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Integrin Clipping: A Novel Adhesion Switch?

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Abstract During human prostate cancer progression, the majority of normally expressed integrins are suppressed with the exception of the α6, α3, and β1 integrins. We have shown that in prostate cancer, the α6 integrin is found paired with the β1 integrin and that a novel form of the α6 integrin that lacks a large portion of the extracellular domain (α6p) exists. The α6pβ1 integrin is found in human prostate cancer tissue specimens as well as tissue culture cell lines and is formed on the cell surface. This review discusses the mechanism of α6pβ1 production and the potential functions of this integrin variant. Our current working model predicts that the α6pβ1 integrin maintains the intracellular cytoskeletal connections associated with the heterodimer while allowing for an alteration in cell adhesion. The mechanism provides a selective advantage for cancer cell metastasis. J. Cell. Biochem. 91: 26–35, 2004. © 2003 Wiley-Liss, Inc.

Key words: adhesion; integrin; cancer progression; protease

Integrins are a large family of transmembrane glycoproteins found in a wide range of animal species. They function as heterodimers composed of α and β subunits and each αβ combination has its own binding specificity and signaling properties. At least eight different β subunits and eighteen α subunits associate to form at least twenty-four different receptors [van der Flier and Sonnenberg, 2001]. It is important to note that there is a tissue specificity to many of the integrin heterodimers. Several excellent reviews have been published detailing the regulatory features of these interesting molecules [Hogg et al., 2002; Hynes, 2002; Liddington and Ginsberg, 2002].

INTEGRIN α6 IN PROSTATE CANCER PROGRESSION

In adult normal tissues of the human prostate gland, the integrin expression reflects the complexity of the components of the basal lamina. The basal lamina of the normal human prostate gland is composed of collagen IV, VII, entactin, laminin containing the α3 chain (laminin 5, 6, 7) and the α5 chain (laminin 10–11), fibronectin, vitronectin, and tenascin [Knox et al., 1994; Brar et al., 2003]. The corresponding integrin heterodimers are present in normal prostate tissue and consist of α2, 3, 4, 5, 6, v, β1, and β4. These integrin units are polarized at the base of the cells adjacent to the basal lamina. The integrin α6β4 in the human prostate gland is associated with hemidesmosomal-like structures [Cress et al., 1995].

In human prostate carcinoma, the expression loss of all the normal components of the basal lamina are observed with the exception of laminin 10–11 (containing α5 chain) and collagen IV. These basal lamina components remain expressed in the tumor tissue [Knox et al., 1994; Brar et al., 2003]. Within the invasive prostate carcinomas, the majority of the integrin subunits are not observed on the tumor cell surfaces [Cress et al., 1995], consistent with earlier reports that many human carcinomas down-regulate the surface expression of integrin subunits [Albelda, 1993].

A notable exception is the persistent expression of the laminin receptors, α3 (10% of the cases expressing) and α6 (69% of the cases expressing) integrins, observed in the invasive human prostate carcinomas. More recent work by our group investigating 163 cases of prostate carcinoma in biopsy samples has revealed approximately 84% of the cancers express either the α3 or the α6 integrin, independent of stage [Schmelz et al., 2002].

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Since our initial observations in prostate carcinoma, many other human epithelial malignancies have been investigated and found to have persistent or elevated expression of the laminin (α3, α6 integrin) receptors. These include a variety of invasive human tumors, namely, squamous cell carcinomas, endometrial adenocarcinomas, colorectal carcinoma, bladder cancer, renal carcinoma, and pancreatic carcinoma [Rabinovitz and Mercurio, 1996]. Further, within these studies that investigated metastatic lesions, all have found the presence of the α6 integrin.

