase in message for, and secretion of, type IV collagenase enzyme – suggesting that signal transduction through this integrin might underlie the elevated expression of this metalloproteinase.

Montgomery *et al.* [31] recently provided an elegant example of how the interplay of this integrin, when expressed as a part of a tumorigenic phenotype, could suppress an apoptotic response through a specific interaction with the extracellular matrix. These researchers, using an αv-deficient melanoma line, found that the variant melanoma cells underwent apoptosis when grown within three-dimensional dermal collagen. However, transfection with αv cDNA restored αvβ3 expression, and subsequent cell survival, dependent on the ligation of this complex within the collagen. It was shown that attachment to the collagen was initially mediated through the collagen receptor α2β1, and the cells were then able to degrade this collagen to expose cryptic αvβ3 binding sites. That ligation of αvβ3 within collagen suppresses apoptosis and promotes melanoma cell growth allowed these authors to propose that this mechanism may be fundamental to the association between αvβ3 and melanoma tumorigenesis [31]. How such signaling through αvβ3 might relate to the survival of normal, non-tumor, cells is not known; but it may be significant that cultured embryonic fibroblasts rapidly lose expression of αvβ3 upon reaching confluence, and display alternative members of the αv subfamily on their cell surface [7]. It may be, therefore, that αvβ3 signaling promotes survival only in active migratory cells and might provide quite a different signal in cells that have ceased to migrate.

Intercellular contact as a regulator of apoptosis

Not surprisingly, in addition to signals derived from the extracellular matrix, cell survival can depend on signals provided from other cells. This 'social control' view, as Raff [32] points out, indicates that just as an individual cell requires signals provided by other cells in order to proliferate, a cell may activate a suicide programme in the absence of specific intercellular survival signals. This 'cell death by default' provides a homeostatic control that would be important during development, and also a potentially effective tumor control mechanism effected by the deletion of misplaced cells.

Heterotypic examples of intercellular contact in promoting cell survival have emerged in the last few years. Manabe *et al.* [33] found they could circumvent the rapid apoptotic cell death exhibited by freshly isolated B-lineage acute lymphoblastic leu-

