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GLYCOBIOLOGY OF LAMININ-INTEGRIN INTERACTION AND THE METASTATIC PHENOTYPE

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Metastasis, the spread of cancer cells throughout the organism, is a multistep and selective process (Price et al., 1986). In this process, neoplastic cell interaction to extracellular matrix (ECM) components is unequivocally essential, allowing us to delineate some metastatic phenotype features on the grounds of ECM recognition (Brentani, 1989).

BASEMENT MEMBRANES AND METASTASIS

Basement membrane, a specialized ECM separating parenchymal cells from their supporting connective tissue (Leblond & Inoue, 1989), represents one of the selective barriers to invasion process. Following the fate of carcinoma cells, from the primary tumor to the onset of metastasis, we would observe that neoplastic cells must disrupt their basement membrane in order to invade the stromal connective tissue (Albrechtsen et al., 1981), where there are vascular elements which vehiculate neoplastic cells through the organism. Once in the circulation, malignant cells must evade host immune response. Evading mechanisms include neoplastic cell interaction with platelets and leukocytes, embolization and adhesion to endothelial cells as well as to subendothelial basement membranes, all of them mediated by ECM components (Blood & Zetter, 1990). Circulating cells are purged easier than adhered ones. Adhered cells must degrade basement membranes in order to get into the interstitial stroma, where they may proliferate, forming a metastatic nodule.

Recognition and adhesion to laminin - a glycoprotein specific from basement membranes (Timpl et al., 1979; Buck et al., 1990) - propitiate delivery of specific collagenases, which will favour the invasion process (Turpeenniemi-Hujanen et al., 1986). Recent data suggest that signals transduced by ECM receptors induce collagenase and stromelysin gene expression (Werb et al., 1989).

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INTEGRINS AS LAMININ RECEPTORS

Specific cell surface receptors mediate recognition and adhesion to laminin. Multiple distinct laminin receptors have been described in mammalian cells until now (Table). Among these receptors some are members of the integrin family (Hynes, 1987; Ruoslahti & Giancotti, 1989).

As discussed elsewhere in this issue (Brentani, 1991), integrins are integral glycoproteins, heterodimeric in composition, which present a dual function. Their cytoplasmic domain interacts with cytoskeletal proteins, such as vinculin (Burridge & Feramisco, 1980), talin (Burridge & Connel, 1983), actin (Tamkun et al., 1986) and fibulin (Argraves et al., 1989); their extracellular domain is related to cell-cell recognition or extracellular matrix protein binding.

The term integrin was coined in 1986 by Hynes and colleagues (Tamkun et al., 1986), and it was proposed to denote the role of these integral proteins in the association between extracellular matrix and cytoskeleton. Indeed, matrix fibrils containing fibronectin or laminin are organized in bundles in the extracellular space (Fig. 1), aligned with microfilament bundles containing actin (Hynes & Destree, 1978) and also with the molecules mentioned above, as illustrated in Fig. 2.

As pointed in Table, laminin binding integrins are related to VLA (very late antigen) subfamily. As discussed in this issue, this interaction seems to be independent on Arg-Gly-Asp (RGD) sequence, differently from almost all integrin-ECM components interactions. Thus, it seems clear that must be at least two different mechanisms for integrins interactions with their ligands.