α6β4 INTEGRIN AS A DOMINANT RECEPTOR IN NORMAL TISSUE; α6β1 DOMINATES IN PROSTATE TUMOR TISSUE

The α6 integrin can pair with either a β1 or β4 subunit. Both of these receptors will bind to laminin [Sonnenberg et al., 1991; Lee et al., 1992; Niessen et al., 1994; Nielsen and Yamada, 2001]. The integrin β4 subunit appears to be the dominant pairing unit for α6. In normal epithelial systems, the α6β4 integrin is essential for hemidesmosome formation and is associated with intermediate filaments whereas the α6βl integrin is associated with focal contacts and vinculin [Jones et al., 1991; Kurpakus et al., 1991]. Developmental studies have stressed the importance of the integrin α6 and the ligand, laminin during embryogenesis [Hierck et al., 1993]. The α6 and β4 "knockout" experiments in mice show that allellic loss of either of these subunits will result in a blistering phenotype that is a lethal event [Dowling et al., 1996; Georges-Labouesse et al., 1996; van der Neut et al., 1996].

The α6 integrin is one of the few alpha subunits that can pair with more than one beta subunit, i.e., β1 or β4. In normal prostate glands, as well as prostatic intraepithelial neoplasia (PIN), the α6β4 integrin is dominant [Davis et al., 2001a] (Fig. 1). The PIN lesion is regarded as carcinoma in situ. In invasive prostate carcinoma, the α6 integrin is paired exclusively with the β1 subunit [Cress et al., 1995]. The β4 subunit expression is selected against during prostate tumor progression (Fig. 1). Hemidesmosome-like structures detectable by electron microscopy within the normal prostate gland are not present during prostate tumor progression [Nagle et al., 1992, 1994, 1995]. These data suggest that during the progression of PIN to invasive carcinoma, loss of the α6β4 hetrodimer expression occurs. It is important to note that this is a universal defect in prostate cancer whereas genetic alterations in prostate cancer indicate a multi-clonality of the disease [Cheng et al., 1998]. The biological consequence of the loss of β4 expression is the loss of a dominant adhesive structure to the basal lamina. By analogy to the results observed with skin blistering diseases [Niessen et al., 1996], we suspect that in prostate carcinoma, the loss of the α6β4 integrin results in utilization of alternative adhesion structures to the basal lamina.

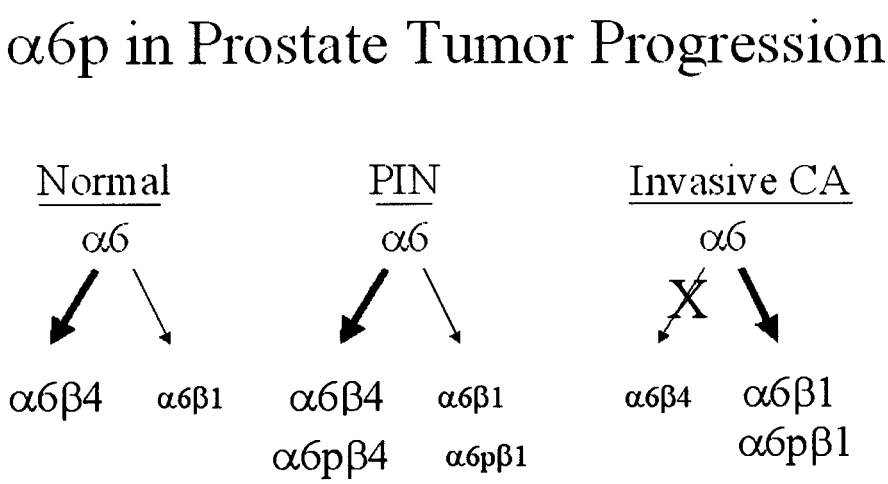

Fig. 1. The relative abundance of α6β1 and α6β4 integrins in human prostate cancer progression. Normal, prostatic intraepithelial neoplasia (PIN) and invasive cancer have either a dominant integrin pair as indicated by the bold arrow or suppression of integrin expression as indicated by (X). The α6p variant can be present as α6pβ1 or α6pβ4.

