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The Role of P13-Kinase on Melanoma Cell Adhesion and Migration

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Abstract : Integrins are responsible for anchoring cells to the extracellular matrix and also can initiate intracellular signal pathways. The interaction between integrins and the extracellular matrix results in activation of PKC, PTKs, p21Ras, MAPK and PI3-kinase. The $\alpha4\beta1$ integrins are expressed mainly on haematopoietic cells and also appear on several tumour types. The adhesion of $\alpha4\beta1$ to VCAM-1 is an essential step for lymphocyte extravasation during the inflammatory response. Furthermore, the interaction of $\alpha4\beta1$, expressed on tumour cells, with VCAM-1, might have a role in tumour cell extravasation.

We examined the role of PI3-kinase activation in $\alpha4\beta1$-mediated melanoma cell adhesion and migration. Inhibition of PI3-kinase did not have any effect on cell adhesion. However, the migration of HMB2-$\alpha4$ and DX3 cells on fibronectin, but not VUP-$\alpha4$, was inhibited by PI3-kinase inhibitor. $\alpha v\beta3$-mediated migration in VUP cells which had been transfected with $\beta3$ cDNA to create expression of the $\alpha v\beta3$, did not involve PI3-kinase activity, suggesting that this was not the case. On the other hand, VUP-$\alpha4$ cells were found not to express p110α, a specific form of PI3-kinase, which may be involved in melanoma migration, whereas the HMB2-$\alpha4$ cells expressed this isoform.

Key Words: integrin, PI3-kinase, migration, melanoma, adhesion

P13-Kinaz'ın Melanoma Hücre Bağlanması ve Göçündeki Rolü

Özet : İntegrinler hücreler arası proteinlere bağlanmalarının yanısıra hücre içi sinyal yollarını da aktive etme yeteneğine sahiptirler. İntegrinlerin extrasellüler proteinler ile etkileşmesi sonucu protein kinaz C, protein tyrozin kinaz, p21ras, MAPK ve PI3-kinaz gibi sinyal moleküllerinin aktivasyonu mümkün olmaktadır. $\alpha4\beta1$ integrini çoğunlukla kan hücrelerinde bulunmaktadır bunun yanında birkaç çeşit tumor hücresinde de varlığı tespit edilmiştir. Dokularda oluşan hasarın tamiri sırasında lökositlerin hasarlı dokuya geçişindeki en önemli basamaklardan bir tanesi $\alpha4\beta1$-VCAM-1 ilişkisidir. Bu ilişkiyi kullanan tumor hücrelerinin de kan damarlarından dokulara geçtiği düşünülmektedir.

Bu çalışmada PI3-kinaz moleküllünün melanoma hücrelerinin bağlanma ve göç yeteneklerine olan etkileri araştırılmıştır. PI3-kinazın baskılanması hücre bağlanmasını etkilememiştir. Fakat, HMB2-$\alpha4$ ve DX3 melanoma hücrelerinin fibronektin üzerindeki göçü PI3-kinaz inhibitörü tarafından bloke edilmiştir. PI3-kinaz inaktivasyonu VUP-$\alpha4$ hücrelerinin göçünde ise etkili olmamıştır. $\alpha v\beta3$ integrini transfer edilmiş VUP hücreleriyle yapılan deneyler ise bu integrininde etkili olmadığını göstermiştir. Diğer bir yandan, VUP-$\alpha4$ hücrelerinde p110α'in bulunmadığı tespit edilmiş ve melanoma hücre göçünde bu formun etkili olabileceği fikrine varılmıştır.

Anahtar Sözcükler: integrin, PI3-kinaz, göç, hücre bağlanması

Introduction

Integrins are a major family of cell surface receptors composed of heterodimers, α and β subunits, that are responsible for anchoring cells to the extracellular matrix (ECM). They also can interact with cellular signal transduction molecules and transmit signals to the nucleus following stimulation received from interaction with the ECM (1,2). Integrin signalling regulates various important events during embryogenesis and development, normal cell proliferation, differentiation, migration and survival and, in cancer cells, uncontrolled cell growth and metastasis (3). The signals generated by integrin-ECM interactions result in the activation of a number of signal pathways, including inositol lipid synthesis (4), activation of protein kinases (5), PKC pathways (6) and changes in intracellular pH and Ca^{2+} levels (7,8). Recently, the integrin-mediated activation of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3-kinase) and p21Ras (9,10,11) have also been demonstrated, firmly linking the adhesive mechanisms of the cell with its proliferation machinery.

The α4β1 integrins, which are members of the integrin family, are particularly interesting due to their involvement in various developmental and physiological processes (12). Two α4 integrins, α4β1 and α4β7, interact with an alternative-spliced segment of fibronectin (V25 or CS1) and with the counter-receptors, VCAM-1 and MAdCAM-1. The α4 integrins are suggested to have a role in haematopoiesis, lymphoid and myeloid cells, lymphocyte homing and metastasis (13,14,15). The α4β1 integrin is expressed primarily on various leukocytes such as normal peripheral blood B and T cells and monocytes, but not on neutrophils (16), and also α4 integrin may appear on some nonhaematopoietic cells such as muscle cells and nervous system cells (17). Several tumour types other than melanoma have also been shown to express α4β1, including neuroblastoma, renal cell, rhabdomyosarcoma and osteosarcoma (18,19,20).