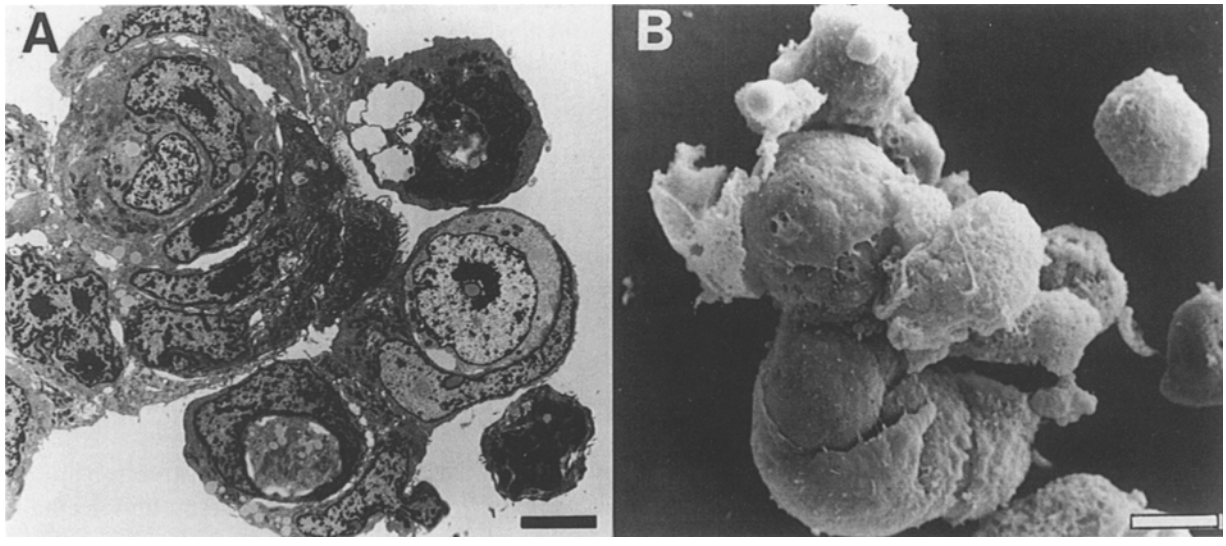

Fig. 1. Organoid Reformation. Ultrastructural examination of reforming LIM 1863 cells show that this event involves sophisticated arrangements of cells, not merely aggregation. A) Transmission electron microscopy. Initial adhesion involves the engulfment of one enterocyte by another, followed by concentric cell attachment to these doublets of cells. Bar represents 5 μm. (Reproduced from The Journal of Cell Biology, 1994, 125, 403–415 by copyright permission of The Rockefeller University Press.) B) Scanning electron microscopy. In three-dimensions the close proximal contacts generated between cells by membrane extensions become apparent. Bar represents 5 μm.

kemia cells if these cells were seeded on allogeneic bone-marrow (BM) derived stromal cells. The apoptosis could not be prevented by the addition of IL-7, nor by the substitution of the feeder layer with murine BM stromal cells, fibroblast lines, or other human-derived BM cell lines – all confirming the specificity of the non-transformed BM stromal cells [33]. The actual mechanism underlying this specificity had not been determined. Similarly, Burkitt lymphoma cells survival and growth was promoted by seeding on irradiated fibroblasts, again by preventing apoptotic cell death [34]. Once more the actual mechanics of this anti-apoptotic pathway were not elucidated, however the prolonged survival did not depend on the induction of bcl-2. More importantly, cell supernatants from feeder cells and from lymphoma cells growing autonomously at high density could not substitute for the survival and growth-promoting effects of the fibroblast feeder cells [34], implicating quite strongly a role for intercellular adhesion, as opposed to soluble factor signalling, in the anti-apoptotic response. Another example of cell adhesion as a suppressor of apoptosis in a heterotypic cell system is provided by Fujita *et al.* [35], wherein growth and survival of a mouse T cell lymphoma line depended on contact with lymph stromal cells – in this case, antibodies to undefined lymphoma cell adhesion molecules not only disrupted the intercellular contact, but caused apoptosis as a consequence.

Studies with the LIM 1863 colon carcinoma cell line provide an example of homotypic cellular interactions in protecting against apoptosis [36]. This line grows in suspension as a three dimensional spheroid or 'organoid' structure, exhibiting morphological and functional organization with features of normal colonic crypts [37, 38]. Within the spheroid, polarized cells are arranged around a central lumen. Maintainence of the organoid structure is dependent on the presence of calcium ions within the culture medium – suspension cultures of single dissaggregated cells can be grown in medium containing less than 100 μm calcium: upon readdition of calcium ions the cells reform the organoid structure by way of a complex series of re-arrangements that follow initial cell contact (Fig. 1) [36, 38]. The reformation event, which relies on active cell participation, appears likely then to involve calcium-dependent cell adhesion molecules such as cadherins and integrins. This was substantiated when it was

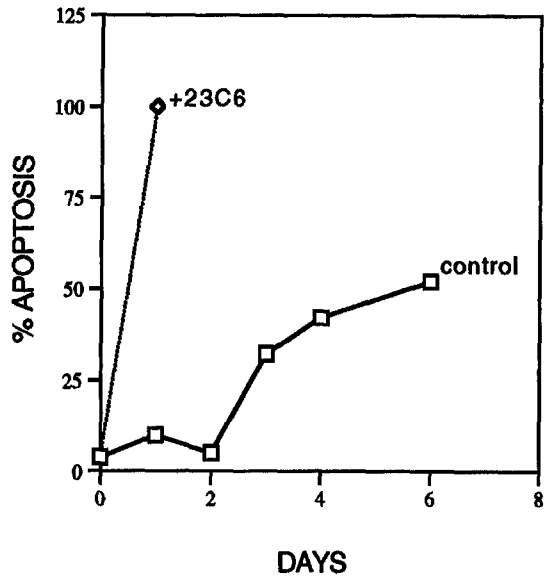

Fig. 2. LIM 1863 single cells were diluted to 100 cells/well in a 96-well plate in the presence or absence of 100 μg/ml 23C6 Fab fragments. Cells were stained with Hoechst and 150–250 cells counted for each time point to determine the percentage of apoptotic cells.