Other epithelial malignancies either have a mixture of α6β1 and α6β4 expression or as in human colon carcinoma, dominant expression of the α6β4 integrin [Mercurio and Rabinovitz, 2001]. Although the unique absence of the α6β4 heterodimer in prostate cancer was originally controversial, the observation has been validated by other groups [Allen et al., 1998; Bonkhoff, 1998]. More recently DNA microarray based expression profiles also confirm the suppression of β4 mRNA in human prostate carcinoma whereas the expression level remains elevated in human colon carcinoma [Su et al., 2001]. Taken together, these data suggest that either the α6β4 integrin is selected against (as in prostate carcinoma) or its function altered in the case of other epithelial malignancies. Investigations by others to understand the functions of the α6β4 integrin in cell motility and invasion should prove interesting. In prostate carcinoma, our attention is focused primarily on the α6β1 or α3β1 integrins and their potential role in facilitating invasion on laminin coated structures.

EVIDENCE TO SUGGEST A ROLE OF α6β1 IN PROSTATE CANCER INVASION

Previous studies using tissue culture prostate cell lines and an in vitro model have suggested the participation of α3β1 and α6β1 in the prostate cancer invasion process [Dedhar et al., 1993; Witkowski et al., 1993]. The α6 integrin has been proposed as an invasive factor in basal cell and squamous cell carcinoma [Rossen et al., 1994]. Of particular interest was that the α6 expression in carcinoma was not polarized but appeared diffusely distributed on the membrane. A high expression level of α6β1 integrin in human breast carcinoma is correlated with reduced survival [Friedrichs et al., 1995]. Developmentally, the α6 integrin is required during the apical ectodermal ridge formation, an organogenesis in the mouse [Georges-Labouesse et al., 1996]. Recently in keratinocytes, the role of the α6 integrin in migration has been suggested as it associates laterally with CD9 [Baudoux et al., 2000] and CD151 [Kazarov et al., 2002; Lammerding et al., 2003].

The persistence of the α6βl integrin in prostate carcinoma is a consistent finding in tumor tissues and suggests that some adhesion structures are maintained. Experimentally, using our mouse model system, we have found that the α6 integrin is associated with tumor cell invasion through the mouse diaphragm and migration of the cells into and through the muscle layer [Rabinovitz et al., 1995]. Taken together, these data suggest a shift in the ECM adhesion structures during prostate tumor progression.

α6P INTEGRIN VARIANT: A CLIPPED INTEGRIN

During the course of our investigations, we discovered an α6 integrin variant called α6p [Davis et al., 2001b]. This is a 70 kDa structural variant that is missing the extracellular β-propeller domain and can be found paired with either the β1 or β4 subunits [Davis et al., 2001b]. The variant is also recognized by antibodies (J1B5 and GOH3) specific for the full length α6 [Sonnenberg et al., 1987; Damsky et al., 1994]. These antibodies are used for the immunohistochemical localization of the full length α6 integrin. Detection of the variant is accomplished using non-reducing PAGE, followed by Western blotting techniques to detect the 70 kD form using an antibody specific for the cytoplasmic light chain. Metabolic labeling experiments indicated that the α6p variant has a three-fold increase in biological half-life on the cell surface as compared to the full length α6 integrin and no precursor product type relationship exists between the α6 integrin forms [Davis et al., 2001b; Davis et al., 2002]. Taken together, these data indicated that α6p was not a degradation product.

Several experiments suggested that the α6p variant arises while on the cell surface. First, all the α6p within a cell can be surface biotinylated whereas approximately only one-half of the full length α6 can be labeled in this fashion. Approximately 40% of the α6 integrin on the surface of DU145 cells existed in the α6p form. The α6p variant contained the expected glycosylations indicating it has been trafficked through the endoplasmic reticulum and the golgi to reach the cell surface. [Davis et al., 2002]. More recently, experiments abundantly expressing the recombinant form of the α6p integrin showed that although the α6p can be abundantly expressed, it cannot pair with either the β1 or β4 subunits (data not shown).