In this paper, our aim is to describe the effect of PI3-kinase on α4β1-mediated melanoma cell migration and adhesion. PI3-kinase is composed of 110 and 85kDa subunits (21) and the p110 subunit of PI3-kinase has been shown to be a catalytic domain of this enzyme containing the lipid kinase activity (22,23). The p85 subunit is a regulatory domain which has many sequence similarities to the protein-protein interaction domains known to occur in other signalling enzymes. The p85 subunit contains two SH2 domains, a SH3 domain and a break point cluster region (BCR) domain. Members of the PI3-kinase family showed high homology in the lipid kinase domain and the catalytic domain but the p110γ PI3-kinase form was shown to lack the p85 binding domain.

Some reports have suggested that PI3-kinase is involved in integrin-mediated signal transduction cascades as well as in regulating integrin-mediated cellular events, such as cell migration (24) and adhesion (25). Platelet aggregation, induced by thrombin or activating peptides, is mediated by activated αIIbβ3 integrin and can be inhibited by blocking PI3-kinase activity with specific inhibitors such as Wortmannin (26). Wortmannin was also shown to inhibit β1 integrin-mediated adhesiveness following stimulation of the CD7 antigen on T cells. In

neutrophils, β2-mediated adhesion stimulates PI3-kinase activity (25) and, in osteoclasts, αvβ3-mediated adhesion stimulates activation and association of PI3-kinase with c-Src (27). In human melanoma cells, migration on fibronectin, αvβ3 integrin-mediated adhesion on vitronectin and actin stress fibre formation were also inhibited by Wortmannin in a dose-dependent manner (28), while a possible role for PKC also has been described in experiments on PI3-kinase induced cell motility (24).

Materials and Methods

Cell Lines and Cell Culture

Various human melanoma cell lines were used in this study; HMB2, α4-expressing HMB2-α4 and DX3 human malignant melanoma cell lines are cutaneous melanoma lines, originally derived from metastases. In addition, VUP, which is an ocular melanoma line derived from a primary choroidal tumour and α4-expressing derivative VUP-α4 were also used.

The cells were cultured in sterile plastic tissue culture flasks in E4 medium supplemented with 10% (v/v) heat inactivated foetal calf serum (GIBCO BRL Paisley, Scotland) and L-glutamine (1mM final concentration) as adherent monolayers. Melanoma cells were incubated at 37ºC in 8% CO_2/92% air in the humidified atmosphere provided by an incubator.

Antibodies

The monoclonal antibodies which were used here are as follows: anti-α4, 7.2 antibody, the function of which is unknown (Marshall et al., unpublished; produced in-house); anti-α4, HP2/1 blocking antibody (Dr. F. Sanchez-Madrid); anti-α5, P1D6 blocking antibody (Life Technologies, UK); anti-αvβ3, LM609 blocking antibody (Chemicon International, UK); anti-αv, 17E6 blocking antibody (29, E. Merk, Germany); anti-α3, P1B5 blocking antibody (Life Technologies, UK).

Synthesis of Purified Substrates

CS1 Peptide

The α4β1 binding site in the alternatively spliced type III connecting segment of fibronectin is a 24-amino acid sequence (CDELPQLVTLPHPNLHGPEILDVPST) called CS1 peptide that was synthesised by the Peptide Synthesis Laboratories under the direction of Dr. Gerard Evan at ICRF Lincoln's Inn Fields, London. Amino acids shown in bold represent the minimal sequence required for cell binding.

rs VCAM-1

Pure recombinant soluble VCAM-1 was supplied by British Biotechnology Ltd., Abingdon, Oxford, UK.

Adhesion Assay

Human melanoma cells were cultured as detailed previously. Cell adhesion assays were performed in 96-well plates (Falcon 3912; Beckton Dickinson). Wells were coated for 60 min at 37ºC with 50µl aliquots of various concentrations of human fibronectin, (Sigma Chemicals Ltd., Poole, UK), vascular cell adhesion molecule (rsVCAM-1 British Biotech., UK) or CS1 peptide diluted with Dulbecco's PBS. Any potential nonspecific cell adhesion sites were blocked for 1h at 37ºC with 100µl of 0.1% (w/v) heat-denatured BSA in PBS. The cells were detached with 0.25% trypsin/EDTA, resuspended in 400µl complete medium containing 0.37MBq [^{51}Cr] and incubated for 45 min at 37ºC. After the incubation time, the cells were washed three times with Tris-buffered saline (TBS) pH 7.4 (-24mMTris/HCL, 137mM-NaCl, 2.7mM-KCl, 0.1%BSA, 2mM-Glucose) and resuspended to a final density of $3x10^5$ cell/ml in Tris buffer. Cell suspensions containing $1.5x10^4$ cells treated with or without PI3-kinase inhibitor (LY294002) were added to each well of the 96-well plates and allowed to attach to the substrata for 60 min at 37ºC in an incubator. Non-adherent cells were removed by rinsing the plates in a bath of TBS (supplemented with 1mM-$CaCl_2$ and 0.5mM-$MgCl_2$). After two washes, the 96-well plates were cut into individual wells and the radioactivity associated with each well TS determined in a gamma counter (1261 Multigamma; LKB Wallac, Bromma, Sweden). The percentage of adherent cells was determined by a comparison of the residual radioactivity with the total input.

