Published in final edited form as:

Prostate. 2001 February 15; 46(3): 240–248.

Unique Expression Pattern of the $\alpha 6 \beta 4$ Integrin and Laminin-5 in Human Prostate Carcinoma

Tracy L. Davis¹, Anne E. Cress^{1,2}, Bruce L. Dalkin³, and Ray B. Nagle^{4,*}

¹ Department of Radiation Oncology, University of Arizona, Tucson, Arizona

² Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona

³ Department of Surgery, University of Arizona, Tucson, Arizona

⁴ Department of Pathology, University of Arizona, Tucson, Arizona

Abstract

BACKGROUND—The $\alpha 6 \beta 4$ integrin and its ligand, laminin-5, are essential gene products for the maintenance and remodeling of a stratified epithelium. Apparent loss of polarized $\alpha 6 \beta 4$ integrin and laminin-5 protein expression in invasive prostate cancer as compared to normal prostate glands is known to occur. It is unknown whether these alterations occur in prostatic intraepithelial neoplasia (PIN) lesions and whether this combined defect occurs in other epithelial cancers.

METHODS—Human prostate tissues containing both normal, PIN, and cancerous regions and normal and cancer tissue from breast and colon were obtained at surgery and examined for $\beta 4$ integrin and laminin-5 using standard immunofluorescence staining methods.

RESULTS—Both normal prostate glands and PIN lesions contain $\beta 4$ integrin and laminin-5. Prostate carcinoma was unique in that both $\beta 4$ integrin and laminin-5 expression was uniformly absent. In contrast, the $\beta 4$ integrin and its ligand, laminin-5 were detected in all of the colon carcinoma cases and in 60% of the breast carcinomas.

CONCLUSIONS—The $\beta 4$ integrin and its ligand, laminin-5 are altered during the transition of PIN lesions to invasive prostate carcinoma. These data suggest the loss of these proteins during cancer progression. In both prostate and breast carcinoma, the normal expression pattern of the $\beta 4$ integrin and laminin-5 is interrupted, in contrast to the persistent $\beta 4$ integrin and laminin-5 expression detected in colon carcinoma.

Keywords

integrin; prostate; epithelial; laminin; carcinoma; $\alpha 6 \beta 4$; colon; breast; tissue

INTRODUCTION

Prostate cancer, the most common visceral neoplasm in males [1] is variable in its clinical progression. Many cases present with slow-growing, clinically inapparent forms of the invasive carcinoma confined to the prostate, while others present with a rapidly growing, aggressive tumor that quickly metastasizes. The cause of this variability is still unknown, but is due in part to differences in the ability of a given carcinoma for cellular invasion and metastatic spread.

*Correspondence to: Dr. Ray B. Nagle, MD, PhD, Department of Pathology, University of Arizona Health Sciences Center, 1501 N. Campbell Avenue, P.O. Box 245043, Tucson, AZ 85724-5043. rnagle@u.arizona.edu.

During invasion, tumor cells can make an extracellular matrix which differs from that found in the normal structures. The invading cells interact with the new basal lamina to promote migration [2,3]. Prostate carcinomas synthesize a new basal lamina lacking the $\beta 3$ and $\gamma 2$ subchains of laminin-5 [4,5]. Colorectal carcinomas produce a laminin-5-rich basal lamina, the presence of which was correlated with a high degree of metastasis to the liver. These metastatic lesions often had intact, well-defined basal lamina [6]. Gastric carcinomas also have been shown to increase their expression of laminin-5 at the invasive edge [3,7].

Invasive prostate carcinoma also is associated with changes in cell adhesion receptors. In particular, loss of E-cadherin [8–11], gain of N-cadherin, loss of hemidesmosomes [4,12], and integrin alterations occur [10,13–17]. The $\alpha 6 \beta 4$ integrin which is expressed mainly in stratified epithelial tissues, is the predominant integrin pair found in normal prostate epithelium and is associated with the hemidesmosome, laminin-5 and intermediate filaments [18]. The $\alpha 6 \beta 4$ integrin appears to be downregulated in prostate carcinoma [2,12,13,17] and some breast carcinomas. Other studies indicate that it is persistent in head and neck tumors [19], colon carcinoma [20,21] and breast carcinoma [22–24].

