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Modulation of the urokinase-type plasminogen activator receptor by the β6 integrin subunit

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Abstract

Over-expression of components of the urokinase system is well documented in cancer and is thought to enable tumour cells to migrate and invade. Changes in integrin expression are also a common feature of tumours and have been linked to changes in protease activity. It has been shown that the αvβ6 integrin is neo-expressed in a number of epithelial carcinomas and in wound healing situations. We therefore investigated whether αvβ6 is able to modulate a key regulator of proteolysis, the urokinase receptor. We report that epithelial cells expressing full-length αvβ6 exhibit decreased urokinase receptor expression and function. Furthermore, this novel modulation requires the C-terminal 11 amino acids of the cytoplasmic tail of the β6 integrin subunit. Cells expressing αvβ3, however, did not affect urokinase receptor expression. De novo expression of β6 by melanoma cells and β3 by epithelial cells did not influence urokinase receptor expression or function, suggesting that modulation of urokinase system is both integrin subunit and cell-specific.

Keywords: Urokinase; αvβ6; Integrin; Cytoplasmic tail; Cancer; Epithelial cells; Melanoma cells

Tumour progression involves the disruption of anatomical barriers and penetration of tumour cells into normal adjacent host tissues, as well as the infiltration of normal host cells into the tumour. Such migratory and tissue remodelling events involve complex interactions between integrins and matrix degrading protease systems, such as the plasminogen activator system [1].

Urokinase-type plasminogen activator (uPA) converts enzymatically inactive plasminogen abundant in the extracellular fluid into plasmin, which can degrade most components of the extracellular matrix (ECM) either directly or via activation of matrix metalloproteases (MMPs), thus promoting cellular migration (for review, see [2]). uPA is secreted as an inactive precursor (pro-uPA) that binds with high affinity to a specific cell-surface glycosylphosphatidylinositol-anchored receptor (uPAR) [3]. Concomitant binding of pro-uPA to uPAR and plasminogen to non-specific binding sites at the cell surface strongly enhances plasmin generation [4]. Tumour cells and their surrounding stromal cells show high expression of uPA and uPAR, which are both independent prognostic markers in human cancer. Somewhat paradoxically, high levels of plasminogen activator inhibitor-1 (PAI-1) are also seen in tumours [5].

Integrins are cell-surface heterodimeric molecules that are receptors for the extracellular matrix. Integrins translate messages from the ECM into changes in cytoskeleton and intracellular signalling pathways [6]. Integrins have been implicated in various biological processes including angiogenesis, wound healing, and tumour cell invasion [7]. For example, expression of the fibronectin-binding αvβ6 integrin has been shown in malignant oral and colonic epithelium but not in normal epithelium [8,9]. More recently several studies have found high expression of αvβ6 in oral squamous cell and

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other carcinomas such as colon, lung, and advanced stages of breast carcinoma and ovarian carcinoma [10–12]. A role for αvβ6 in tissue repair and remodelling has been suggested since increased αvβ6 expression is seen on wound keratinocytes [13]. Recently, we have shown that heterologous expression of αvβ6 in oral keratinocytes led to enhanced MMP-9 secretion, which was functional in mediating cell migration [14] and invasion [15].

Recent studies have suggested a direct link between the plasminogen activator system and integrins. uPAR has been recognised as a multi-functional protein that can directly interact with integrins to initiate intracellular signalling events and may influence multiple biological events such as cell adhesion, migration, and proliferation [16]. In this study we demonstrate that in oral keratinocytes, αvβ6 plays an important role modulating cell-surface uPA proteolytic activity by regulating uPAR expression. The β6 integrin subunit mediates decreased uPAR expression, through its cytoplasmic domain. This suggests that precisely regulated levels of proteolysis, preventing excessive plasmin proteolysis in cancer, may be regulated by integrins. Moreover, this effect appears to be both cell and integrin subunit specific since melanoma cells transduced with β6 and epithelial cells over-expressing β3 do not modulate uPAR expression or function.