Migration Assay

Cell migration assays were performed using Transwell chambers (Costar, UK) containing 8µm-diameter pore size polycarbonate membranes. The undersurface of the membrane was coated by incubation with a 200µl volume of various extracellular matrix proteins (10µg/ml of Fn, 20µg/ml of VCAM-1, 50µg/ml of CS1) for 1h at 37ºC and then the substrate solution was discarded and inserts were washed twice with sterile PBSA. After a final wash, PBSA was replaced with 0.1% (w/v) BSA in PBSA to block non-specific binding and the wells were incubated for 30 min to 1h at 37ºC. While the plates were in the incubator, cells were harvested with 0.25% trypsin/EDTA and washed three times with E4 containing 0.5% heat-denatured BSA. The same medium was also placed in the lower chamber. Cells ($1x10^5$/100µl) treated with or without LY294002 in the assay medium were added to the upper chamber and incubated at 37ºC for 24h. The cells in both the upper and the lower chambers were detached with 0.25% trypsin/EDTA and counted on a CASY-II cell analyser (Scharfe System, Germany). The total cell number was determined and the percentage of migration was calculated.

Inhibition of Migration Assay

The cells were incubated with various antibodies (HP2/1 (anti-α4, 10µg/ml), 17E6 (anti-αv, 2µg/ml), P1D6 (anti-α5, 1:50)) on ice for 20 min and then washed three times with 0.5%

BSA in E4. After the last wash, the cells were resuspended in assay medium and 100µl of cells and antibody complex were placed into the upper chamber of a transwell insert. Similar concentrations of antibodies were added into the lower chamber and the migration assay was carried out as described above.

Flow Cytometry

The expression of integrins was assayed by indirect immunofluorescence using a flow cytometric technique. Melanoma cells were harvested with 0.25% trypsin/EDTA, resuspended in complete medium and washed twice with cell wash buffer (0.1% BSA, 0.1% Azide in PBSA). Cell suspensions containing $2x10^5$ cells/50µl were added to each well of 96-well plates and 50µl of control or experimental antibodies were also added to each well. After a 30 min incubation at 4ºC, cells were washed three times with cold wash buffer and resuspended in wash buffer containing FITC-RAM (DAKO). After another 30 min incubation at 4ºC, cells were washed with wash buffer four times and resuspended in 10% (v/v) formaldehyde in PBSA. Those preparations analysed the next day were kept at 4ºC overnight. Cells were resuspended into 400µl of PBSA and analysed with a flow cytometer (FACScan; Becton Dickinson) using Consort 30 software.

MTT Dye Reduction Assay

Monolayer HMB2-α4 and VUP-α4 melanoma cells in exponential growth phase were harvested and incubated with various dilutions of LY294002 (Calbiochem, UK) for 1h at 37ºC. The excess drug was washed off with culture medium, a single cell suspension was prepared and the cell number determined using a haemocytometer. Samples were resuspended in fresh medium to give a density of $2.5x10^4$/ml for HMB2-α4 and $5x10^4$/ml for VUP-α4 cells. Aliquots (200µl) of the cell suspensions were placed into each of 88 wells of a 96-well microtitre plate. The initial row of 8 wells was filled with 200µl of medium alone to serve as a blank. Plates were then incubated in an 8% CO_2 atmosphere at 37ºC. The number of living cells was measured each day. At the end of the exposure time, 20µl of MTT dye solution (5mg/ml in sterile PBSA) was added to each well and the plates were incubated for a further 2 h. The medium containing MTT solution was then gently removed from the wells by aspiration leaving the reduced tetrazolium salt present as blue crystals in the wells. The tetrazolium salt was then solubilised by addition of 200µl of DMSO to each well, followed by agitation using a plate shaker for 10 min. Absorbance at 540 nm was determined by use of a Dynatech. MR5000 (Dynatech Lab, USA) plate reader with a reference beam of 690nm. Values obtained for the medium blanks automatically were subtracted. Each drug concentration was repeated 4 times per experiment. The results of repeat wells within the same experiment were averaged and the SD within each experiment was always <10%.

Results

Effect of PI3-kinase on Melanoma Cell Adhesion and Migration

The consequence of PI3-kinase inhibition on melanoma cell adhesion and migration was investigated by using the specific PI3-kinase inhibitor LY294002. The effect of this agent, which inhibits ATP-binding activity of PI3-kinase, is shown in Figure 1. Basically the treatment of VUP-α4 cells with this inhibitor at concentrations ranging from 5 to 10μg/ml had no effect upon either adhesion or migration, on a range of substrates.