shown that an antibody, 23C6, directed against a conformational-dependent epitope on the αv integrin subunits, totally inhibited the reformation process when used as Fab fragments. More importantly, it was further shown that such antibody-mediated inhibition of reformation in the presence of calcium ions lead rapidly to cell death by apoptosis [36]. Since the antibody did not induce apoptosis when added to cells already engaged in reformation or to intact organoids and, if washed out early enough, the LIM 1863 cells could reform viable organoids even after exposure to the antibody, it was concluded that the cells were undergoing apoptosis as a result of the loss of intercellular contact [36]. The LIM 1863 cells express both αvβ5 and αvβ6 but it was not determined which integrin was involved in this process.

Integrin engagement and the promotion of apoptosis

Integrin-mediated signaling may be implicated in the regulation of apoptosis much more profoundly than simply providing relatively non-specific survival signals. It has been suggested that intercellular contact may also 'prime' a cell for apoptosis [39]. MDCK cells, if grown at low density, are resistant to suspension-induced cell death when trypsinized and replated onto polyHEMA (an attachment inhibiting substrate), whereas cells from confluent cultures will quickly succumb to apoptosis when separated from their matrix [13]. Similarly, pretreatment with TPA or scatter factor, both of which cause the breakdown of intercellular junctional complexes [40–42], protect these cells from apoptosis.

In our own studies with the LIM 1863 cell line, in addition to showing that inhibition of intercellular contact by Fab fragments of the 23C6 antibody lead to cell death by apoptosis [36] it was found that simply seeding the single cells in a calcium rich medium at low density – such that they were unable to form cell-cell contacts – also lead to apoptosis. More recent data suggest that the αv integrin may play a more direct role in the apoptotic process, and that binding of the 23C6 antibody is doing more than simply blocking intercellular adhesion. Thus, when single cells are seeded into calcium-rich medium at low density (100 cells/well to ensure that no cellular contact occurs) they will slowly undergo apoptosis over a period of several days (Fig. 2). Figure 3 shows that even after a period of 24 hours as diluted single cells, the cells were viable and able to reform the organoid structures. In contrast, addition of Fab fragments of the 23C6 antibody to these diluted cells caused an apoptotic response that occured very rapidly – with all cells dead within 24 hours (Fig. 2). These data suggest two signals in the induction of apoptosis: loss of intercellular contact and rounding of the cells, together with a second signal delivered by αv integrin engagement by the cells in suspension.

Mechanisms of integrin involvement in apoptosis

Integrins as signaling molecules

The ubiquitous nature and diversity of the integrins places this family as the primary candidates for the

delivery of apoptotic (or anti-apoptotic) signals from the ECM. Direct or indirect associations with kinases and cytoskeletal components suggests β subunits as the key players in signaling, with the associated α subunits playing a supporting role by providing the substrate specificity of the heterodimeric receptor complex. However, as will be discussed below, the shared structural motifs present in the α subunits may have a part to play in the regulation of some forms of apoptosis.

A detailed review of integrin-mediated signaling is given elsewhere in this issue but, from the viewpoint of the role of integrins in the regulation of apoptosis, emphasis is given here on the capacity of different integrins to trigger discrete intracellular phosphorylation events [43] and to initiate gene transcription. Recent work with integrins by Kapron-Bras *et al.* [44] has shown that ligand engagement or triggering with anti-integrin antibodies activates protein tyrosine kinases resulting in the phosphorylation of several proteins on tyrosine and in the activation of ras to a GTP-bound form [44]. More recently this same group demonstrated that cross-linking of different integrin complexes (through antibody-mediated ligation and clustering of individual α subunits) stimulated tyrosine kinase activity, leading to the phosphorylation of distinct, discrete proteins, which were also different from those observed when the cells were permitted to spread on matrix [43].