Materials and methods

Cell lines. VB6 [17] is a high αvβ6-expressing oral SCC population generated by retroviral infection of V3 SCC cells [8] with pBabe puro retroviruses encoding wild-type β6 cDNA. C1 cells are the null-infectants and express 5–10% of the β6 expressed by VB6. V3 cells were separately retrovirally infected with a mutant-β6 construct lacking the C-terminal 11 amino acids to create the VB6Δ11aa line [18]. These cells were sorted with magnetic beads coated with the anti-β6 antibodies, 10D5 and E7P6 (Chemicon, Harrow, UK), until αvβ6 expression was similar to that of VB6 [18]. V3 cells were also transfected with full-length β3 integrin to create an αvβ3-expressing cell line, V3B3 [19]. Three human melanoma cell lines MeWo, DX3, and A375P [20] were also infected with pBabe puro β6 (wild-type) or pBabe puro retroviruses. After three rounds of magnetic bead sorting with anti-β6 antibodies, E7P6 and 10D5 (Chemicon [17]), DX3β6, MeWoβ6, and A375Pβ6 and their puromycin-resistant counterparts (DX3puro, MeWopuro, and A375Ppuro) were generated. Cell-surface expression of αvβ6 was monitored by flow cytometry using mouse monoclonal antibody E7P6 (Chemicon).

Cell culture. Oral keratinocytes were maintained in keratinocyte growth medium (KGM) comprising three parts Dulbecco's modified Eagle's medium (DMEM) and one part Hams's F12 medium (Life Technologies, Paisley, UK) containing 10% foetal calf serum (FCS) (PAA Labs, Yeovil, UK). Medium was supplemented with 10 ng/ml epidermal growth factor (EGF), 0.5 μg/ml hydrocortisone, 5 μg/ml insulin, 10^{-10} M cholera toxin, 1.8×10^{-4}M adenine (Sigma Chemical, Poole, UK), 2.5 μg/ml fungizone, 100 IU/ml penicillin, and 100 IU/ml streptomycin (Life Technologies). Flow cytometric analyses to confirm the integrin profile of the cells were performed (results not shown but described previously [17]). Cells were used from sub-confluent cultures from early passages (<10). Melanoma cells were maintained in growth medium comprising DMEM containing 10% FCS and supplemented with 100 IU/ml penicillin, 100 IU/ml streptomycin, and 1 μg/ml puromycin (Sigma Chemical).

Flow cytometry. Flow cytometric analysis was used to determine the dual expression of αvβ6 and uPAR expression on the β6-transfected melanoma cells. Following trypsinisation cells were washed three times with PBS + 10% FCS and then incubated with anti-αvβ6 mAb, E7P6 at 10 μg/ml on ice, followed by an anti-mouse RPE-conjugated secondary antibody (Dako, Ely, UK). The cells were then stained with a rabbit polyclonal anti-uPAR antibody, 399R (American Diagnostica, Axis-Shield Diagnostics, Upton, UK), at 10 μg/ml, followed by an anti-rabbit FITC-conjugated secondary antibody (Dako). Between each antibody incubation step cells were washed in PBS + 10% FCS. Cells were then analysed for expression of αvβ6 and uPAR using a FACScaliber cytometer (Becton–Dickinson, Cowley, UK) running Cell Quest software.

Northern blotting. Total cellular RNA was isolated using the acid guanidinium thiocyanate–phenol–chloroform method of Chomczynski and Sacchi [21]. The RNA was purified through isopropanol and ethanol washes and 5 μg of RNA was electrophoresed in 1% agarose gels containing 10% formaldehyde and transferred to a nylon membrane (Hybond N, Pharmacia Biotech, Amersham, UK) by capillary action using 10× sodium chloride/sodium citrate (SSC). The filter was probed for 2 h at 68 °C with ^{32}P radiolabelled cDNA specific for the full-length uPA mRNA. This probe was generated by restriction digestion of fragments from pcDNA3.1 vectors. Three stringency washes using 2× SSC, 0.1% SDS, followed by a high stringency wash with 0.2× SSC, and 0.1% SDS were carried out to eliminate non-specific probe interactions. Filters were autoradiographed using X-ray films with intensifying screens at −80 °C. Bands were quantified using NIH image software and normalised to 18S ribosomal RNA bands.

