THE WALTER HERBERT LECTURE

Control of cell motility and tumour invasion by extracellular matrix interactions

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Summary Integrins are heterodimeric transmembrane proteins with large ectodomains and a short cytoplasmic tail inside the cell. They mediate cell adhesion to extracellular matrix proteins and to the surfaces of other cells. In many cases the sequence recognised by the integrins in the extracellular matrix proteins is the tripeptide Arg-Gly-Asp (RGD). Short synthetic peptides containing this sequence can inhibit invasion in vitro and tumour dissemination in vivo. Thus, the αβ₃ fibronectin binding integrin appears to be the key integrin in the invasion of at least melanoma, osteosarcoma and glioblastoma cells. Modulation of the level and activities of this integrin can suppress invasion, whereas the αβ₁ vitronectin binding integrin appears to be associated with increased invasiveness. There is increasing evidence that some of these effects are mediated through signals elicited by the binding of integrins to their target proteins. This possibility has generated a great deal of interest in the cytoplasmic molecules that might mediate the integrin-associated signalling.

Extracellular matrices can control cell motility and tumour cell invasion by forming tissue barriers and by serving as adhesive substrates. They also transmit signals to cells, both directly through the receptors that mediate adhesion and through growth factors and other cytokines bound to the matrix.

It has been long suspected that cell adhesion plays an important role in tumorigenicity and invasiveness. This notion is based in part on the observation in the early 1970s that fibronectin, a major component of the extracellular matrix, was absent from the matrix of many tumorigenic cell lines. Normal cells deposit an extracellular matrix around themselves and then become anchored to it. Tumour cells (and migrating embryonal cells) fail to deposit matrix, and this allows such cells to remain less attached and more mobile than normal cells. Thus, reinforced interaction of tumour cells with matrix has an inhibitory effect on cell proliferation and migration and converts the cells into non-tumorigenic cells (Giancotti & Ruoslahti, 1990; Schreiner et al., 1991). Moreover, the cell-cell adhesion molecule E-cadherin can curb the invasiveness of epithelial cells that express it (Behrens et al., 1989; Chen & Öbrink, 1991; Vleminkx et al., 1991). Finally, a gene termed DCC that appears to encode for an adhesion protein is lost in metastatic colon cancer cells (Vogelstein et al., 1989), suggesting that DCC also prevents cell invasion as well as possibly the relocation of cells to distant sites.

While the observations discussed above establish a role for cell adhesion in immobilising cells, it is clear that cell adhesion can also promote migration of normal as well as tumorigenic cells. Various kinds of cultured cells migrate on extracellular matrix substrates and cell-matrix, and cell-cell adhesion is thought to provide guidance and traction for cell migration in vivo (see Ruoslahti & Pierschbacher, 1987). Thus, for example, leukocytes lacking a group of integrin-type cell adhesion receptors are incapable of moving from the circulation into tissues (see Springer, 1990). Tumour dissemination and invasion can be prevented with peptides that can inhibit cell adhesion (Ruoslahti, 1991). It may be that strong adhesion immobilises a cell but moderate adhesions is needed for a cell to migrate (invade), because it provides the traction necessary for movement. Alternatively, it may be that the cell adhesion receptors generate regulatory signals in cells and that it is those signals that control cell migration and invasion.

There is another important way extracellular matrices control cell behaviours. Increasing evidence suggests that many, if not all, growth factors and cytokines are immobilised through binding to extracellular matrices or to cell surfaces (Ruoslahti & Yamaguchi, 1991). This short review discusses some of the new developments in the work dealing with the signalling aspect of cell-matrix interactions mediated by the integrin family of adhesion receptors.

Integrin-type adhesion receptors as signalling molecules

Integrins are a family of membrane glycoproteins consisting of two subunits α and β. Their properties have been extensively reviewed (Hemler, 1990; Springer, 1990; Ruoslahti, 1991; Hynes, 1992). Thirteen integrin α subunits and eight β subunits have been reported which, as far as it is known, combine to form at least 19 different heterodimers (Figure 1).

The recognition site for many of the integrins that bind to extracellular matrix and to platelet adhesion proteins is the tripeptide Arg-Gly-Asp, or RGD (Ruoslahti & Pierschbacher, 1987). A peptide sequence entirely different from RGD (GPEILDVPST) has been identified as the target sequence of the α₅β₃ integrin in the alternatively spliced CS-1 segment of fibronectin (Mould et al., 1990; Guan & Hynes, 1990).

At least six of the known integrins function in an RGD-dependent fashion (Figure 1). The remaining integrins are likely to recognise other sequences, such as the one from the CS-1 segment.

The presence of multiple integrins endows a cell with the capacity to recognise the cell's own extracellular matrix and matrices secreted by other cells. This recognition system is probably responsible for much of the positional information cells need for anchorage, polarity, differentiation and directed migration. Moreover, as discussed below, the ability of tumour cells to invade also appears to depend on the interaction of integrins with their ligands.

Adhesion peptides inhibit tumour dissemination

Tumour cell migration through amnion membrane tissue can be inhibited with synthetic RGD peptides in an in vitro tumour cell invasion assay (Gehlsen et al., 1988). This effect

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of the peptides in the invasion assay is receptor-specific; peptides that inhibit the fibronectin receptor (αβ integrin) best are most active, whereas a peptide that inhibits the vitronectin receptor (αβ integrin) better than the fibronectin receptor (Pierschbacher & Ruoslahti, 1988) has much less of an effect on the invasion. This is regardless of the fact that the test cells possess the vitronectin receptor.