Clustering of integrins has also been shown to induce tyrosine phosphorylation of numerous 120–125 kDa proteins [45]. One protein in particular has been identified in focal contacts and has been termed pp125 Focal Adhesion Kinase ($pp125^{FAK}$) [46, 47]. It requires both integrin attachment and cell spreading in order to become activated [48] and has been implicated as one of the major mediators of integrin signalling. This is further supported by the finding that suspended endothelial cells will remain viable if treated with sodium orthovanadate, an inhibitor of protein phosphatases. This results in a protein phosphorylation profile similar (but not identical) to that obtained when cells attach to the ECM [12].

The link between integrin signaling and the inhibition or initiation of gene transcription may be

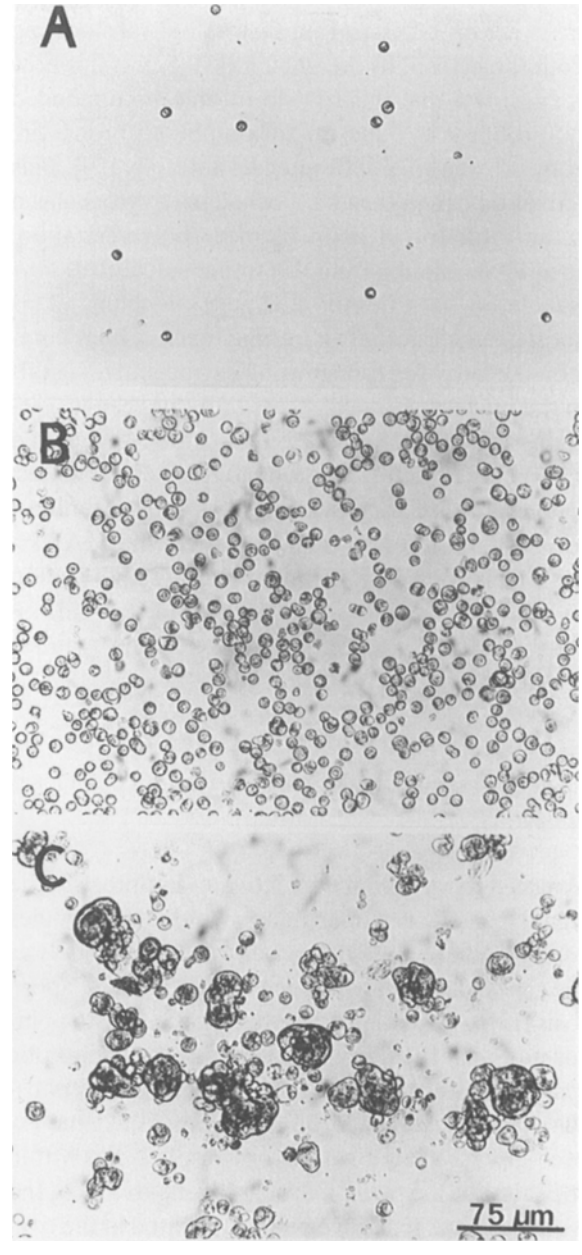

Fig. 3. A) LIM 1863 single cells were diluted to 500 cells/well in 24-well plates and incubated for 24 hours at 37° C, 10% CO_2. B) The cells from A were then harvested, concentrated by centrifugation and replated at a density of 2.4×10^4 cells/well in a 96 well plate. C) After 24 hours, these same cells have reformed the organoid structure.