Our goal was to study the $\alpha 6 \beta 4$ integrin and its ligand, laminin-5, in prostate carcinoma during the normal to PIN cancer progression and compare the expression pattern between human prostate, breast and colon carcinoma.

MATERIALS AND METHODS

Tissue Samples

Surgical samples of normal, PIN and malignant human prostate, breast and colon tissues were embedded in OCT medium (Miles, Elkhart, IN), and immediately snap-frozen in an isopentane bath cooled by Freon. Cryostat sections were stained with hematoxylin and eosin, and examined in order to select areas for study. Sections used for immunohistochemistry (IHC) were fixed for 5 min in 4°C acetone, and stored at –20°C until used.

Antibodies

Five anti-human $\beta 4$ integrin monoclonal antibodies were used in these experiments: 3E1 (mouse IgG1, used 1:2000, obtained from GIBCO-BRL), [25], A9 (mouse IgG2a, used 1:100, was a generous gift from Dr. Art Mercurio), [26], 450-11A (mouse IgG1, used 1:50), [27], 450-10D (mouse IgG2a, used 1:50), [27], and 439-9B (rat IgG2, used 1:50), [28]. The 450 and 439 antibodies were a generous gift from Dr. Steve J. Kennel. The schematic (Fig. 1) illustrates the position of the epitopes on the $\beta 4$ integrin, modified from [27]. An anti-pan cytokeratin, polyclonal rabbit antibody (18A) which stains prostate basal cells more intensely than luminal cells [29] was used in dual immunofluorescence studies to locate normal and carcinomatous epithelial cells. GB3 is an anti-laminin-5 antibody whose epitope is recognized when all three chains of the molecule are assembled [30] (Seralab, Crawley Down, Sussex, UK). Secondary antibodies used were adsorbed to reduce cross-reactivity against human tissues (Jackson Immuno Research Labs, West Grove, PA): goat anti-rabbit FITC, donkey anti-mouse Cy3, and donkey anti-rat Cy3.

For the colocalization of $\beta 4$ or laminin-5 with cytokeratin, frozen sections were fixed in 4°C acetone for 5 min. Slides were hydrated with PBS for 5 min, incubated with anti- $\beta 4$ integrin antibodies (see above for dilutions) or anti-laminin-5 monoclonal mouse antibodies and anti-cytokeratin polyclonal rabbit antibody for 1 hr at room temperature, washed with PBS, incubated with appropriate secondary antibodies and rinsed in PBS. Coverslips were placed on slides, sealed with Mowiol and stored at 4°C until examined using a Zeiss 410 confocal scanning laser microscope. The resulting images were processed using Adobe Photoshop 5.5.

Grading of the Tissue and Analysis by Immunofluorescence Microscopy

The prostate carcinomas (n = 11) were graded 1–5 using the Gleason system [31]. The breast carcinomas (n = 5) were classified as infiltrating, lobular or ductal and graded 1–3 based on Elston's criteria [32]. The colon carcinomas (n = 6) were graded as well, moderate, or poorly differentiated.

Basal polarization of the $\beta 4$ integrin was determined by direct comparison of the field to a normal gland within the same tissue, which showed a distinct linear organization of protein along the basal aspect of the cells composing the gland. IHC staining was semi-quantitatively evaluated by the following scale of intensity: (0) no staining above background, ($\pm$) weak staining, (+1) positive staining up to intense staining (+2). A photograph which included these five levels of staining was used as a standard reference of comparison for scoring individual cases. Cases which scored (+1) to (+2) were recorded as positive.

RESULTS

Of the 11 prostate carcinomas examined, 10 were classified as Gleason grade 3 and one was Gleason grade 4. Four of the five breast carcinomas were infiltrating ductal, the remaining one was an infiltrating lobular carcinoma. Three of the four infiltrating ductal carcinomas were classified as Elston grade 2 lesion, and one was Elston grade 3. Of the six colon carcinomas examined, four were well-differentiated and two were moderately differentiated.