In contrast, substantial effects on one of these characteristics were obtained when HMB2-α4 cells were used in similar experiments. Thus, as shown in Figure 2, panel A, there was no effect of LY294002 on cell adhesion but, as documented in Figure 2, panel B, there was a substantial reduction in the migration of these cells on a fibronectin substrate. This meant that at a concentration of 10μg/ml, the migratory capacity of these cells was reduced by about 75% on this substrate. Interestingly there was no significant effect of equal doses in the migratory capacity on VCAM-1 (Figure 2, panel B). Experiments using an additional melanoma line known to express α4 at its surface (DX3) gave similar though less dramatic results (Figure 3). The

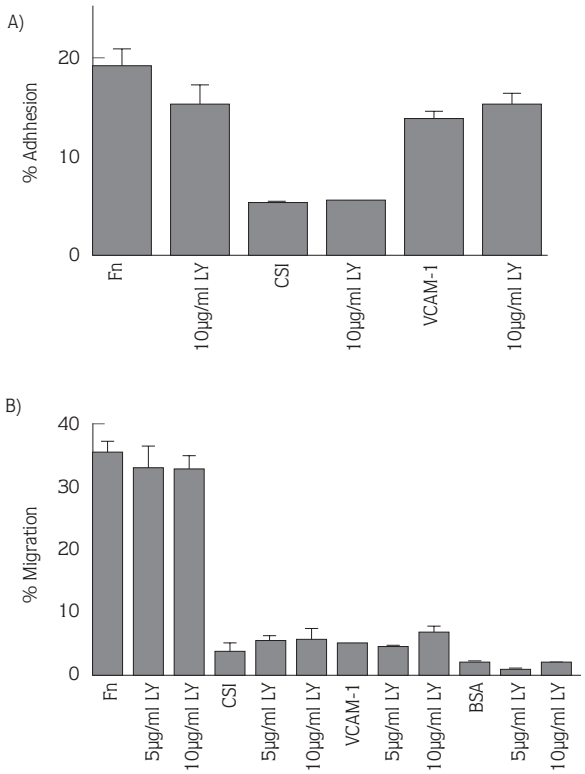

Figure 1. Effect of PI3-kinase inhibition on α4β1-mediated adhesion (A) and migration (B) in VUP-α4 melanoma cells. 5-10μg/ml LY294002 was added to cells (A) that were plated out onto 96-well plates coated with 10μg/ml Fibronectin, 20μg/ml CS1 and 10μg/ml Vascular cell adhesion molecule-1 overnight. After labelling cells with ^{51}Cr, an adhesion assay was carried out as described in Materials and Methods. (B) After addition of 5-10μg/ml LY294002, cells were transferred onto Transwell inserts and migration assay was carried out. Data represent three independent experiments. $p<0.05$.

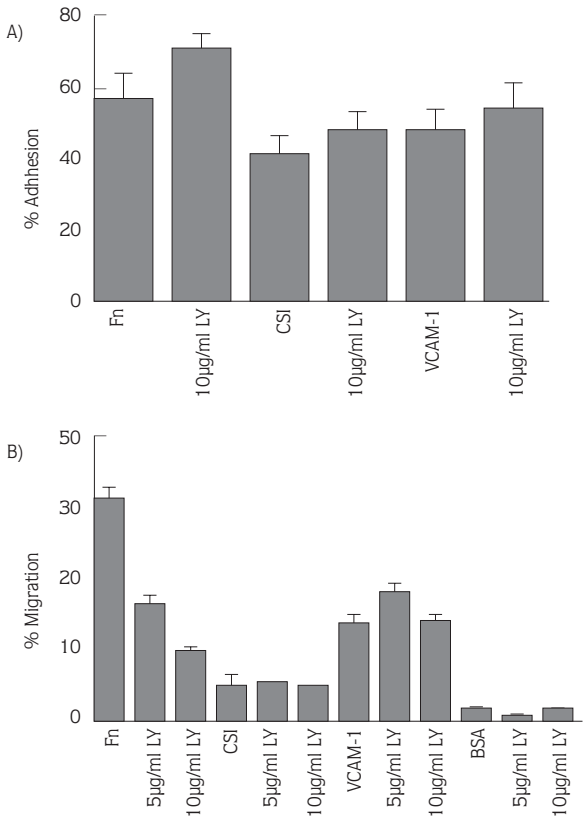

Figure 2. Effect of PI3-kinase inhibition on $\alpha 4\beta 1$-mediated adhesion (A) and migration (B) in HMB2-$\alpha 4$ melanoma cells were treated with 5-10µg/ml LY294002. Data represent three independent experiments. Figure A) $p<0.05$ (significant), figure B) $p<0.02$.

migration of these cells on fibronectin was reduced by 30% ($p<0.05$) in the presence of 10µg/ml LY294002 (Figure 3, panel B). The demonstration that LY294002 inhibited specifically the migratory capacity of HMB2-$\alpha 4$ cells, and to a lesser extent DX3 cells, on fibronectin substrates but not on other $\alpha 4$ substrates suggested that integrins other than $\alpha 4\beta 1$ might be involved in this response. The refractory nature of VUP-$\alpha 4$ cells to this specific inhibitor could also indicate that this cell type does not express the requisite integrin which relies upon PI3-kinase activity. To address this possibility we examined patterns of integrin expression on the small panel of melanoma cell lines shown in Table 1. The most obvious difference between HMB2-$\alpha 4$ and DX3 cells on the one hand and the VUP-$\alpha 4$ cells on the other, appeared to be the lack of expression of the $\alpha v\beta 3$ heterodimer by VUP-$\alpha 4$ cells. Therefore, VUP-$\alpha v\beta 3$ transfected cell lines were used to determine the effect of $\alpha v\beta 3$ integrin. As shown in Figure 4, PI3-kinase inhibitor did not have any effect on melanoma cell migration, even in the present of $\alpha v\beta 3$ integrin. To evaluate which integrins regulated migration on the fibronectin substrate, we performed a series of Transwell migration assays in the presence of various anti-integrin antibodies. The results are shown in Figure 5. It can be seen (Figure 5,