Western blotting. Cell membrane extracts were obtained by hydrophobic detergent phase separation using Triton X-114, as previously described [22], which serves to concentrate membrane proteins such as uPAR. Ten micrograms of protein, as determined by DC protein assay (Bio-Rad, Hemel Hempstead, UK), was separated on a 10% acrylamide gel under non-reducing conditions and transferred to nitrocellulose membrane. Soluble uPAR (from M. Ploug, Finsen Laboratory, Copenhagen, Denmark) was loaded as a positive control. The blot was blocked with PBS/0.5% Tween 20/5% milk powder and washed 3 × 15 min in PBS/0.5% Tween 20. The blot was then probed with 10 μg/ml R4 monoclonal anti-uPAR antibody (from G. Hoyer-Hansen, Finsen Laboratory, Copenhagen, Denmark) for 1 h, washed as before, and incubated with horseradish-peroxidase-conjugated secondary antibody (Dako) for 30 min, followed by a further wash in PBST. Blots were developed using Enhanced Chemiluminescence according to the manufacturer's protocol (Amersham–Pharmacia, Little Chalfont, UK).

Cell-surface plasmin generation. Cell-associated plasminogen activation was measured spectrophotometrically using a plasmin-specific fluorogenic substrate [23]. Confluent monolayers were washed in serum and additive free medium and pre-incubated with 2% BSA/50 mM Tris/100 mM NaCl/0.01% Tween 80 for 5 min at 37 °C. Human Lys-plasminogen (1.8 μg/ml, Enzyme Research Labs, Swansea, UK) was added together with the plasmin-specific peptide substrate H-D-Val-Leu-Lys-7-amido-4-methylcoumarin (HDVLK-AMC, 250 μM, Bachem, St. Helens, UK); this substrate is continuously hydrolysed by the generated plasmin to liberate the highly fluorescent 7-amido-4-methylcoumarin (AMC) group, which was quantified spectrophotometrically. Plasminogen activation was followed kinetically for 30 min at 37 °C, using a SPECTRAmaxTM GEMINI XS, Dual Scanning Microplate Spectrofluorimeter (Molecular Devices, Wokingham, UK) at excitation/emission wavelengths of 360/440 nm, respectively. Results were analysed using SOFTmax PRO software.

Migration assays. Haptotactic cell migration assays were performed using matrix-coated polycarbonate filters (8 μm pore size, Transwell,

Becton–Dickinson). The filter under-surface was coated with fibronectin (10 μg/ml) in PBS for 1 h at 37 °C and blocked with migration buffer (0.5% BSA in DMEM) for 30 min at 37 °C. For blocking experiments, cells were incubated with 10 μg/ml aprotinin (Sigma Chemical) for 20 min prior to seeding. The lower chamber was filled with 600 μl migration buffer (with the inhibitors if appropriate). The cells were plated in the upper chamber of triplicate wells, at a density of 1×10^5 in 100 μl migration buffer, and incubated at 37 °C for 4 h (keratinocytes) and 3 h (melanoma cells). Following incubation, Transwell inserts were fixed in 10% formalin, stained with 0.5% crystal violet in 10% ethanol for 10 min, and rinsed briefly in H_2O and dried. Cells in the upper compartment were removed using a cotton wool swab and the filter was mounted on a microscope slide. Cells that had migrated to the lower surface of the filter were counted by microscopy, using multiple random high-powered fields (five fields per filter).

Results

Increased expression of wild-type β6 integrin subunit reduces uPAR expression

Urokinase receptor expression was determined by Western Blotting of hydrophobic (membrane) protein extracts from the panel of SCC cell lines. Fig. 1A shows that VB6, which has 20 times the amount of αvβ6 on its surface as C1 cells, has significantly less uPAR than C1 cells. Fig. 1B shows graphically the summary of three separate experiments and indicates that VB6 expresses 50% less uPAR than C1. Fig. 1 also shows that VB6 expresses significantly less uPAR than VB6Δ11aa, a cell line that expresses a similar amount of αvβ6 on its surface as VB6 but which is composed of a mutant β6 lacking the C-terminal 11 amino acids. These data suggest that the ability of αvβ6 to down-regulate uPAR requires the C-terminal 11 amino acids of the β6 subunit. The effect was specific for the β6 subunit as cells over-expressing β3 did not show this effect.