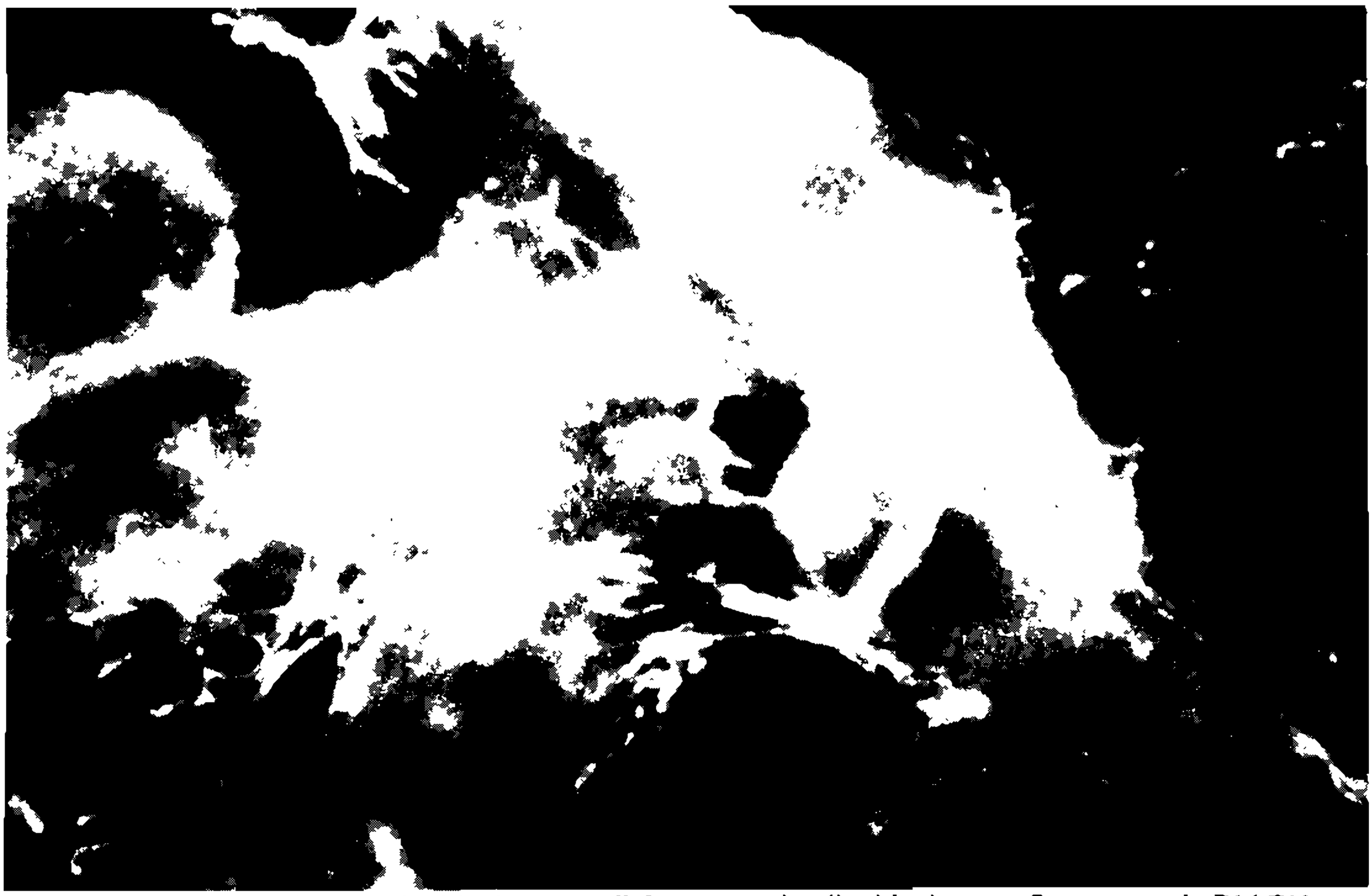

Fig. 1: fibronectin bundles organized in the extracellular space, visualized by immunofluorescence in B16-F10 murine melanoma cells.