provided by the finding that integrin engagement can lead to the activation of mitogen-activated protein (MAP) kinases [49, 50]. Normally triggered by serum or growth factors, these serine/threonine ki-

nases act on transcription factors by translocating from the cytosol to the nucleus [51, 52]. It has now been shown that this translocation can be induced in fibroblasts by adhesion to laminin or fibronectin, or by crosslinking of β1 integrin antibody [50]. This activation can in turn be blocked with cytochalasin D, an inhibitor of actin filament polymerization, once again highlighting the importance of the cytoskeleton in integrin-mediated signaling. This finding suggests that there may exist a final common pathway for transmitting extracellular signals to the nucleus. How FAK fits into this scenario remains to be determined, but it can be speculated that it may be acting upstream of the MAP kinases. Indeed, preliminary evidence has shown similarities between integrin-mediated FAK and MAP kinase activation – both are induced rapidly upon ECM attachment, inhibited by the same tyrosine kinase inhibitors, and require cytoskeletal organization for activation [49].

Gene expression and signal transduction in apoptosis

Coupled to the signal that activates apoptosis is the signal transduction machinery – and the molecules which mediate these responses can vary as widely as the types of signaling molecules that regulate apoptosis [19]. Consequently, there is now a wealth of information derived from a diverse array of apoptotic systems on the role of calcium fluxes [53, 54], phosphorylation events [55, 56] and changes in gene expression associated with apoptosis. It is not within the scope of the present article to rehearse all of the gene products that have been implicated in the control of apoptosis, and there are several excellent reviews recently published on this [57–59]. While it is possible, and even likely, that integrin-mediated signaling will influence the subcellular localization and function of such molecules as Rb protein and c-*fos* that are associated with apoptosis [60, 61], and may also serve to induce the transcription of others such as bcl-2 [58, 62], this review will focus on molecules for which there is evidence for integrin involvement in the delivery of the apoptotic signal.

ICE

The mammalian protein ICE (interleukin-1β converting enzyme) shares sequence and functional similarity to the product of the ced-3 gene of *Caenorhabditis elegans* [63]. In the nematode, ced-3 is required for programmed cell death, and both ICE and Ced-3 induce apoptosis when expressed in rodent fibroblasts [64]. In the murine mammary gland, ICE mRNA is expressed by tissues undergoing involution but is not expressed by lactating tissue [25]. Since the (proteolytic) function of ICE can be inhibited by the viral protein crmA, Boudreau *et al.* [25] transfected a construct encoding this gene product into CID-9 mammary epithelial cells and demonstrated protection from cell death caused by loss of ECM anchorage. Hence, in this system, engagement of β1 integrins somehow suppresses the expression of ICE, an event that may also occur *in vivo*. As well as suppressing the expression of ICE, it is possible that cellular interactions with the ECM and resultant cell stretching influence the capacity of ICE to promote apoptosis by the proteolytic release of active DNAase 1 – the nuclease thought to fragment DNA during apoptosis. In many cells, DNAase 1 exists in an inactive state complexed with actin [65] and it has been proposed as a substrate for ICE proteolysis [59]. The relative degree of actin polymerization within cells is regulated by integrin engagement with the ECM, and this is likely to influence the accessibility of the inactive endonuclease to proteolysis.

p53

The tumor suppressor gene product p53 has been extensively analysed in terms of structure/function and many of its functional properties can be located to specific domains [reviewed in 66]. In addition to being able to induce growth arrest in cells, the wild-type (wt) form of the protein, at least, has been shown to participate in the regulation of apoptosis. Oren's group [67] induced expression of p53 in a murine myeloid leukaemic cell line that lacked endogenous p53. The use of a temperature sensitive mutant allowed them to analyse the introduced p53 with either wild-type or mutant properties. The result was that these cells were triggered to undergo apoptosis by the wild-type, while the mutant form

was incapable of producing this response. It has been generally considered that such apoptosis may be a secondary consequence of (wt) p53-induced growth arrest, exacerbated in certain cell types that had suffered DNA damage. Further investigation revealed that this may not always be the case, since the leukaemia cells continued to progress through the cell cycle even as their chromosomal DNA was being fragmented [68]; the authors do, however, suggest that a relationship exists between the cell cycle and p53-mediated cell death. The involvement of p53 in apoptotic death is not restricted to myeloid cells. Transfection of wt p53 into colon carcinoma cells could also induce apoptosis [69].