The RGD peptides can also affect tumour dissemination in vivo. Several laboratories have published experiments in which dissemination of intravenously injected tumour cells in mouse tissues has been inhibited by a simultaneous injection of an RGD peptide (Humphries et al., 1986; Tressler et al., 1989; Saiki et al., 1989a,b). The dissemination and/or subsequent function of intravenously injected lymphocytes is also inhibited by RGD peptides (Ferguson et al., 1991), suggesting that a likely side effect of RGD peptide therapy might be perturbation of some immune functions.

The mechanism whereby the RGD peptides inhibit tumour invasion, dissemination and cell proliferation in the experimental systems described above is not clear, but the effect could be due to inhibition of cell adhesion. The loss of adhesion would deny the cells anchorage as well as traction for migration.

Another increasingly intriguing explanation is that the binding of the peptide to the fibronectin receptors could deliver a regulatory signal into the cells. That this could be the case is suggested by several observations. Increasing evidence from studies with lymphocytes, macrophages and other cells shows that the β integrins including αβ participate in signalling (Shimizu & Shaw, 1991; Schwartz et al., 1991). This seems to apply to various types of tumour cells also. Thus, cells grown in the presence of fibronectin are less tumorigenic than those grown in the presence of laminin (Terranova et al., 1984). Colon cancer cells respond to being grown in a collagen gel by differentiating, an effect that appears to be mediated by the αβ integrin (Pignatelli & Bodmer, 1989). Moreover, tumorigenic cells lacking the αβ integrin are incapable of migrating even on surfaces where this integrin plays no adhesive role (Bauer et al., 1992). The migratory behaviour can be restored by reintroducing the αβ integrin through gene transfer.

In contrast, ligands of the αβ integrin can enhance tumour cell invasion in a basement membrane invasion assay (Seftor et al., 1992). A possible mechanism for this effect is the induction of proteolytic enzymes capable of degrading extracellular matrix (Werb et al., 1989). Interestingly, an elevated expression of the αβ integrin has been found to coincide with the transition of melanomas into an invasive mode (Albelda et al., 1990). These observations strongly suggest the existence of an integrin-associated signalling system in which the various integrins mediate distinct signals (Figure 2). They also suggest ways of improving adhesion peptide design.

Considering the effect of the αβ integrin in invasion, an ideal anti-invasive compound might be one that is specific for the αβ integrin and affects it in such a manner that the result for the cell is the same as the lack of this integrin in the CHO cells. This would result in a migratory paralysis and the effect would not be neutralised by an invasion-promoting effect elicited through binding of the peptide to the α integrin. It is possible that the existing RGD peptides have that kind of an effect because they not only inhibit tumour cell (melanoma, glioma, sarcoma) invasion through amniotic membranes (Gehlsen et al., 1988), but also through matrigel (Seftor et al., 1992), which does not contain fibronectin. These observations suggest two modes of RGD peptide action in cancer therapy; they can be competitive inhibitors of adhesion or deliver inhibitory signals by acting as integrin agonists.

Possible mechanisms of integrin signalling

As discussed above, a vast body of evidence suggests that integrin ligation does not only mediate adhesion but sends signals into the interior of a cell. However, little is known about what might happen inside the cell as a result of integrin ligation. The integrin cytoplasmic tails are short (30–50 amino acids) and their sequences give few clues as to what molecules they might interact with. A possible exception is an alternatively spliced cytoplasmic domain of the β subunit we have found recently (Languino & Ruoslahti, 1992). It has some homology with the phosphotyrosine

![Figure 1 Integrin subunit combinations and the ligand specificities of the resulting integrin heterodimers.](image-url)
binding sequence motif, SH2, which is present in many regulatory proteins. Protein phosphorylation appears to be involved, because several of the integrin subunits are phosphorylated (Hirst et al., 1986; Freed et al., 1989; Hibbs et al., 1991; Hillery et al., 1991). Moreover, a 120–130 kDa protein has been identified recently, the phosphorylation of which in tyrosine residues occurs when cells spread on fibronectin (Guan et al., 1991; Kornberg et al., 1991). This protein may be present in the adhesion plaques where the fibronectin-binding integrin is also concentrated when cells attach to a fibronectin surface. The function of the 120–130 kDa protein is not known, but it is a good candidate for mediating some of the intracellular effects of fibronectin-induced (and perhaps other) cell adhesion.

We have recently found a new protein, which we have named peregrin, that is a also a possible messenger of signals originating at the integrins (Thompson et al., unpublished results). We discovered peregrin because it copurified with the αβ1 integrin in affinity chromatography. Peregrin is a nuclear protein that has several features in common with various transcription factors; it may transmit signals from alterations in cell adhesion to the nucleus.

**Conclusion**

Recent information on integrins suggests that, in addition to serving as the physical ‘hooks’ for cell adhesion, they transmit signals into cells. Much of the current work on integrins is directed at deciphering the molecular workings of these signalling events inside the cell. Two intracellular proteins that may be important in this regard have been isolated and others are undoubtedly in the pipeline. Progress in this area of cell adhesion will make important contributions to the understanding of contact inhibition and contact induction of differentiation and is also likely to be helpful in the design of cell adhesion-based approaches to anti-invasive tumour therapies.

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