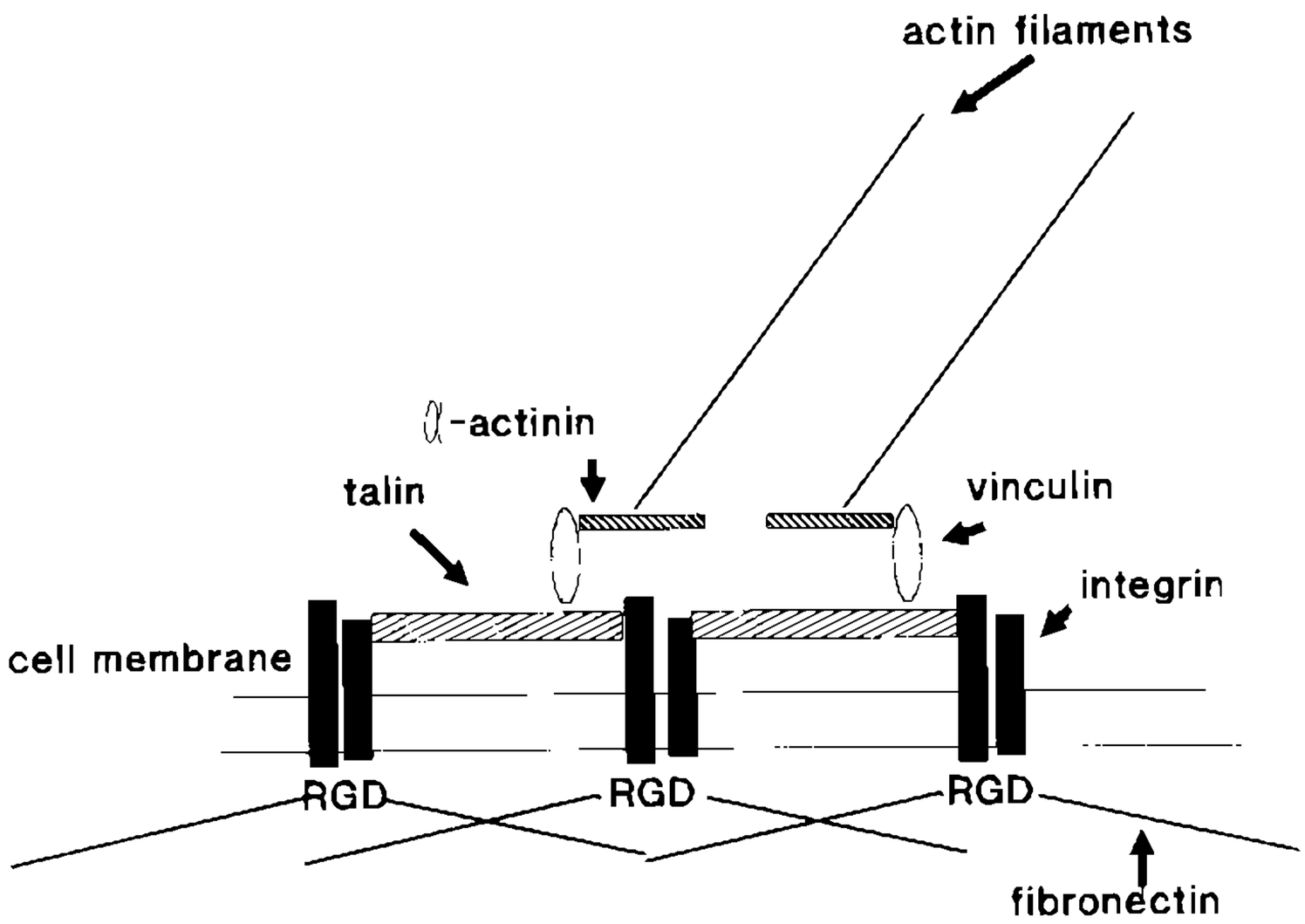

Fig. 2: model representing the association between extracellular matrix proteins and cytoskeleton, mediated by integrins.

ON THE GLYCOSYLATION OF LAMININ BINDING INTEGRINS

Analyzing the Table carefully, we can observe the existence of glycoconjugates among non-integrin laminin receptors (sulfatides and heparan-sulfate proteoglycan). Laminin interaction with these receptors is clearly dependent on receptor sugar moieties, pointing to laminin as a lectin-like molecule, providing one more example of the role of carbohydrate in cell recognition. This, among several examples, reveals a current tendency on re-evaluating oligosaccharide function in glycoproteins, besides conferring physical properties as solubility, protease resistance and conformational and thermal stability (Rademacher et al., 1988; Paulson, 1989).

We have adressed here the role of sugar moieties in laminin interaction to a mouse melanoma integrin. The model studied is the B16-F10 cell line. B16-F10 cells do not metastasize spontaneously, however they present a high lung colonization potential in experimental metastasis assays. This model is suitable for approaching the second step of the metastatic pathway: circulating neoplastic cell arrest and invasion. It was possible to characterize at least two different VLA integrins among

TABLE

Mammalian Laminin Receptors

Receptor	Model	Reference
Non-integrin laminin receptors		
68 kDa	fibrosarcoma cells	Malinoff & Wicha, 1983
	mammary gland tumor cell	Rao et al., 1983
	macrophages	Huard et al., 1986
120 kDa	NG 108-15 (neural hybrid cells), 3T3 fibroblasts,	
	embryonic chicken brain	Smalheiser & Schwartz, 1987
67 and 120-140 kDa	PC-12 (pheochromocytoma),	
	neuron cells	Douville et al., 1988
69 kDa	endothelial cells	Yanariello-Brown et al., 1988
Galactosyltransferase (60 kDa)	B16-F10 (melanoma cells)	Runyan et al., 1988
CBP-35 (Mac 2)	macrophages	Woo et al., 1990
Heparan-sulfate proteoglycan	placental cells	Isemura et al., 1987
Sulfated glucolipids (sulfatides)	melanoma cells	Roberts et al., 1985
Integrin laminin receptors		
VLA (very late antigen) Subfamily		
VLA-1	neurons	Ignatius & Reichardt, 1988
VLA-2	LOX cells (melanoma cells)	Elices & Hemler, 1989
	endothelial cells	Languino et al., 1989
	colon carcinoma cells	Lotz et al., 1990
VLA-3	MG-63 (osteosarcoma cells)	Hynes et al., 1989
	keratinocytes	Carter et al., 1990
VLA-6	platelets	Sonnenberg et al., 1988
	melanoma cells	Ramos et al., 1990
	macrophages	Shaw et al., 1990
$\alpha 6\beta 4$	colon carcinoma cells	Lotz et al., 1990