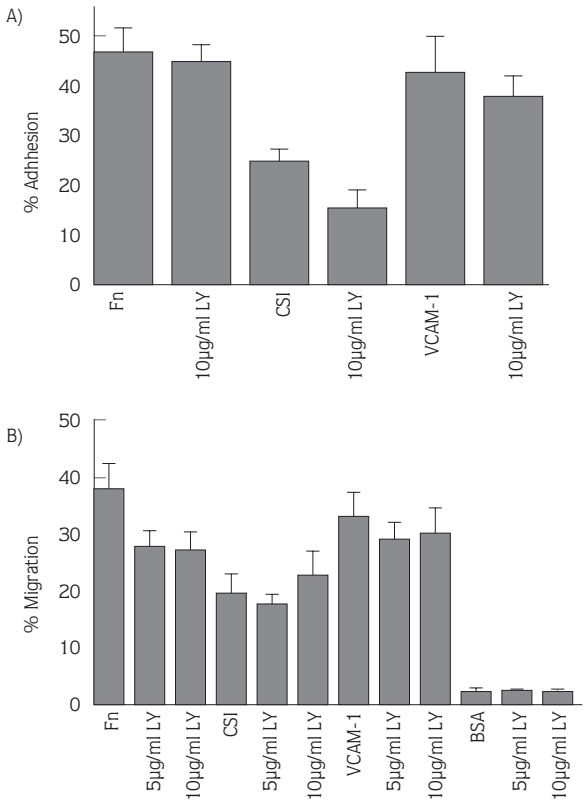

Figure 3. Effect of PI3-kinase inhibition on $\alpha 4\beta 1$-mediated adhesion (A) and migration (B) in DX3 melanoma cells. Data represent three independent experiments.

Table The level of integrins expression was investigated in melanoma cells by flow cytometer. The cells were incubated with anti-integrin antibodies and then coupled with secondary antibody, FITC-RAM. After 30 min incubation, cells were washed and fixed with 10% formaldehyde in PBSA. Cells were analysed with a flow cytometer using Consort 30 software. "+" shows the level of integrin expression.

Cells	DX3	HMB2	HMB2-α4	VUP	VUP-α4
Integrins					
αv	++	++	++	+	+
α3	+++	+++	+++	++	++
α5	++	+	+	++	++
α4	++	-	++	-	++
αvβ3	++	++	++	-	-

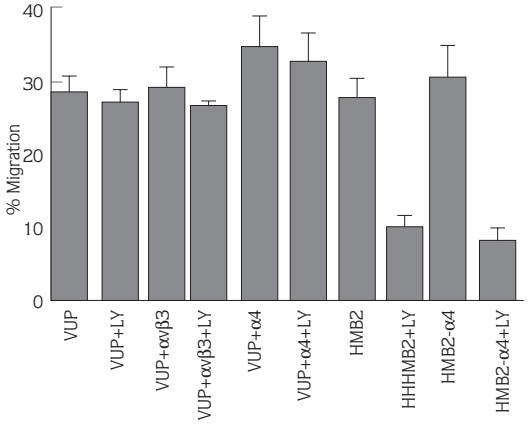

Figure 4. VUP, VUP-α4, VUP-αvb3, HMB2 and HMB2-α4 melanoma cells were plated on transwell inserts coated with 10µg/ml fibronectin. After 24 hours, cells were harvested and % of migration was calculated. Data represent three independent experiments.