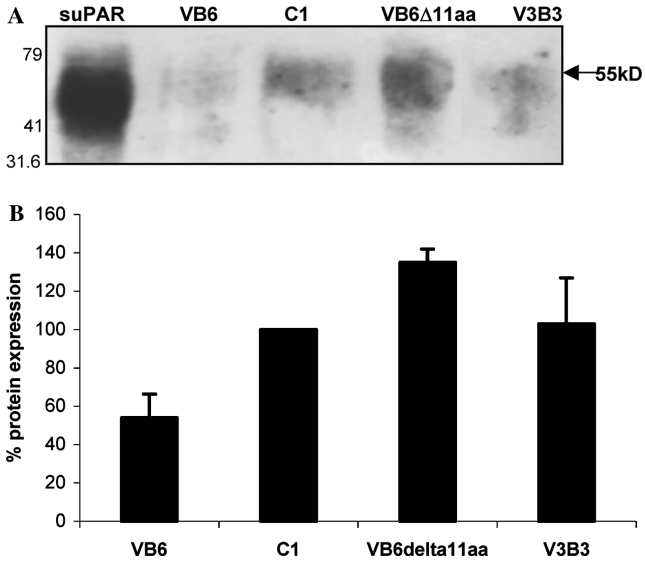

Fig. 1. Expression of uPAR in cell membrane extracts. Expression of full-length αvβ6 in oral keratinocytes decreases uPAR expression. (A) Western blot for uPAR expression. Ten micrograms of protein was electrophoresed under non-reducing conditions and probed for uPAR using 10 μg/ml mAb clone R4. Blots were developed using a peroxidase-conjugated secondary antibody and ECL. A representative result is shown. (B) Blots were subjected to densitometric analysis using Scion image software. Results were normalized to the control C1 cells and graph represents the mean (±SEM) percentage protein expression of three experiments.

Increased expression of αvβ6 down-regulates cell-surface plasmin generation

Optimal plasminogen activation on the cell surface requires an intact urokinase receptor, with binding sites for both uPA and plasminogen. To assess whether enhanced expression of αvβ6 on the surface influenced uPAR function, a cell-surface plasminogen activation assay was performed. The results in Fig. 2 are from cells plated in normal growth medium. However, cells were also stripped of endogenously bound uPA by incubation with a low-pH buffer, followed by incubation with exogenously added excess pro-uPA [22] to saturate uPA receptors, and this produced identical results (data not shown). Thus, the results are not influenced by endogenous uPA production. The pattern of cell-surface plasminogen activation was similar to that of membrane expression data, showing that VB6 generated half the activity of C1 or VB6Δ11aa cells. The rate of plasmin generation was determined by interpolation from a plasmin standard curve and expressed as nM plasmin/min: VB6 = 0.35 nM/min, C1 = 0.6 nM/min, VB6Δ11aa = 0.58 nM/min, and V3B3 = 0.65 nM/min. These data further support the role of the C-terminal 11 amino acids of β6 in regulating uPAR expression and therefore uPA activation. Cells over-expressing β3 did

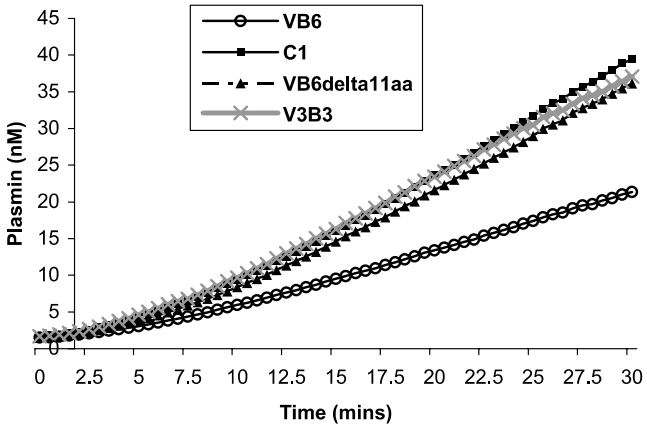

Fig. 2. Cell-surface plasmin generation. Cells expressing full-length αvβ6 show reduced cell-surface plasmin generation. Plasminogen activation on the cell surface was measured kinetically for 30 min using the fluorogenic plasmin substrate HDVLK-AMC and plasmin generation was determined by interpolation from a plasmin standard curve. Figure shows the combined results of two experiments in triplicate.

not show reduced plasminogen activation, suggesting that this effect was specific for the β6 subunit.