Expression of the $\beta 4$ Integrin in Normal, PIN and Carcinoma of the Prostate

The dual IHC staining of normal, PIN, and invasive prostate carcinoma tissues (Fig. 2A–C stained with 18A anti-cytokeratin; Fig. 2D–F stained with 3E1, anti- $\beta 4$ integrin) indicated that the loss of the $\beta 4$ protein epitope could be traced through these progressive lesions. In normal prostate glands, the $\beta 4$ integrin was abundantly present in the basal cells (Fig. 2A,D). In PIN, a hypothesized premalignant lesion, the $\beta 4$ integrin, was retained by the residual basal cells, but was not present in areas in which the basal cells were lost or where transformed PIN cells abut the basal lamina of the gland (Fig. 2B,E). The $\beta 4$ integrin also was observed in the vascular endothelium (v) (Fig. 2E). In carcinomatous prostate glands, the $\beta 4$ integrin was not expressed (Fig. 2C,F).

Expression of Laminin-5 in Normal, PIN and Carcinoma of the Prostate

Dual IHC staining of normal, PIN, and carcinoma prostate tissues (Fig. 3A–C stained with 18A anti-cytokeratin; Fig. 3D–F stained with GB3, anti-laminin-5 monoclonal antibody) indicated that the loss of assembled laminin-5 protein could be traced through these progressive lesions. In normal prostate glands, the assembled laminin-5 was expressed by the basal cells (Fig. 3A,D). In the PIN lesion, assembled laminin-5 protein was localized within and adjacent to the residual basal cells, and was not seen elsewhere in the gland (Fig. 3B,E). In the carcinoma glands (Fig. 3C,F), assembled laminin-5 was no longer detectable. The punctate staining within these images in the tissue sections represents the autofluorescence of lysosomal pigments.

Detection of the $\beta 4$ Integrin Differed in Prostate, Breast and Colon Carcinoma

Human epithelial tissue samples were dual-stained for cytokeratin and $\beta 4$ integrin using IHC. Normal and carcinomatous cells were detected by an anti-cytokeratin polyclonal rabbit antibody, 18A (Figs. 4A–C) [29]. The $\beta 4$ integrin was detected using the 3E1 anti-human $\beta 4$ integrin monoclonal antibody, which recognizes an extracellular epitope of the $\beta 4$ protein (Fig. 4D–F). The $\beta 4$ integrin was detectable at the epithelial stromal interface in normal prostate glands (Fig. 4D), breast lobules (Fig. 4E). Invasive prostate and breast carcinoma (Fig. 4D,E) adjacent to normal tissue, did not contain detectable $\beta 4$ integrin. In contrast, in the colon tissue,

both normal crypts and carcinoma showed strong basal polarization of the $\beta 4$ integrin (Fig. 4F) (data for normal colon not shown).

Expression of Laminin-5 Protein in Prostate, Breast and Colon Carcinoma

The presence of the ligand for the $\alpha 6\beta 4$ integrin, laminin-5, was investigated using the three types of human epithelial tissue samples. The samples were dual-stained for cytokeratin and assembled laminin-5 using IHC. Normal and carcinomatous cells were detected by an anti-cytokeratin polyclonal rabbit antibody, 18A (Fig. 5A–C). Laminin-5 was detected using the GB3 anti-laminin-5 antibody whose epitope is recognized when all three chains of the molecule are assembled (Fig. 5D–F) [30]. Extracellular laminin-5 protein was detectable in normal prostate glands, breast lobules, and colon crypts. Invasive prostate carcinoma adjacent to the normal structures did not contain detectable laminin-5 (Fig. 5D). Invasive breast carcinoma did not contain strong polarized staining of the laminin-5 but did contain significant cytoplasmic staining (Fig. 5E). The breast carcinoma cases were observed to have diffuse cytoplasmic staining. In contrast, colon carcinoma showed strong extracellular polarized staining of the laminin-5 protein (Fig. 5F) (results for normal colon not shown).