The observation that α6p is produced while on the cell surface led to the hypothesis that α6p was produced by proteolytic processing after it

is displayed on the cell surface. Several proteases were considered as likely candidates to mediate the cleavage, including matrix metalloproteases (MMPs), ADAMS (Disintegrin and A Metalloprotease), and the urokinase-type plasminogen activator (uPA).

MECHANISM OF α6P PRODUCTION: CLIPPING THE INTEGRIN AT THE CELL SURFACE

We have recently shown that α6p was produced by proteolytic cleavage of the full length α6 integrin by uPA [Demetriou et al., submitted]. This cleavage by uPA was specific to the α6 integrin since cleavage products are not produced for the α3β1, α5β1, or αvβ3 integrins (unpublished data). Mass spectrometry of the α6 integrin variant indicates that the amino acid fragment closest to the extracellular domain was RVNSLPEVLPILNSDEPKTAHID as indicated on Figure 2. The most probable site of cleavage in α6 integrin lies within a loop region within the thigh domain of the molecule (amino acids 572–595), just ahead of the "genu" or highly flexible site described for the αV integrin subunit [Xiong et al., 2001] (Fig. 2). Given that the three dimensional structure of the integrin molecule requires cysteine residues for maintenance of the loop structure and that the molecular weight of the variant is 70 kD, the potential cleavage residues are K576, R574, R578, R594, or R595. Theoretical predictions of serine protease sensitive sites in the molecule also suggest these sites.

The reported urokinase-type plasminogen activator receptor (uPAR) binding site resides at positions 272–298 in domain IV of the molecule [Wei et al., 2001]. Experimental data confirms that the full length α6 subunit interacts with uPAR, whereas the α6p variant, that is missing this portion of the molecule, does not interact (Demetriou et al., submitted). In contrast, both the full length and α6p variant contain membrane proximal regions sufficient to support the predicted lateral membrane association with CD151 [Kazarov et al., 2002]. The extracellular side of CD151 engages in strong lateral association with the α3 integrin and is predicted for the α6 integrin according to the transmembrane linker model [Kazarov et al., 2002]. Figure 3A indicates that while the full length α6 interacts with CD151, the α6p form of the integrin does not. This raises the possibility that the CD151 is released after the integrin is clipped. Alternatively, the interaction with CD151 may prevent cleavage of α6 to α6p. These questions remain to be answered.

The α6p integrin is present not only in tissue culture cell lines but also in human prostate cancer tissue, mouse squamous cell carcinomas induced by treatments with DMBA and TPA and invasive mouse melanomas induced by