B16-F10 surface glycoproteins. One binds laminin, and the other fibronectin (Fig. 3). This result is in line with other data, and confirms the existence of different VLA integrins in the same cell type (Ramos et al., 1990). Laminin binding integrin was characterized as a lectin-like molecule, since its interaction with laminin was significantly reduced after ligand deglycosylation.

Tunicamycin, an inhibitor of biosynthesis of N-linked oligosaccharide chains (Elbein, 1987) and carbohydrate oxidizing agents, as periodate (Bobbit, 1956) were used to assess the role of glycoconjugates on cell adhesion.

Tunicamycin-treated cells undergo important morphological alterations, becoming round in appearance, which suggests altered cytoskeletal organization. A significant decrease in lung colonization potential was observed in these treated cells (Irimura et al., 1981). Adhesion to laminin was significantly impaired (Humphries et al., 1986b). Interestingly, we could observe that B16-F10 laminin binding integrin is tunicamycin-sensitive, as it was not exposed at the cell surface level after this treatment. These results could tentatively explain changes observed on cell shape, lung colonization and capacity of adhesion to laminin.

Cells exposed to oxidizing agents (periodate, e.g.) were less adherent on extracellular matrix proteins relative to control cells (Cheresh et al., 1986), reinforcing the role of glycoconjugates, as gangliosides, on cell adhesion. However, glycoprotein sugar residues are also periodate-sensitive. Analysis of cell lysates, separated electrophoretically and transferred onto nitrocellulose, defined laminin interaction to its integrin as a periodate-sensitive binding and fibronectin interaction to its integrin as a periodate-resistant one (Fig. 4). These results demonstrate an alternative mechanism for interaction between integrins and their ligands, which is dependent upon sugar residues, besides the cannonical RGD dependent one. In this regard, glycosylation processes should be considered as a mechanism for variable specificity among integrin family members.

Glycoprotein processing inhibitors, generally glycosidase or mannosidase inhibitors, which are less toxic than tunicamycin, have been used to assess the role of oligossacharides in cell-cell or cell-ECM interaction (Elbein, 1987; Trudel & Holland, 1989). Swainsonine, a mannosidase II inhibitor, has been widely studied. Swainsonine-treated B16-F10