Polarized Staining Pattern of $\beta 4$ Integrin and Laminin-5 in Epithelial Carcinomas

Since the different types of carcinoma appeared to vary in the amount and distribution of $\beta 4$ integrin and laminin-5, five different anti- $\beta 4$ antibodies, representing epitopes present within the extracellular and cytoplasmic portions of the integrin were used (see Materials and Methods). Serial sections of normal and carcinoma tissues were examined by IHC. Normal prostate, breast tissue, and colon tissue showed positive polarized staining for the five different $\beta 4$ antibodies (data not shown), similar to the pattern shown in Figure 4D, E.

Polarized staining was recorded and distinguished from total positive staining within the carcinomas using the criteria set forth in the Materials and Methods. The results are shown for prostate carcinoma ($n = 11$) (Fig. 6A), breast carcinoma ($n = 5$) (Fig. 6B) and colon carcinoma ($n = 6$) (Fig. 6C). Using five different antibodies specific for the $\beta 4$ integrin, no cases of prostate or breast cancer contained the $\beta 4$ integrin basally polarized, in contrast to that found in colon carcinoma. Colon carcinoma consistently showed strong, basally polarized localization in all cases, independent of the antibody used to detect the $\beta 4$ integrin (Fig. 6C). In prostate cancer, although none of the antibodies detected polarized staining of the $\beta 4$ integrin, two antibodies (439-9B, 450-10D) detected diffuse cytoplasmic staining in 82 and 80% of the cases (Fig. 6A). These two antibodies recognized extracellular and intracellular epitopes of the integrin respectively. Diffuse cytoplasmic staining was also detected by three other $\beta 4$ -specific antibodies (3E1, A9, and 450-11A) in 20, 9, and 18% of the cases, respectively. None of the 11 prostate cases retained all five $\beta 4$ epitopes. One of the five breast cases retained all five $\beta 4$ epitopes examined (data not shown). In breast cancer, three antibodies specific for $\beta 4$ integrin (A9, 439-9B, and 450-10D) detected 75% of the cases and two antibodies (3E1, 450-11A) detected 40% of the cases (Fig. 6B).

These same cases were also examined for laminin-5 expression using GB3, a monoclonal mouse antibody that recognizes the fully assembled laminin-5 protein. The results obtained for each of the three epithelial carcinomas are shown in Figure 6D. Laminin-5 was absent in all nine cases examined of prostate carcinoma. There was variable cytoplasmic staining in three of five breast carcinomas and positive basally localized staining in all six colon carcinomas examined.

DISCUSSION

We reported previously that in normal prostate glands, hemidesmosomes were present but in prostate carcinoma the structures were lost [12]. Correlated with this ultrastructural observation was the finding that several proteins associated with the hemidesmosomes, including the $\beta 4$ integrin as well as two chains of its ligand, laminin-5 and the anchoring filament collagen VII [2,13], were absent in prostate carcinoma. In the present study, we further investigated the $\beta 4$ integrin and laminin-5 expression pattern within normal, PIN lesions, and prostatic carcinoma. Our results indicate that the loss of the $\beta 4$ and laminin-5 expression occurs during the normal to PIN to carcinoma transition. Focal loss of the $\beta 4$ and laminin-5 was observed in PIN, consistent with the known loss of the basal cells which characterize these lesions. We noted in particular that within the PIN lesion, the remaining basal cells often demonstrated extended processes containing $\beta 4$ integrin that occurred between the luminal cells and was not confined to the normal location, i.e., at the basal aspect of the cells.

The loss of $\alpha 6\beta 4$ integrin in prostate carcinoma could be due to the loss of the normal basal cell population as is progressively seen through PIN and cancer lesions. Alternatively, previous studies indicate that the basal cells may play an important proliferative role in the normal and neoplastic human prostate [33]. Recently, a stem cell model has been described in which stem cells in the basal cell population acquire secretory luminal characteristics after androgen stimulation and thus prostate cancer ultimately may be derived from these transformed stem cells [34,35].