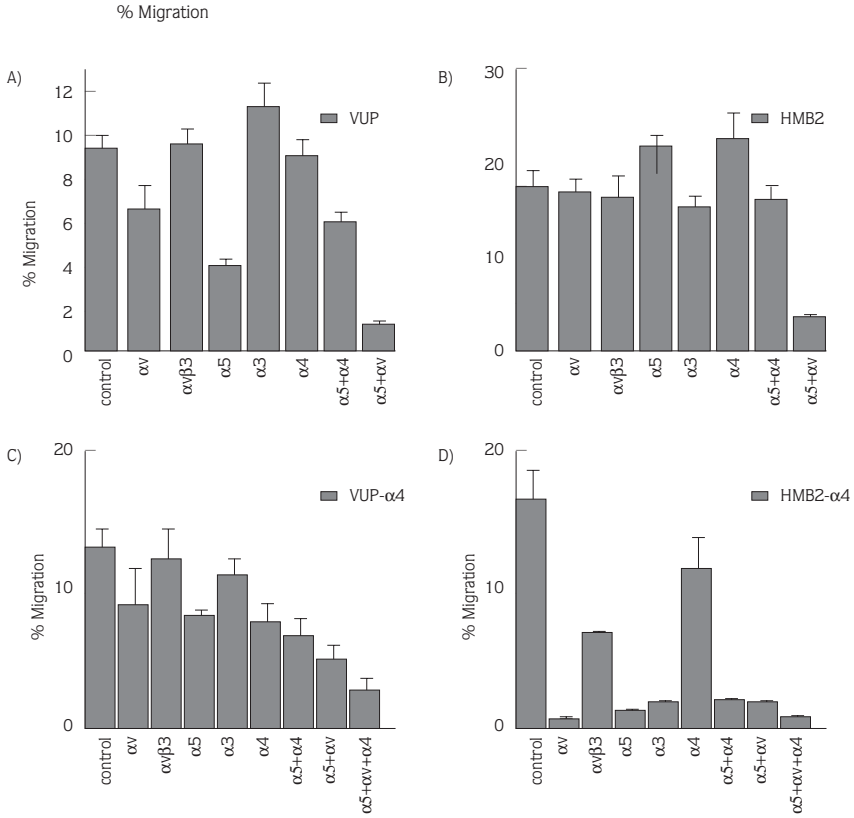

Figure 5. Panel A, VUP cells; Panel B, HMB2 cells; Panel C, VUP-α4 cells; Panel D, HMB2-α4 cells. Experiment was repeated three times.

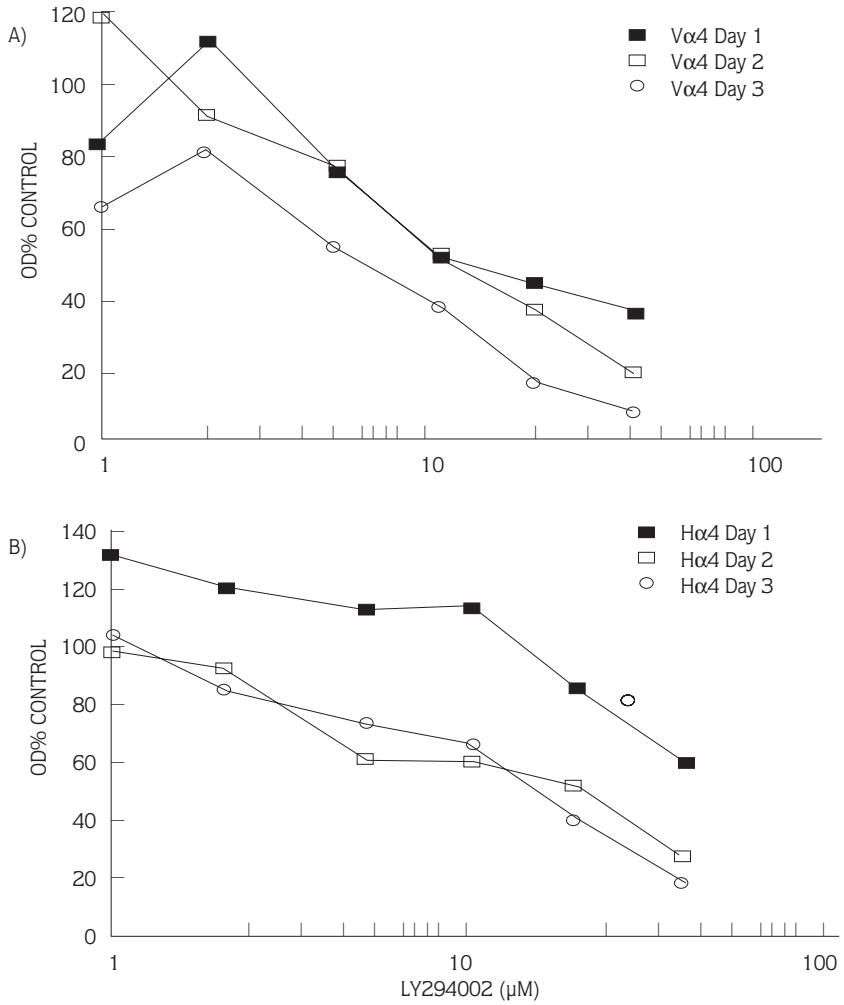

Figure 6. Toxicity of LY294002 was determined by MTT assay for both VUP-α4 (Panel A) and HMB2-α4 (Panel B) melanoma cells. Predetermined cell numbers were incubated with various concentrations of LY294002 during the experiment at 37ºC. Excess drug was washed off with 10% FCS and cells were plated out onto 96-well plates. After each day, 20μl of 5mg/ml MTT was added to each well and incubated for a further 2h. Medium was discarded and 200μl of DMSO was used to dissolve the dye. Density of dye was measured by plate reader (ratio between OD of plates at 570nm). Results are the mean of quadruplicate wells. Standard deviation less than 10%.

panel A) that the majority of the migratory capacity in VUP cells was provided by α5, with perhaps a minor component from an αv-containing heterodimer (VUP cells express αvβ1), and that, broadly speaking, this pattern of activity was preserved in the VUP-α4 cells (Figure 5, panel C). In contrast, the HMB2 cells appeared to depend for their migration on an interaction between α5 and αv in that significant inhibition was achieved only in the combined presence of antibodies to these two subunits (Figure 5, panel B). The pattern of behaviour was very different in the HMB2-α4 cells. Now antibodies to αv, α5 and α3 were as effective in blocking migration as were the combinations of αv+α5 antibodies (Figure 5, panel D). These results appear to indicate that, in this cell line, de novo expression of α4, while not affecting the levels of expression of other integrins, has an effect upon their functional capacity. The blocking activity of LY294002 on the migratory capacity of cells of this sub-line may then be a reflection of the involvement somehow of PI3-kinase in this process.