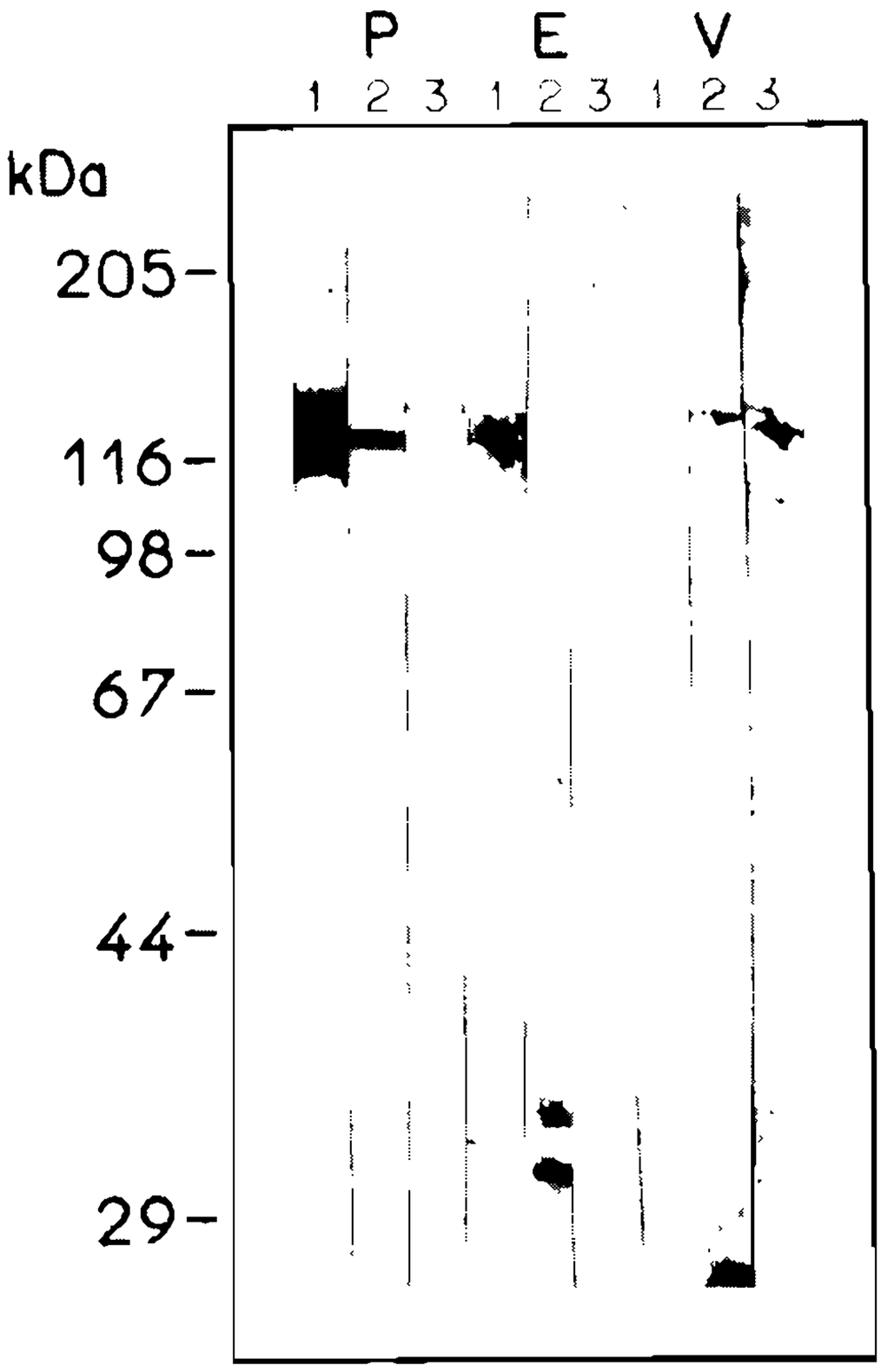

Fig. 3: characterization of different VLA integrins in B16-F10. Cell lysates were purified through affinity chromatography on laminin-Sepharose columns, separated on SDS-PAGE and electrotransferred onto nitrocellulose filters. P represents cell lysate before chromatography (Pre-elution); E represents eluted material; and V represents the pass through material (Void). Laminin and fibronectin binding activity are represented in lanes 1 and 3, respectively. Immunoreactivity to anti- β1 chain is represented in lane 2. Note that there are at least two different β 1 integrins. E2 binds laminin and V2 binds fibronectin.

cells presented a decrease in their lung-colonizing ability, related to a reduced cell retention at lung during the first 24 h after intravenous injection (Humphries et al., 1986a). A decrease on cell adhesion on laminin-coated surfaces was also observed (Humphries et al., 1986b). In the same model, we could determine a small but significant decrease on swainsonine-treated cell adhesion to laminin, although no differences were observed relative to the integrin expression at the cell surface level. Nevertheless, an increase in the global dissociation constant at equilibrium, determined on direct binding assays was observed, suggesting a role for oligosaccharides as affinity modulators.

Swainsonine inhibits complex-type oligosaccharide formation (β 1-6 branched oligosaccharides),