Increased αvβ6 down-regulates mRNA encoding for uPAR

In order to determine whether the observed changes in uPAR expression by αvβ6 were transcriptionally or post-transcriptionally regulated, mRNA extracted from C1, VB6, V3B3, and VB6Δ11aa cells was probed in Northern blots for uPAR. Fig. 3A shows that VB6 cells have only 50% uPAR mRNA than the C1, V3B3 or $VB6\Delta_{11aa}$ cells, a highly reproducible observation as shown in the summary of three separate experiments in Fig. 3B. Thus, αvβ6 promotes a down-regulation of uPAR expression on the cell surface by transcriptional down-regulation of uPAR mRNA.

De novo expression of αvβ6 in melanoma cells does not modulate uPAR expression or function

The integrin αvβ6 is only expressed by epithelial cells [24]. To determine whether αvβ6 could down-regulate uPAR expression or activity in non-epithelial cells, we transduced three human melanoma cell lines with pBabe retroviruses encoding puromycin-resistance alone (to create DX3 puro, A375 puro, and MeWo puro) or wild-type β6 (to create DX3β6, A375β6,and MeWoβ6).

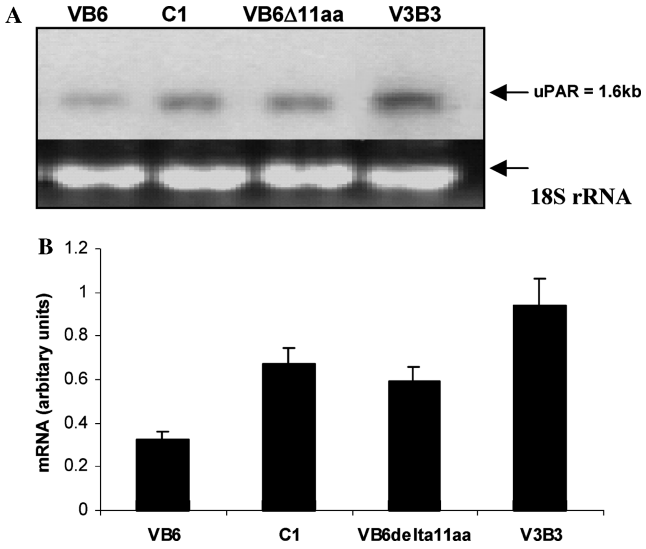

Fig. 3. Expression of uPAR mRNA. Epithelial cells expressing full-length αvβ6 show reduced stable uPAR mRNA expression compared to control cells. Total cellular RNA was extracted and subjected to Northern blotting. Filters were probed with ^{32}P-labelled full-length uPAR cDNA. (A) Top panel: stable uPAR mRNA expression. Bottom panel: ethidium bromide stain of 18S ribosomal band. A representative blot is shown. (B) Blots were subjected to densitometric analysis using Scion image software, normalized to 18S ribosomal bands, and expressed in arbitrary units. Figure shows the combined data of two experiments of six samples each.

Fig. 4A shows a flow cytometric analysis of αvβ6 expression by the transduced melanoma cell lines. It can be seen that the mean fluorescence intensity of αvβ6 on DX3β6, A375β6, and MeWoβ6 was similar to the levels expressed by VB6. In addition, the de novo expression of αvβ6 on the melanoma cells was functional as shown by a β6-dependent increase in migration toward fibronectin (Fig. 4C). Fig. 4A also shows that expression of uPAR on the β6-positive and their β6-negative counterparts was unchanged. A quantification of this is depicted in Fig. 4B. Similarly, when surface plasmin generation was measured (Fig. 4D) the presence of αvβ6 had no effect. Thus, modulation of uPAR expression by αvβ6 seems to be specific to epithelial cells.

Plasmin promotes αvβ6-dependent migration

Previously we have reported that αvβ6 is functional in promoting cell migration toward fibronectin [14]. To determine whether plasmin affected migration toward fibronectin, an αvβ6 ligand, migration assays were conducted in the presence and absence of the plasmin inhibitor aprotinin. Fig. 5 shows that the high αvβ6-expressing VB6 cells migrate significantly (approximately three times) more toward fibronectin than do the low αvβ6-expressing C1 cells. Aprotinin significantly reduces VB6 migration but did not affect C1 migration.