The reorganization of the $\alpha 6\beta 4$ integrin within the PIN lesion is reminiscent of the loss of polarized $\alpha 6\beta 4$ integrin observed in other epithelial disease states such as junctional epidermolysis bullosa with pyloric atresia (PA-JEB). The $\alpha 6\beta 4$ integrin heterodimer is a major component of the hemidesmosome which is expressed at the basal surface of most stratified epithelial cells and serves to link the intermediate filaments of the cytoskeleton to the anchoring filaments of the basal lamina [36–41]. The $\alpha 6\beta 4$ integrin is required for hemidesmosome formation, adhesion to laminin-5 [18,42,43] and is present in normal prostate tissue [12], forming a link with the extracellular matrix protein, laminin-5. It remains to be determined if a structural rearrangement of other components of the hemidesmosome also occurs in PIN lesions.

The biological consequences of abnormal $\beta 4$ integrin expression may involve increased migration and invasion either via actin-containing motility structures [44], or via reorganization of the hemidesmosome. Normal keratinocyte maturation studies suggest that a loss of the $\alpha 6\beta 4$ integrin attachments to the basal lamina was necessary for basal cell detachment and differentiation [45]. Invasive tumors appear to show considerable variation in $\beta 4$ expression. (For a review, see [46].) Tumors in which there is reduced expression of $\beta 4$ include breast cancer [22–24], basal cell carcinoma [47], bladder cancer [48], and prostate cancer [2,12,13, 17].

In this study we have emphasized that the $\beta 4$ expression pattern is different in other epithelial cancers. Expression of $\beta 4$ integrin and laminin-5 in prostate and breast carcinoma was distinctly different from the strong, polarized staining observed in colon carcinoma. In the absence of the ligand laminin-5, $\beta 4$ is not polarized to the surface of breast and prostate carcinomas, but in the presence of laminin-5, has a polar distribution in colon carcinomas. These data suggest that the presence of the ligand may be linked to surface stabilization of the integrin. We note with interest that the fibronectin receptor, $\alpha 5\beta 1$ integrin, rapidly degrades in the absence of its specific extracellular ligand [49]. In the case of $\alpha 6\beta 4$, the absence of the major ligand laminin-5, may result in the failure of the $\alpha 6\beta 4$ integrin to basally polarize with subsequent internalization

and degradation. The $\beta 4$ integrin has been shown to be specifically cleaved by calpain-like enzymes [50] and matrilysin [51] both of which are present in prostate carcinoma [52].

In prostate carcinoma, the loss of the $\beta 4$ integrin and the continued expression of the $\alpha 6$ integrin, result in a predominance of the $\alpha 6\beta 1$ integrin. We hypothesize that the loss of the $\alpha 6\beta 4$ integrin in prostate carcinoma and retention of $\alpha 6\beta 1$ may result in a loss of stable attachment to the basal lamina via the hemidesmosome and promote adhesion to laminin-coated structures such as vessels and nerves [53].

Reorganization of adhesion structures in prostate cancer may be analogous to that observed in PA-JEB. In migration assays, the PA-JEB keratinocytes are more motile on laminin-5 than normal keratinocytes [54]. Alteration of the hemidesmosome structure may facilitate invasion of prostate cancer by allowing migration through the stroma and along nerves. Extracapsular penetration of prostate cancer occurs along these structures and is correlated with poor prognosis [55]. It would be of particular interest to determine the dependence of the migration of $\alpha 6\beta 1$ containing prostate cancer cells along peripheral nerves using in vitro systems.

Acknowledgments

Special thanks to Virginia Clark, Clinical Pathology, University of Arizona and Karim Sallam, Department of Pathology, University of Arizona for their assistance in slide preparation of human tissue specimens.

Grant sponsor: National Institutes of Health; Grant number: CA56666.