An association between integrin-mediated apoptosis and p53 was suggested in studies with the LIM 1863 cells when a total translocation of p53 protein from the cytosol to the nucleus was demonstrated in disaggregated cells undergoing antibody-mediated apoptosis [36]. This is a rapid process, disappearance of p53 from the cytosol being observed within thirty minutes of the addition of 23C6 antibody and calcium to the disaggregated cells (Fig. 4), long before the onset of detectable apoptosis in these cells. This time, in fact, coincides with the period of reversibility, when the cells survive if the antibody is washed out and they are allowed to re-establish intercellular contact [36]. Nuclear localization and DNA binding are primary events required for p53 function, thus it is likely that in these cells the integrin-mediated signal that promotes apoptosis is operating by inducing this translocation.

Nur 77

One mechanism of apoptosis for which there is no evidence of any integrin involvement at present deserves speculative comment, simply because it provides a very attractive model for illustrating a possible means by which integrins could directly regulate the process. Liu *et al.* [70] found that engagement of the T cell receptor of murine thymocytes with antibody lead to the induction of nur 77, and that the product of this gene induced apoptosis. Nur 77 protein is an orphan member of the steriod hormone receptor family that contains the canonical sequence KGFFKR, shown to be involved directly in DNA binding by all steroid hormone receptors

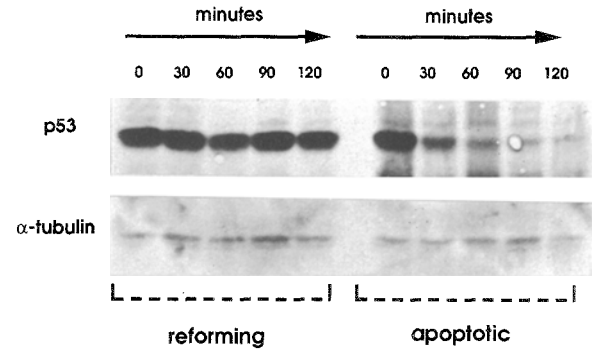

Fig. 4. p53 translocation. Immunoblot of cytoplasmic lysates with the anti-p53 antibody PAb1801. In reforming LIM 1863 cells, the levels of cytoplasmic p53 remain constant. In contrast, cells triggered to undergo apoptosis by 23C6 antibody exhibit a rapid loss of detectable p53 from the cytosol, which we have shown is due to translocation to the nucleus [36]. This translocation occurs rapidly, with a significant reduction apparent within thirty minutes, and total clearance by two hours. An anti-tubulin control immunoblot is shown in the lower panel.

[71, 72]. As pointed out by Dedhar *et al.* [72], all of the integrin α subunits contain the sequence KXGFFKR and, upon integrin activation by engagement of its ligand, the integrins bind and sequester a molecule called calreticulin which binds this sequence [73]. Further, calreticulin, which is mostly to be found in the endoplasmic reticulum can also translocate between the cytosol, plasma membrane and the nucleus and – most importantly – can block the function of several steroid hormone receptors by binding the same shared sequence [71, 73]. It seems possible therefore that one mechanism by which integrin engagement could promote apoptosis is by the sequestering of calreticulin thereby preventing its protective function of binding to Nur 77 and perhaps other steroid hormone receptors.