Discussion

Interactions between specific cell-surface molecules, such as uPAR and integrins, are crucial to processes of tumour invasion and metastasis. The present study demonstrates that in oral squamous cell carcinoma keratinocytes, the αvβ6 integrin influences the urokinase-type plasminogen activator system in a novel manner by down-regulating uPAR expression and thus cell-surface activation of uPA, suggesting that precisely regulated levels of proteolysis can be regulated by integrins.

Integrin activation has been shown to regulate expression of numerous gene products. Recently, there have been a plethora of studies linking the processes of proteolysis and adhesion, via uPAR and integrins. Co-localisation and immunoprecipitation studies have shown that uPAR may physically interact on leukocytes with β2-integrin [25] and on fibrosarcoma cells with β1- and β3-integrins [26]. In addition to the physical association of uPAR with various integrin β subunits, functional interactions between uPAR and the integrins β1 [27] and αvβ5 [28] have been shown to promote uPAR directed cell migration towards vitronectin. In addition to integrin regulation of the urokinase system, uPA or uPAR may in a reciprocal manner modulate integrin expression or function. For example, uPAR has been shown to influence integrin activity, both by

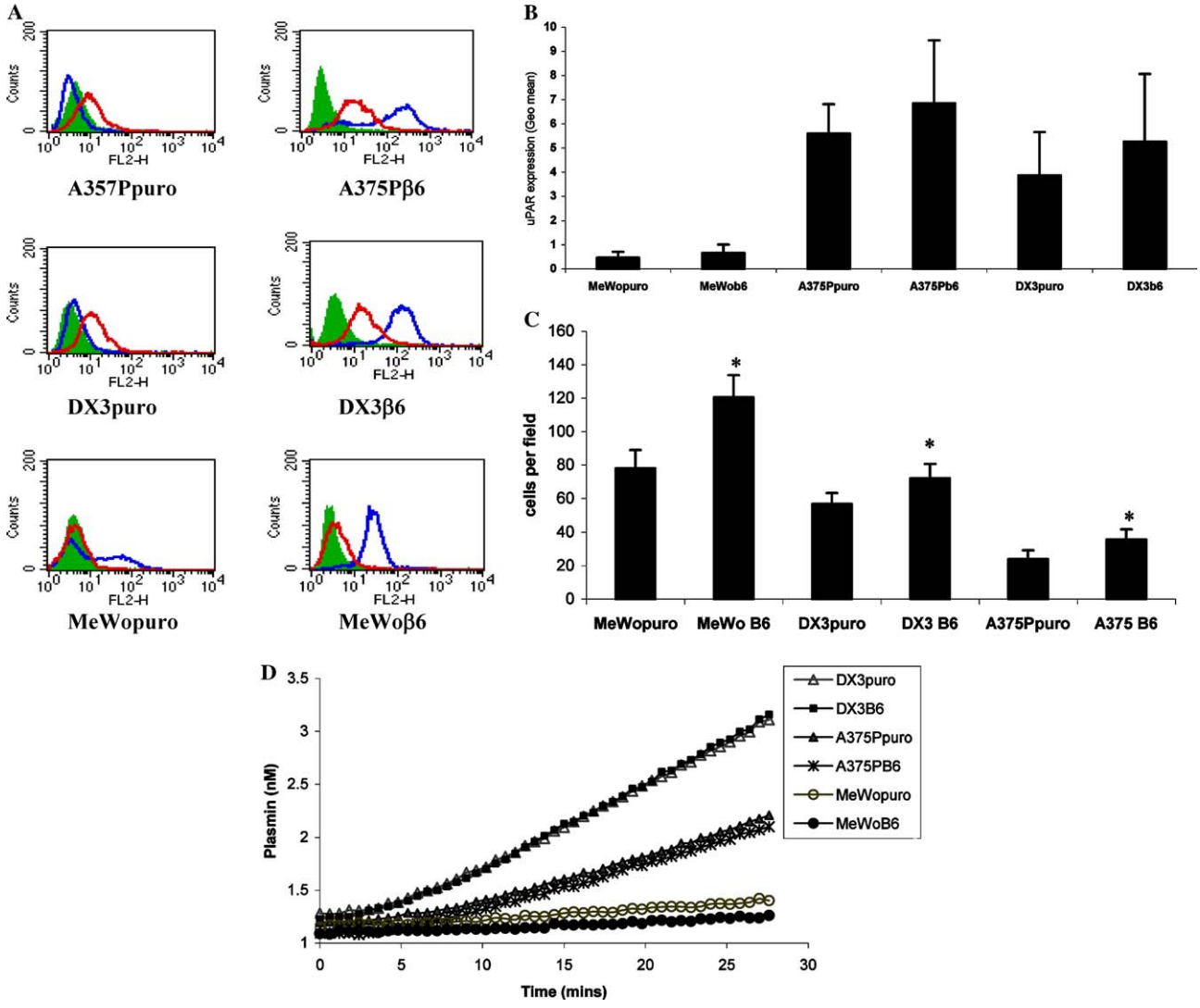

Fig. 4. αvβ6 expression in melanoma cells does not have effect on uPAR levels. (A) Double stain FACS analysis for αvβ6 and uPAR expression in β6-transfected melanoma cells. Cells were stained with the anti-αvβ6 mAb E7P6 at 10 μg/ml, followed by an RPE-conjugated secondary antibody. Cells were washed and then incubated with the anti-uPAR primary antibody, 399R at 10 μg/ml, then with a FITC-conjugated secondary antibody. Expression of cell-surface αvβ6 (blue) and uPAR (red) was determined by FACS analysis. Figure shows a representative result of three independent experiments in duplicate. (B) uPAR expression in the transfected melanoma cell lines. The flow cytometric data were for uPAR expression quantified using the geometric mean values. Figure represents the combined mean ± SD of three independent experiments. (C) Melanoma cell migration towards fibronectin. In all of the three cell lines, the αvβ6-expressing cells showed a 20–25% increased migration towards fibronectin. Cell migration assays were performed using plasma