Discussion

Effects of PI3-kinase on α4β1-Mediated Melanoma Cell Adhesion and Migration

A few recent reports have suggested that PI3-kinase is required for integrin-mediated cell migration and adhesion (24). In human melanoma cells, αvβ3-integrin-mediated cell adhesion and migration on vitronectin was inhibited by a PI3-kinase inhibitior, suggesting a role for PI3-kinase in the regulation of the affinity of αvβ3 integrin (28). PI3-kinase, acting on adhesion and actin reorganisation in interleukin-8 induced neurophils, has been documented (28,30). The stable association of PI3-kinase with FAK also was described in NIH 3T3 cells that were stimulated by cell adhesion and PDGF *in vivo* and by autophosphorylation of recombinant FAK *in vitro*.

The inhibition of PI3-kinase had no effect on the adhesion and migration of VUP-α4 cells plated on fibronectin, CS1 or VCAM-1 (Figure 1). Both of these cellular functions were also unaffected in HMB2-α4 (Figure 2) and DX3 (Figure 3) cells plated on either CS1 or VCAM-1, indicating that α4β1-mediated cell adhesion and migration may not require the activation of PI3-kinase in these two melanoma cell lines. However, the migration of HMB2-α4 and DX3 cells on fibronectin, but not their adhesion, was inhibited significantly by PI3-kinase inhibition. These results suggest that different integrins, or possible different types of PI3-kinase, may differentially regulate the migration of HMB2-α4 and DX3 cells rather than VUP-α4 cells. The expression of the αvβ3 integrin was shown by FACS analysis to be lacking in VUP-α4 cells but to be present in HMB2-α4 cells. Initially, therefore, it was thought that the αvβ3-mediated cell migration in HMB2-α4 may require PI3-kinase activity. However, the inhibition of PI3-kinase activity in VUP cells transfected with β3 cDNA did not have any effect on cell migration on fibronectin suggesting that this was not the case (Figure 4). Interestingly, the expression of different types of PI3-kinases in these two melanoma cell lines was observed such that clone o of the (p110) catalytic subunit of PI3-kinase was expressed in HMB2-α4 cells but barely

detected in VUP-α4 cells (Dr. B. Vanhaesbrook, personal communication). Experiments involving the transfection of VUP cells with p110 cDNA may reveal whether this type of PI3-kinase is responsible for the differences in migration of HMB2-α4 and VUP-aα4 cells on fibronectin. On the other hand, the investigation of the involvement of different integrins in fibronectin migration using inhibitory antibodies showed that in VUP-α4 cells, migration was regulated mainly by α5β1, α4β1 and αvβ1, or the combination of these three integrins. In HMB2-α4 cells, the integrins αv, α5 and α3 showed a significant regulatory function in cell migration which was not evident in the parental cells, suggesting that transfection of HMB2-α4 cells with a human α4 cDNA has a functional effect on other integrins. This may be one explanation for the altered behaviour of HMB2-α4 cells in either adhesion or migration or it may be that effects on these parameters require inhibition of various signal pathways.

References

1. Schwartz, M.A., Integrins, oncogenes and anchorage independence. J. of Cell Biology, 139: 575-578, 1997.
2. LaFlamme, E.S. and Auer, L.K., Integrin signalling. Cancer Biology, 7: 111-118, 1996.
3. Varner, J.A. and Cheresh, D.A., Integrins and cancer. Current Opinion in Cell Biology, 8: 724-730, 1996.
4. McNamee, H.P., Ingber, D.E. and Schwartz, M.A., Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown. J. Cell Biology, 121: 673-678, 1993.
5. Cary, L.A., Han, D.C., Polte, T.R., Hanks, K.S. and Guan, J-L., Identification of $p130^{cas}$ as a mediator of focal adhesion kinase-promoted cell migration. J. of Cell Biology, 140: 211-221, 1998.
6. Newton, A.C., Regulation of protein kinase C. Current Opinion Cell Biology, 9: 161-167, 1997.
7. Schwartz, M.A., Lechene, C., Adhesion is required for protein kinase C-dependent activation of the Na^+/H^+ antiporter by platelet-derived growth factor. Proc. Natl. Acad. Sci. USA, 89: 6138-6141, 1992.
8. Leavesley, D.I., Schwartz, M.A. and Cheresh D.A., Integrin β1- and β3-mediated endothelial cell migration is triggered through distinct signalling mechanisms. Journal Cell Biology, 121: 163-170, 1993.
9. Chen, Q., Kinch, M.S., Lin, T.H., Nuridge, K. and Juliano, R.L., Integrin mediated cell adhesion activates mitogen activated protein kinases. Journal Biology Chemistry, 269: 26602-26605, 1994.
10. Guinebault, C., Payrastre, B., Racaud-Sultan, C., Mazarguil. H., Breton, M., Mauco, G., Plantavid, M., and Chap, H., Integrin-dependent translocation of phosphoinositide 3-kinase to the cytoskeleton of thrombin-activated platelets involves specific interactions of p85α with actin filaments and focal adhesion kinase. Journal Cell Biology, 129: 831-842, 1994.
11. Kapron-Bras, C., Fitz-Gibbon, L., Jeevaratnam, P., Wilkins, J. and Dedhar, S., Integrin clustering stimulates p21ras activation. Journal Biology Chemistry, 268: 20701-20704, 1993.
12. Yang., J.T., Rayburn, H. and Hynes, R.O., Cell adhesion events mediated by α4 integrins are essential in placental and cardiac development. Development, 121: 549-560, 1995.
13. Williams, M., Hughes, P.E., O'Toole T.E. and Ginsberg, M.H., The inner world of cell adhesion: integrin cytoplasmic domains. Trends Cell Biology, 4: 109-112, 1994.