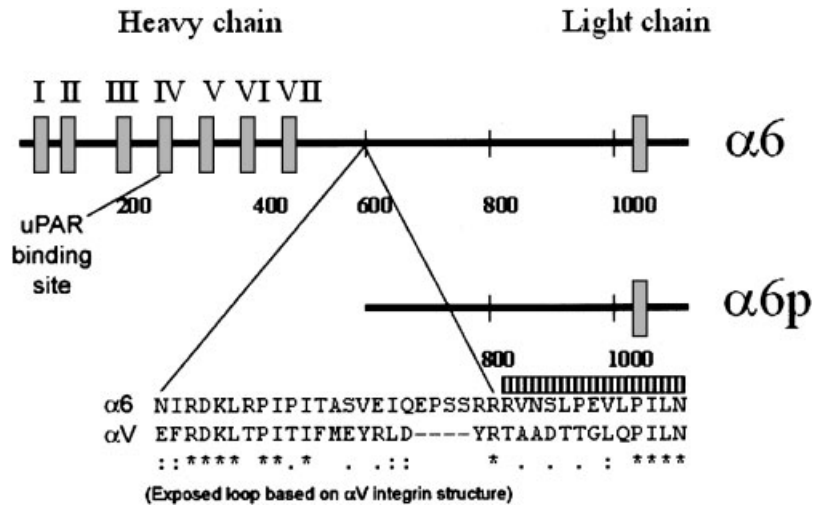

Fig. 2. Schematic representation of the α6 and α6p integrins. Repeated domains (shaded rectangles) are indicated by Roman numerals I–VII (I = 42–79, II = 113–145, III = 185–217, IV = 256–292, V = 314–352, VI = 375–411, VII = 430–470). The putative ligand and cation binding domains are contained between repeated domains III and IV, and V and VI, respectively. The site of urokinase-type plasminogen activator receptor (uPAR) interaction is at residues 272–298. The exposed loop (as indicated by triangle) in the thigh domain contains the predicted cleavage sites. The aminoacids under the striped bar were detected by mass spectrometry in α6p. Conserved amino acids (*), conservative substitutions (:) are as indicated. The membrane spanning region (shaded rectangle) occurs at residues 1012–1037.

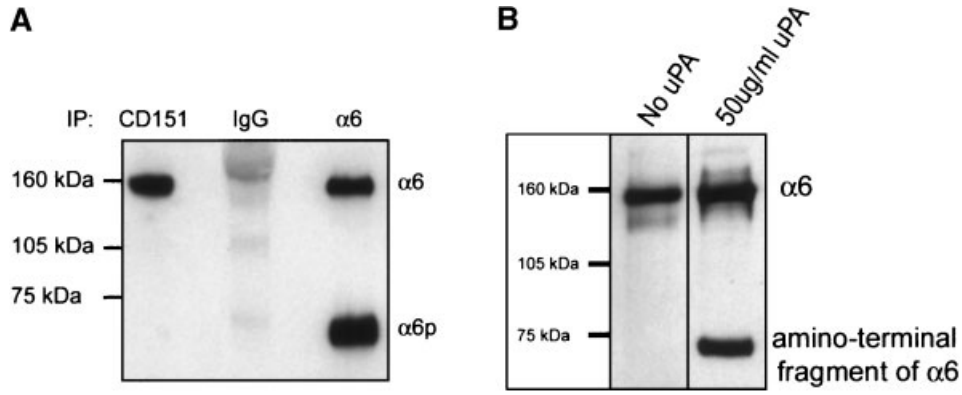

Fig. 3. Experimental detection of the α6p variant and the amino-terminal fragment by Western blot analysis. **A**: CD151 interacts with the α6 but not the α6p integrin in DU145H cells. DU145H cells were lysed with RIPA buffer and proteins were immunoprecipitated for CD151 (using antibody 5C11), control IgG, or α6 (using antibody J1B5). Proteins analyzed by SDS–PAGE and a Western blot was performed for the α6 integrin using the AA6A rabbit polyclonal antibody. **B**: The amino-terminal fragment of the cleaved α6 integrin is released in the ECM. MCF10A cells were grown for 3 days and then treated with 50 μg/ml uPA for 3 h. The MCF10A matrix was prepared according to the procedure previously described [Gospodarowicz, 1984]. The samples were analyzed by SDS–PAGE and a Western blot was performed for the α6 integrin using the A33 rabbit polyclonal antibody.

activated Ras (submitted and unpublished data). These data suggest that the clipping of the integrin is an event found in tissue as well as under conditions of tissue cultured cell lines.

Since α6p can remain paired with either β1 or β4, it would suggest that the shortened version of α6 contains the information required for maintaining the heterodimer interaction. Interestingly, expression of the α6p subunit by transfection does not allow heterodimer formation, again indicating that the α6p forms only after the full length form has reached the cell surface. In our recent experiments, the clipping of α6 to α6p does not alter the recovery of other elements of the hemidesmosome. These data would suggest that clipping the extracellular domain after the establishment of the hemidesmosome does little to affect the structures once they are formed. We would expect that the clipping of the α6 integrin would not alter the assembly process since recent experiments suggest that the cytoplasmic domain of the β4 subunit is sufficient for hemidesmosome formation [Nievers et al., 1998]. Our prediction is that the clipping of the α6 integrin to the α6p form would allow an alteration in cell adhesion without affecting the formation or the stability of the HD. An alternative hypothesis is that the lateral membrane associations with the α6 subunit, i.e., CD151 may be altered with the integrin clipping. Experiments are underway to distinguish these possibilities.

