Adhesion molecules in melanoma—more than just superglue?

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J R Soc Med 1996;**89**:393–395 SECTION OF ONCOLOGY, 14 JUNE 1995

SUMMARY

Malignant melanoma is increasing in incidence, and, though early lesions are readily treatable, systemic therapy for metastatic disease remains disappointing. Integrins are a family of cell-surface molecules that mediate adhesion between the cell and the extracellular matrix. One member of the integrin family, the $\alpha v\beta 3$ integrin, is associated with progression of melanomas, in that the most malignant cells express the highest levels of $\alpha v\beta 3$. Like many members of the integrin family, $\alpha v\beta 3$ recognizes the sequence Arg-Gly-Asp (RGD) in its ligands, and other molecules that contain this sequence will compete with the natural ligands (such as vitronectin) for binding. There is growing evidence that integrins function as receptors for signal transduction, and that integrin-mediated signalling can affect cell behaviour and even cell survival. Under certain circumstances, loss of integrin-mediated signalling will induce apoptosis, or programmed cell death, and we have demonstrated that melanoma cells treated with a cyclic peptide with high affinity for the $\alpha v\beta 3$ integrin will undergo apoptosis within three days. This mechanism might be exploited therapeutically.

INTRODUCTION

For cells to survive in an organized tissue they must adhere, first to each other, to form a functional 'sheet' as in epithelia, but secondly to their underlying extracellular matrix, the 'soup' in which cells are suspended. Adhesion is mediated by specialized organelles and by specific types of molecule. For example, cell-cell adhesion is mediated by cadherins, and cell-matrix adhesion by a family of specialized adhesion molecules called integrins[1]. Why should so many different types of adhesion molecule have evolved? Their diversity argues that their function is more than merely structural, and we shall illustrate this viewpoint with special reference to one type of integrin molecule closely associated with malignant melanoma.

INTEGRINS—A FAMILY OF SPECIALIZED ADHESION MOLECULES

The integrin family are among the best characterized adhesion molecules. They consist of an α and a β subunit, which link together to form heterodimers. The association of different α subunits with different β subunits accounts for the diversity of integrins, at least 20 different types of which are known to exist. Nevertheless, many have in common the property of recognizing and binding to a specific amino-acid sequence, Arg-Gly-Asp (RGD) which is found in a number of matrix molecules including fibronectin, vitronectin, and the von Willebrand factor. Although the recognition sequence is the same in many matrix molecules, the molecular conformation is probably different between different molecules, hence the differences in binding specificities between different integrins and different matrix molecules[2].

One integrin, formed by the association of αv and $\beta 3$ subunits, has particular affinity for vitronectin (and is often referred to as the vitronectin receptor). This integrin, $\alpha v\beta 3$, is found on vascular endothelium, in certain organs such as the kidney, and on melanoma cells, in which context its possible functions will be discussed here.

MALIGNANT MELANOMA—A MODEL OF TUMOUR PROGRESSION?

Melanomas might provide a particularly useful model of tumour progression to study in the laboratory. They yield cells which display a range of metastatic potential in laboratory studies, and clinically, they also exhibit a wide spectrum. This extends from pre-malignant lesions such as dysplastic naevi, tumours in a horizontal growth phase (superficial spreading melanoma and lentigo maligna), tumours in a vertical growth phase (nodular melanoma), to overtly metastasizing cells. We know that invasive melanoma cells are associated with the integrin $\alpha v\beta 3$, and that there is a correlation between metastatic potential and

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αvβ3 levels[3]. Why this should be so is unclear. It seems likely that the processes of invasion and metastasis require specific cell-adhesive functions to be competent, but there are other properties of integrins such as αvβ3 which might also contribute to the phenomenon.

INTEGRINS—ATTACHMENT OR SIGNALLING

As well as mediating cell attachment integrins are now thought to have important roles in signal transduction. This has been suggested by studies of the organization of integrins in cells, where they participate in complex structures known as focal adhesion complexes. These structures are found at points of attachment between cells and matrix, and connect the extracellular matrix indirectly to actin filaments, major components of the cytoskeleton[4]. Among the molecules which congregate with integrins into focal adhesion complexes is a specific tyrosine kinase, known as focal adhesion kinase (FAK)[5]. Since tyrosine kinases are associated with signal transduction[6], e.g. from the epidermal growth factor receptor, the existence of FAK suggests that integrins, too, might initiate or mediate signals to the cell interior, perhaps stimulated by cellular attachment to a specific matrix molecule.