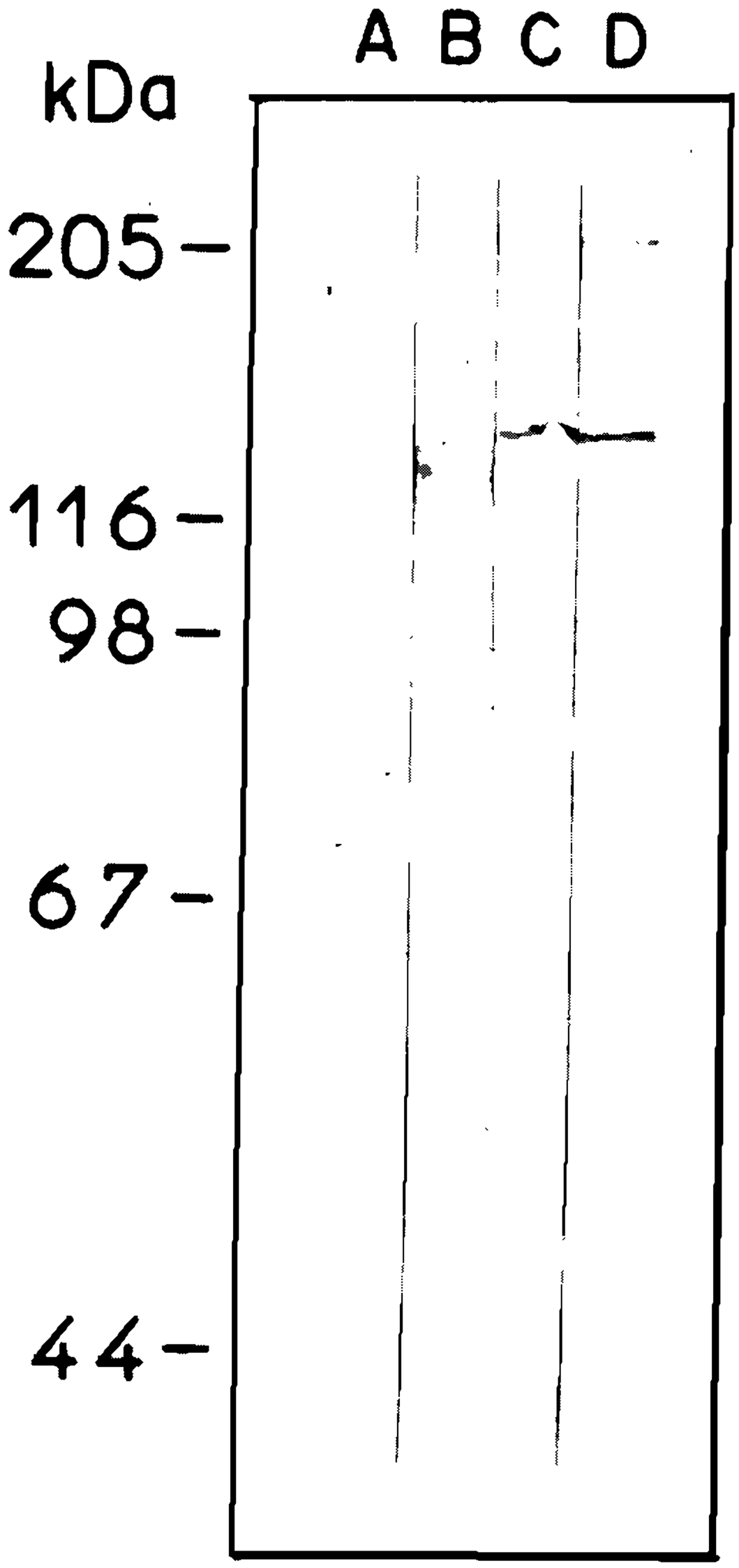

Fig. 4: the integrin which binds laminin is periodate-sensitive. Lane B represents control laminin binding activity. Lane A was treated with periodate before incubation with laminin (note the abolishment of laminin binding activity). Lane C represents fibronectin binding activity. Lane D was periodate-treated, denoting fibronectin binding resistance to receptor sugar residues oxidation.

which are recognized by leukoagglutinin from *Phaseolus vulgaris* (L-PHA). This oligosaccharide formation is dependent upon glycosyltransferase (GlcNAc-transferase V) activity (Dennis, 1988). This enzymatic activity is significantly increased in malignant relative to benign human breast lesion (Dennis & Lafert, 1989), and it can be assessed by L-PHA reactivity. The integrin we studied presents L-PHA reactive oligosaccharides, suggesting it as a substrate for GlcNAc-transferase V activity. We can therefore speculate that metastatic cells presenting enhanced GlcNAc-transferase activity could express a laminin binding integrin glycoform displaying greater affinity to laminin relative to that presented by non-metastatic cells. Experimental evidence supports the idea that β 1-6 branched Asn-oligosaccharides favour invasion of basement membranes (Yagel et al., 1989). Further studies are necessary in order to evaluate the role of these oligosaccharides in metastasis, as well as to determine their potential diagnostic or prognostic value.

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