ECTODOMAIN SHEDDING: A MECHANISM FOR INTEGRIN CLIPPING?

Ectodomain shedding is the proteolytic release of the extracellular domain of transmembrane proteins from the cell surface. The release may allow alteration of the cell surface phenotype rapidly in response to environmental changes. Alternatively, this process is developmentally regulated during branching morphogenesis and may reflect the necessity for dynamic membrane remodeling [Peschon et al., 1998; Zhao et al., 2001].

Ectodomain shedding is known as the release of the extracellular domain of transmembrane receptors by limited proteolysis [Arribas and Borroto, 2002]. This process is thought to regulate the function of the receptors. This process occurs near or at the cell surface and it can occur in non-stimulated or stimulated cells [Arribas and Borroto, 2002]. The best characterized way to stimulate ectodomain shedding is the use of phorbol esters [Arribas and Borroto, 2002]. Interestingly, the α6p integrin is produced by cleavage close to the cell surface and it was shown that the phorbol ester 12-*o*-tetradecanoyl-phorbol-13-acetate (TPA) induces α6p production (Demetriou et al., submitted). Ectodomain shedding leading to the generation of soluble domains of receptors can modulate the functions of the ligands [Rose-John and Heinrich, 1994].

Several different types of membrane proteins undergo protein ectodomain shedding and include TNFα [Schedin et al., 1996], kit ligand [Yee et al., 1993], TNF receptors [Madge et al., 1999], growth factor precursors such as heparin binding epidermal growth factor [Tokumaru et al., 2000], and hepatocyte growth factor receptor [Nath et al., 2000]. Of particular significance to this work is that adhesion molecules are known to be shed from the cell surface [Beer et al., 1999]. In addition a protein within the hemidesmosome, BP180, is proteolytically processed on the cell surface. Collagen XVII/BP180, an epidermal adhesion molecule, exists as a full-length transmembrane protein and as a soluble 120 kd ectodomain that is shed from the keratinocyte surface by furin-mediated proteolysis [Schumann et al., 2000]. This is an intriguing observation since this is a protein involved in the assembly of the hemidesmosome, a structure known to be absent in prostate cancer.

The shedding response is accelerated under physiological conditions such as inflammation, apoptosis, and arthritis [Subramanian et al., 1997]. Experimentally one can use PMA, calcium ionophores, chemotactic peptides, cytokines, and growth factors to induce shedding. In our previous work, the integrin αvβ3 was induced to shed from the cell surface in response to heat shock [Majda et al., 1994], although the integrin itself is not clipped. Our work more recently with the α6p variant indicates that it can be induced by conditions in normal cells that result in membrane adhesion remodeling [Davis et al., 2001b]. These events are calcium-induced differentiation in epidermal keratinocytes or androgen-induced differentiation in prostate cells. The switching of integrin function by post-translational clipping is a unique aspect of integrin function.

FUTURE STUDIES

A current model of integrin function suggests that the α subunit cytoplasmic domain will inhibit specific functions of the β cytoplasmic domain. Binding of the extracellular domain to the ligand relieves this inhibition by allowing the two subunits to swing apart like a hinge [Burridge and Chrzanowska-Wodnicka, 1996; Hughes et al., 1996]. The α6p integrin variant and its release from ligand binding interactions may affect the signaling potential of the α6 integrin. Proposed signaling studies using normal human keratinocytes tranfected with the full length α6 integrin and the subsequent rendering of the α6p form should answer these questions.