fibronectin-coated polycarbonate filters. Cells migrating to the underside of the filter after 3 h were counted using multiple random fields (200× magnification). Figure represents the mean ± SD of two experiments in triplicate. β6 expression in all of the cell lines significantly increased migration towards fibronectin ($p < 0.05$, Mann–Whitney U test). (D) Cell-surface plasmin generation in melanoma cells. Cells were incubated with a fluorogenic plasmin substrate HDVLK-AMC and human Lys-plasminogen both in excess. Plasminogen activation was measured kinetically at 37 °C for 30 min and the plasmin generated was determined by comparison with a plasmin standard curve. αvβ6- and non-αvβ6-expressing cells of each cell line produced the same levels of cell-surface plasminogen activation when saturated with exogenous pro-uPA.

inhibiting [27] and promoting [29–31] β1 integrin function. In breast carcinoma cell lines, engaging uPAR with catalytically inactive urokinase up-regulates the expression of αv and β5 integrin chains in a time- and concentration-dependent manner, which is dependent on protein kinase C activity and leads to enhanced tumour cell migration and invasion [32]. There are even in vivo data to suggest that uPAR–integrin complexes are functionally involved in tumour progression [33]. These data suggest there is a molecular cross-talk between stromal and epithelial cells involving integrins and uPA/uPAR which may influence tumourigenesis.

The present study shows that increased expression of the αvβ6 integrin after retroviral transfer of cDNA into oral carcinoma cells down-regulates uPAR expression. This regulation occurs at the transcriptional level, since the αvβ6-expressing cells show 2-fold less uPAR mRNA expression than the control cells that express negligible

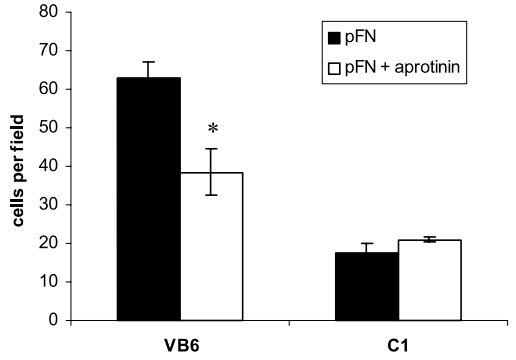

Fig. 5. Epithelial cell migration towards plasma fibronectin. Cell migration assays were performed using fibronectin-coated polycarbonate filters, in the absence and presence of 10 μg/ml aprotinin, a plasmin inhibitor. Cells migrated to the underside of the filter after 4 h were counted using multiple random fields (200× magnification). Figure represents the mean ± SD of two experiments in triplicate. Cells expressing αvβ6 showed a 3-fold increased migration towards plasma fibronectin compared to the control cells, which was plasmin-dependent ($p < 0.05$, Mann–Whitney U test).