References

1. Haas GP, Sakr WA. Epidemiology of prostate cancer. *CA Cancer J Clin* 1997;47(5):273–287. [PubMed: 9314822]
2. Cress AE, Rabinovitz I, Zhu W, Nagle RB. The alpha 6 beta 1 and alpha 6 beta 4 integrins in human prostate cancer progression. *Cancer Metastasis Rev* 1995;14(3):219–228. [PubMed: 8548870]
3. Tani T, Karttunen T, Kiviluoto T, Kivilaakso E, Burgeson RE, Sipponen P, Virtanen I. Alpha 6 beta 4 integrin and newly deposited laminin-1 and laminin-5 form the adhesion mechanism of gastric carcinoma. Continuous expression of laminins but not that of collagen VII is preserved in invasive parts of the carcinomas: implications for acquisition of the invading phenotype. *Am J Pathol* 1996;149(3):781–793. [PubMed: 8780383]
4. Hao J, Yang Y, McDaniel KM, Dalkin BL, Cress AE, Nagle RB. Differential expression of laminin 5 (alpha 3 beta 3 gamma 2) by human malignant and normal prostate. *Am J Pathol* 1996;149(4):1341–1349. [PubMed: 8863681]
5. Fuchs ME, Brawer MK, Rennels MA, Nagle RB. The relationship of basement membrane to histologic grade of human prostatic carcinoma. *Mod Pathol* 1989;2(2):105–111. [PubMed: 2657718]
6. Hida J, Matsuda T, Kitaoka M, Machidera N, Kubo R, Yasutomi M. The role of basement membrane in colorectal cancer invasion and liver metastasis. *Cancer* 1994;74(2):592–598. [PubMed: 8033038]
7. Pyke C, Salo S, Ralfkiaer E, Romer J, Dano K, Tryggvason K. Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res* 1995;55(18):4132–4139. [PubMed: 7664291]
8. Umbas R, Isaacs WB, Bringuier PP, Schaafsma HE, Karthaus HF, Oosterhof GO, Debruyne FM, Schalken JA. Decreased E-cadherin expression is associated with poor prognosis in patients with prostate cancer. *Cancer Res* 1994;54(14):3929–3933. [PubMed: 7518346]
9. Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HE, Debruyne FM, Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. *Cancer Res* 1992;52(18):5104–5109. [PubMed: 1516067]
10. Murant SJ, Handley J, Stower M, Reid N, Cussenot O, Maitland NJ. Co-ordinated changes in expression of cell adhesion molecules in prostate cancer. *Eur J Cancer* 1997;33(2):263–271. [PubMed: 9135498]