INTEGRIN-MEDIATED SIGNALLING AND CELL SURVIVAL

If integrins do mediate signalling, could they have more profound effects on cell growth than simply facilitating attachment? Several lines of evidence suggest that this is so. Mutant melanoma cells which lack the αv gene lose their ability to form tumours when injected into nude mice. However, if the intact αv gene is replaced by transfecting the mutant cells, such that they are able once again to express αvβ3, they regain their ability to form tumours[7]. A useful *in vitro* model of αvβ3-dependent melanoma cell growth can be constructed, since melanoma cells bind to type 1 collagen. Initially, the adhesion is mediated by integrins of the β1 family, but after a short time in culture these are succeeded by αvβ3, which binds to a cryptic RGD site that is only exposed after degradation of the collagen molecule by enzymes produced by the melanoma cells. Melanoma cells growing in 3-dimensional denatured type 1 collagen gels are thus dependent on αvβ3 for attachment. The mutant melanoma cells which lack αv will also bind to type 1 collagen, but in the absence of intact αvβ3 will undergo apoptosis after around three days. This phenomenon has been termed *anoikis*[8,9], from the Greek for 'homelessness'. Upon transfection of the mutant melanoma cells with the αv gene, intact αvβ3 function is restored, and the cells will no longer undergo apoptosis in collagen.

There are other methods by which the function of αvβ3 can be disrupted in melanoma cells. For example, monoclonal antibodies will bind to integrins and can have similar effects to αv gene mutations. However, it has been known for some years that peptides which contain the RGD recognition sequence will bind to integrins in competition with matrix molecules, the extent of the competition depending on their relative affinities[10]. Linear RGD-containing peptides may have only limited affinity for integrins such as αvβ3 compared with intact vitronectin, and if injected will disappear rapidly from the circulation because of protease degradation and renal excretion. Cyclic peptides, however, offer the opportunity to construct a RGD-containing peptide with high affinity for a particular integrin (manipulated by altering the amino acid sequence around RGD), and in a form which is more resistant to protease digestion and excretion[11]. One peptide in particular, abbreviated cRGDfV, has been shown to have high affinity for the vitronectin receptor αvβ3[12]. It can induce regression of αvβ3-negative tumours grown on chick chorioallantoic membrane by an indirect, anti-angiogenic action, via induction of αvβ3-expressing endothelial cells to undergo apoptosis by the anoikis pathway[13].

We have been studying the effects of this cyclic peptide on αvβ3-expressing melanoma cells growth in type 1 collagen gels, and have observed a similar effect to that seen in αv-deficient mutant cells grown in the same way. The melanoma cells initially attach and appear to be healthy. After about three days, control cells continue to grow, but cells treated with cRGDfV begin to round up, and after 2–3 more days will die. These cells exhibit all of the features of death by apoptosis, such as chromatin condensation, formation of apoptotic bodies, and endonuclease activation resulting in characteristic DNA 'laddering' in agarose gels. The mechanism by which anoikis leads to death in these cells is unclear; it has been suggested that *bcl*-2 downregulation may be involved, but so far we have not observed this in our cell system.

FUTURE DIRECTIONS

What might be the therapeutic applications of compounds such as cRGDfV? To treat cancer by selectively inducing apoptosis in the most malignant cells is an enormously attractive idea. However, further studies are required to determine whether these agents have any activity *in vivo*. There may be a number of problems to resolve, for example the affinity of cRGDfV for laminin receptors[12], and the possibility that endothelial αvβ3-expressing endothelial cells might 'mop up' circulating peptide and reduce the amount available to reach a tumour. None the less, it may be possible to design new compounds in the future—could a RGD-containing molecule be fashioned which is conjugated to a radionuclide, or to a prodrug, without affecting its affinity for a target receptor such as αvβ3? Will compounds

which target $\alpha v\beta 3$ be as potent as anti-angiogenesis agents as the initial studies suggest? Targeting of integrin-mediated signalling may one day be therapeutically useful not only in malignant melanoma and other cancers but perhaps in non-cancerous disease as well.

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(Accepted 31 October 1995)