amounts of αvβ6. In addition, we show that the C-terminal 11 amino acids, unique to the β6 integrin subunit, were essential for the β6-dependent down-regulation of uPAR. These observations were confirmed by decreased plasminogen activation at the cell surface indicating reduced uPAR function. Elevated levels of uPA and uPAR are seen in many malignant tumours and are indicative of a poor prognosis [5,34]. In fact, high αvβ6 expression in ovarian cancer cells has recently been shown to be associated with elevated levels of uPA and uPAR [35]. It may therefore seem counter-intuitive that αvβ6, an integrin that promotes invasion, should decrease levels of uPAR. However, similar data exist for the αvβ3 integrin where over-expression in Chinese hamster ovary cells resulted in a transcriptional down-regulation of uPAR. The signal was initiated by the binding of β3-endonexin to NITY sequence on β3 [36]. Contrary to this, our studies indicate that expression of β3 into these oral carcinoma cells (V3B3) did not significantly alter uPAR expression, suggesting that αvβ3 is not a crucial integrin in modulating plasminogen activation in this particular cell system.

The integrin αvβ6 is not detected on resting keratinocytes but up-regulated on keratinocytes during the late stages of wound repair [13]. It is thus possible that one of the roles of αvβ6 in wounds is to regulate uPA activity so as to inhibit a chronic wound response. In fact, excessive degradation of extracellular matrix is incompatible with efficient cellular migration [37,38] since excess plasmin proteolysis may prevent accumulation of ECM components that stimulate growth and proliferation. Data supporting this proposal come from experiments with PAI-1, the primary physiological inhibitor of uPA, which promotes tumour growth and angiogenesis by preventing excess plasmin formation [39].

Our studies also revealed that the C-terminal 11 amino acids of β6 were essential for β6-dependent down-regulation of uPAR. The C-terminal 11 amino acids of β6 have previously been reported to be required for αvβ6-dependent, but ligand-independent, up-regulation of the gelatinase, MMP-9 [40]. Thus, the C-terminus of β6 may be a major site in regulating proteolytic activity of epithelial cells and an attractive target for therapeutic intervention.

It should be acknowledged that there are conflicting data concerning the regulation of the uPA/uPAR system by integrins. For example, increased expression of αvβ3 integrin correlates with an increased expression of uPAR in melanoma cells [41] but a decreased expression of uPAR in ovarian cells [36]. In the present study, αvβ6 decreased uPAR expression, however, αvβ6 expression into melanoma cell lines did not affect uPAR expression or function. These cells, however, showed an increased migration towards fibronectin confirming that the αvβ6 integrin was functional. This suggests that modulation of the urokinase system is both integrin subunit and cell-type specific.

VB6 cells expressing high levels of αvβ6 but low levels of uPAR show an increased migration towards fibronectin, suggesting that uPAR may not be directly involved in promoting cellular migration. uPAR has been shown to act as an adhesion molecule by binding to vitronectin [42]. Thus, down-regulation of uPAR may result in enhanced cell motility. This β6-dependent migration was inhibited by aprotinin, a plasmin inhibitor, suggesting it may be the result of increased plasmin expression or the resultant activation of MMPs. uPAR expression may control the non-proteolytic functions of uPA. In Hep3 carcinoma cells decreased uPAR levels caused a uPA-mediated inhibition of proliferation [43].

In summary, we have shown that increased expression of the epithelial integrin αvβ6 can down-regulate uPAR at the transcriptional level. Furthermore, this capacity requires the C-terminal 11 amino acids of the β6 cytoplasmic tail. These data show for the first time that one of the mechanisms employed by the epithelial integrin αvβ6 to influence tumour cell migration and invasion is by regulating the machinery required for cell-surface proteolysis.

Acknowledgments

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