A previous report has shown that 11 amino acids at the cytoplasmic domain at the carboxy-terminus of the α6A integrin inhibited proliferation and promoted differentiation by regulating β1 integrin signaling [Sastry et al., 1999]. The regulation of β1 integrin signaling by the α6 subunit could be affected by the uPA dependent cleavage of the α6. For example, α6p may prevent signals from being activated by not allowing ligand binding to the integrin. The production of α6p does not depend upon ligand occupancy. Alternatively, α6p may function by allowing signals to persist while promoting release from the ECM adhesion.

Although the α6p variant was discovered in prostate cancer cells, it can be induced in normal cells, raising the possibility that α6p may function in normal tissue remodeling [Davis et al., 2001b]. Tissue remodeling is important for many processes including processes in the adult such as wound repair, uterine and mammary gland changes, trophoblast invasion, neovascularization, menstrual cycle, and pregnancy [Streuli, 1999].

During tissue remodeling, proteases including MMPs as well as the plasminogen activator/plasmin system become important [Preissner et al., 1997]. Moreover, integrins have been shown to be involved in this process (summarized in [Beauvais-Jouneau and Thiery, 1997]). In addition, it has been shown that integrins interact with the uPAR [Chapman and Wei, 2001], and that integrin-dependent adhesion, spreading, and migration can be modulated by the plasminogen activator system [Preissner et al., 1997]. Similar to our observation in prostate cancer, the cleavage of α6 integrin by uPA may function in tissue remodeling by releasing cells from the ECM, allowing cell migration. Ectodomain shedding of L-selectin has been shown to regulate adhesion and migration of leukocytes [Hafezi-Moghadam et al., 2001]. It is important to note one advantage of the integrin clipping process would be the modification of adhesion without activation of the known phenomenon of apoptosis induced by inadequate cell matrix interaction i.e., the anoikis response (reviewed in [Frisch and Screaton, 2001]).

Many cells express several integrins on their cell surface and many of them could be simultaneously engaged [Blystone et al., 1999]. Activation of one integrin can affect the function of other integrins [Blystone et al., 1999], referred to as integrin crosstalk [Blystone et al., 1994, 1995, 1999; Porter and Hogg, 1997]. Examples of integrin crosstalk have been shown in different cells lines (summarized in [Blystone et al., 1999]). Future studies will be to determine if cleavage of the α6 integrin and changing integrin adhesion could activate other integrins in switching to a different substrate or to activate adhesion or signaling through other receptors on the same substrate. In this scenario, "integrin clipping" leads to "adhesion switching." This "clip and switch" mechanism would serve as a transitory adhesion state that would account for the coordinated adhesion switch that must occur in basal cell differentiation within the skin and other glandular epithelium.

Finally, the amino terminal fragment that is being clipped off the integrin could have functional significance. This fragment is present within the ECM (Fig. 3B). Recent work investigating fragments from other molecules such as laminin and fibronectin have shown significant biological activity [Schedin et al., 1996, 2000; Pirila et al., 2003; Schenk et al., 2003; Udayakumar et al., 2003]. Others have shown the deposition of a truncated version of the αIIb integrin lacking the cytoplasmic and transmembrane domains within the ECM. The truncated αIIb arises by alternative splicing of the mRNA rather than by surface cleavage [Trikha et al., 1998]. In our experiments, we know that the cleaved N-terminal α6 fragment contains the ligand binding region of the molecule. We speculate that the N-terminal fragment could function to occupy (i.e., block) the site where other laminin receptors bind, thus participating in the "adhesion switching."

Our current working model is that the integrin clipping observed with the α6 integrin is a process involving the direct cleavage of the integrin by uPA. The α6p remains associated with the beta subunit and remains on the cell surface whereas the N-terminal fragment of the α6p is found in the ECM. Both of these events contribute to the alteration in cellular adhesion to the ECM. It is significant to note that while this process was discovered in cancer, it does occur in normal tissue. Finally, the role of α6p in modifying lateral membrane associations and the corresponding signaling pathways will be of interest. Modifying specific lateral associations mediated through integrin clipping will provide an important new dimension to our understanding of integrin signaling.

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