Implications for tumor biology

The extracellular matrix is known to regulate and direct normal cellular processes such as differentiation, proliferation and cell migration. Most tumorigenic cells, at least in culture, fail to deposit a matrix, or do so to a lesser degree than normal cells [1] and, as a consequence, have an added degree of freedom – their mobility is not limited by adhesion

to their own matrix. Thus, escape from the normal differentiation controls through loss of function and/or synthesis of cell adhesion molecules or modification of the cell/ECM interaction will be strongly favoured by selection and may contribute to the uncontrolled pattern of growth typical of malignant neoplasia [74]. From this reasoning, several studies have sought to link changes in integrin expression with the development of a metastatic potential in a variety of different types of tumor [75, 76]. Such changes that have been identified are then equated to alterations in the capacity of the tumor cells to migrate or to colonize different tissues. The realization that integrin engagement can protect against or promote apoptosis adds a new dimension to an understanding of anchorage-dependence and cell positioning [39].

In the circulation, metastasizing tumor cells adhere to other circulating host or tumor cells to form tumor cell emboli [77]. The reason for this requirement is not known although various suggestions have been made, including protection from the mechanical stresses of blood flow, escape from immune surveillance, and aiding in the arrest in the capillaries. As far back as twenty years ago it was reported that lung colonization by disaggregated cell suspensions of single tumor cells was never as successful as that achieved by preformed aggregates [78, 79]. The recent data suggest that the survival of cells detaching from a primary tumor mass may be seriously compromised unless they can form cell aggregates and thereby suppress apoptosis. This interpretation is also consistent with an alteration of integrin phenotypes on tumorigenic cells potentially being able to impart a more apoptotic resistant character. It is feasible that integrin-mediated signals from the matrix may direct the invasive processes (migration, metalloproteinase production etc) and also promote cell survival, with intercellular signaling within the emboli critical for continued tumor cell viability. Further, not only might the actual integrin expression be a defining factor in the acquisition of a metastatic phenotype, but also changes in downstream signaling effectors.

As is the case with tumor cell proliferation that becomes unrestrained by mutations or oncogenes, similar genetic changes can result in resistance to apoptosis by tumor cells. Thus loss, inactivation or mutation of p53 may provide a mechanism for avoiding some forms of apoptosis [80, 81] but other more subtle changes – perhaps involving integrin function – may also be the case in some cancers. For example, in normal lactating breast tissue, p53 is expressed in the milk-producing epithelial cells, but its pattern of expression is restricted to the cytoplasm with nuclear sparing [82], a pattern the authors suggest may provide a physiological pathway allowing rapid cell division during lactation. The same authors [82] have found that one third of breast cancers studies expressed wild type p53, but this also was sequestered in the cytoplasm. Since translocation of p53 from the cytoplasm to the nucleus appears to be integrin directed, at least in the LIM 1863 colon carcinoma cells, this finding may indicate a failure in integrin signaling as a mechanism of escaping apoptosis as a tumor control in some breast cancers.

It has long been appreciated that many anticancer treatments operate by inducing the target cells to undergo apoptosis. A question now being addressed in this burgeoning area of research is how information on the role of cell adhesion in directing this process can be applied to yield better drug regimes. One possible approach is suggested by recent experiments that have provided a greater understanding of multicellular drug resistance. A major problem in cancer treatment is the expression by tumors of either intrinsic (*de novo*) or acquired resistance to chemotherapeutic drugs [83]. Kobayashi *et al.* [84] showed that the expression of drug resistance in murine mammary tumor sublines to several alkylating agents could be fully recapitulated *in vitro* when the cells were grown as multicellular tumor spheroids, but not when the cells were cultured as monolayers. These findings suggested a method of acquired drug resistance in tumors based on the response of a cell population i.e. 'multicellular', as opposed to classic 'unicellular' mechanisms of resistance [84]. The mechanisms of this multicellular resistance are yet to be fully elucidated; however if it is in part mediated by the supression of apoptosis through signals delivered by homotypic adhesion, then this provides the possibility of more effective therapeutic intervention measures for patients with

cancer. These could be based on the role of potential 'anti-adhesives' (analogous to the antibody 23C6 in our system) which might enhance the ability of conventional cytotoxic drugs to kill tumors by disrupting intercellular contacts, and reversing the acquisition of drug resistance [85].

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