14. Holzmann, B. and Weissman, I.L., Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4 like α chain associated with either of two integrin β chains, one of which is novel. Journal of Embryology, 8: 1735-1741, 1989.

15. Qian, F., Vaux, L.D. and Welssman, L.I., Expression of the integrin α4β1 on melanoma cells can inhibit the invasive stage of metastasis formation. Cell, 77: 335-347, 1994.

16. Rosenblatt, K.A., Koepsell, T.D., Daling, J.R., Lyon, J.L., Swanson, G.M., Greenberg, R.S. and Weiss, N.S., Antigenic-stimulation and the occurrence of chronic lymphocytic-leukemia. American Journal of Epidermis, 134: 22-28, 1991.

17. Rosen, G.D., Sannes, J.R., Lachance, R., Cunningham, J.M., Roman, J. and Dean, D.C., Roles of the integrin VLA-4 and its counter receptor VCAM-1 in myogenesis. Cell, 69: 1107-1119, 1992.

18. Yoshihara, T., Esumi, N., Humphries, M.J. and Imashuku, S., Unique expression of integrin fibronectin receptors in human neuroblastome cell lines. International Journal of Cancer, 51: 620-626, 1992.

19. Tomita, Y., Saito, T. and Saito, K., Oile, T., Shimizu, F. and Sato, S., Possible significance of VLA-4 for haematogenous metastasis of renal cell cancer. International Journal of Cancer, 60: 753-758, 1995.

20. Bednarczyk, L.J. and McIntyre W.B., Expression and ligand-binding function of the integrin α4β1 on neural-crest derived tumour cell lines. Clinical Experimental Metastasis, 10: 281-290, 1992.

21. Otsu, M., Hiles, I., Gout, I., Fry, M.J., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A.D., Morgan, S.J., Courtneidge, S.A., Parker, P.J. and Waterfield, M.D., Characterisation of 2 85 kD proteins that associate with receptor tyrosine kinases, middle-T/pp60c-Src complex, and PI3-kinase. Cell, 65: 91-104, 1991.

22. Hiles, I.D., Otsu, M., Volinia, S., Fry, M.J., Gout, I., Dhand, R., Panayotou, G., Ruiz-Larrea, F., Thompson, A., Totty, N.F., Hsuan, J.J., Courtneidge, S.A., Parker, P.J. and Waterfield, M.D., Phoshatidylinositol 3-kinase-structure and expression of the 110kD catalytic subunit. Cell, 70: 419-429, 1992.

23. Gout, I., Dhand, R., Panayotou, G., Fry, M.J., Hiles, I., Otsu, M. and Waterfield, M.D., Expression and characterisation of the p85 subunit of the phosphatidylinositol 3-kinase complex and a related p85-beta protein by using the baculovirus expression system. Journal of Biochemistry, 288: 395-405, 1992.

24. Derman, M.P., Toker, A., Hartwing, J.H., Spokes, K., Falck, J.R., Chen, C-S., Cantley, L.C. and Cantley, L.G., The lipid products of phosphoinositide 3-kinase increase cell motility through protein kinase C. Journal Biology Chemistry, 272: 6465-6470, 1997.

25. Lofgren, R., Ng-Sikorski, J., Sjolander, A.and Andersson, T., β2 integrin engagement triggers actin polymerisation and phophatidylinositol triphosphate formation in non-adherent neurophils. Journal Cell Biology, 123: 1597-1605, 1993.

26. Zhang, J., Shattil, S.J., Cunningham, M.C. and Rittenhouse, S.E., Phosphoinosite 3-kinase-gamma and p85 phosphoinositide 3-kinase in platelets-relative activation by trombin receptor or beta-phorbol myristate acetate and roles in promoting the ligand-binding function of alpha (IIB) beta (3) integrin. Journal Biology Chemistry, 271: 6265-6272, 1996.

27. Hruska, K.A. and Rolnick, F., Engagement of the osteoclast integrin αvβ3 by osteopontin stimulates phophatidylinositol 3-hydroxyl kinase activity. Endocrinology, 12: 354-359, 1995.

28. Metzner, B., Elsner, J., Dobos, G., Kownatzki, E., Parlow, F., Schraufstatte, I. and Norgauer, J., $[Ca]_i$-transients and catin polymerisation in human neutrophils under stimulation with GROα and complement fragments C5a. Agents Actions, 42: 101-106, 1994.

29. Marshall, J.F., Rutherford, D.C., McCartney, A.C.E., Mitjians, F., Goodman, S.L. and Hart, I.R., αvβ1 is a receptor for vitronectin and fibrinogen and acts with α5β1 to mediate spreading on fibronectin. Journal of Cell Science, 108: 1227-1238, 1995.

30. Van Kessel, K.P.M., Park, C.T. and Wright, S.D., A fluorescence microassay for the quantitation of integrin-mediated adhesion of neutrophil. Journal Immunology Methods, 172: 25-31, 1994.