11. Cheng L, Nagabhushan M, Pretlow TP, Amini SB, Pretlow TG. Expression of E-cadherin in primary and metastatic prostate cancer. *Am J Pathol* 1996;148(5):1375–1380. [PubMed: 8623909]
12. Nagle RB, Hao J, Knox JD, Dalkin BL, Clark V, Cress AE. Expression of hemidesmosomal and extracellular matrix proteins by normal and malignant human prostate tissue. *Am J Pathol* 1995;146(6):1498–1507. [PubMed: 7778688]
13. Knox JD, Cress AE, Clark V, Manriquez L, Affinito KS, Dalkin BL, Nagle RB. Differential expression of extracellular matrix molecules and the alpha6-integrins in the normal and neoplastic prostate. *Am J Pathol* 1994;145(1):167–174. [PubMed: 8030747]
14. Bonkhoff H, Stein U, Remberger K. Differential expression of alpha 6 and alpha 2 very late antigen integrins in the normal, hyperplastic, and neoplastic prostate: simultaneous demonstration of cell surface receptors and their extracellular ligands. *Hum Pathol* 1993;24(3):243–248. [PubMed: 7681030]
15. Fornaro M, Tallini G, Bofetiado CJ, Bosari S, Languino LR. Down-regulation of beta 1C integrin, an inhibitor of cell proliferation, in prostate carcinoma. *Am J Pathol* 1996;149(3):765–773. [PubMed: 8780381]
16. Haywood-Reid PL, Zipf DR, Springer WR. Quantification of integrin subunits on human prostatic cell lines—comparison of nontumorigenic and tumorigenic lines. *Prostate* 1997;31(1):1–8. [PubMed: 9108879]
17. Allen MV, Smith GJ, Juliano R, Maygarden SJ, Mohler JL. Downregulation of the beta4 integrin subunit in prostatic carcinoma and prostatic intraepithelial neoplasia. *Hum Pathol* 1998;29(4):311–318. [PubMed: 9563778]
18. Dowling J, Yu QC, Fuchs E. Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. *J Cell Biol* 1996;134(2):559–572. [PubMed: 8707838]
19. Van Waes C, Kozarsky KF, Warren AB, Kidd L, Paugh D, Liebert M, Carey TE. The A9 antigen associated with aggressive human squamous carcinoma is structurally and functionally similar to the newly defined integrin alpha 6 beta 4. *Cancer Res* 1991;51(9):2395–2402. [PubMed: 1750876]
20. Falcioni R, Turchi V, Vittilo P, Navarra G, Ficari FCF, Sacchi A, Mariani-Costantini R. Integrin beta 4 expression in colorectal cancer. *Int J Pathol* 1994;5:573–578.
21. Chao C, Lotz MM, Clarke AC, Mercurio AM. A function for the integrin alpha6beta4 in the invasive properties of colorectal carcinoma cells. *Cancer Res* 1996;56(20):4811–4819. [PubMed: 8841003]
22. Gui GP, Wells CA, Browne PD, Yeomans P, Jordan S, Puddefoot JR, Vinson GP, Carpenter R. Integrin expression in primary breast cancer and its relation to axillary nodal status. *Surgery* 1995;117(1):102–108. [PubMed: 7809822]
23. Hanby AM, Gillett CE, Pignatelli M, Stamp GW. Beta 1 and beta 4 integrin expression in methacarn and formalin-fixed material from in situ ductal carcinoma of the breast. *J Pathol* 1993;171(4):257–262. [PubMed: 7512643]
24. Natali PG, Nicotra MR, Botti C, Mottolese M, Bigotti A, Segatto O. Changes in expression of alpha 6/beta 4 integrin heterodimer in primary and metastatic breast cancer. *Br J Cancer* 1992;66(2):318–322. [PubMed: 1503905]
25. Hessler H, Sakai LY, Hollister DW, Burgeson RE, Engvall E. Basement membrane diversity detected by monoclonal antibodies. *Differentiation* 1984;26(1):49–54. [PubMed: 6370774]
26. Kimmel KA, Carey TE. Altered expression in squamous carcinoma cells of an orientation restricted epithelial antigen detected by monoclonal antibody A9. *Cancer Res* 1986;46(7):3614–3623. [PubMed: 3708592]
27. Kennel SJ, Epler RG, Lankford TK, Foote LJ, Dickas V, Canamucio M, Cavalierie R, Cosimelli M, Ventura I, Falcioni R. Second generation monoclonal antibodies to the human integrin alpha 6 beta 4. *Hybridoma* 1990;9(3):243–255. [PubMed: 2365382]
28. Falcioni R, Sacchi A, Resau J, Kennel SJ. Monoclonal antibody to human carcinoma-associated protein complex: quantitation in normal and tumor tissue. *Cancer Res* 1988;48(4):816–821. [PubMed: 2448027]
29. Nagle RB, McDaniel KM, Clark VA, Payne CM. The use of antikeratin antibodies in the diagnosis of human neoplasms. *Am J Clin Pathol* 1983;79(4):458–466. [PubMed: 6188361]
30. Hsi BL, Yeh CJ. Monoclonal antibodies to human amnion. *J Reprod Immunol* 1986;9(1):11–21. [PubMed: 2431136]

31. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 1974;111(1):58–64. [PubMed: 4813554]
32. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;19(5):403–410. [PubMed: 1757079]
33. Bonkhoff H, Stein U, Remberger K. The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate* 1994;24(3):114–118. [PubMed: 7509483]
34. Bonkhoff H, Remberger K. Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. *Prostate* 1996;28(2):98–106. [PubMed: 8604398]
35. Bonkhoff H. Role of the basal cells in premalignant changes of the human prostate: a stem cell concept for the development of prostate cancer. *Eur Urol* 1996;30(2):201–205. [PubMed: 8875201]
36. Sonnenberg A, Linders CJ, Daams JH, Kennel SJ. The alpha 6 beta 1 (VLA-6) and alpha 6 beta 4 protein complexes: tissue distribution and biochemical properties. *J Cell Sci* 1990;96 (Pt 2):207–217. [PubMed: 1698797]
37. Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. *Proc Natl Acad Sci USA* 1990;87(22):8970–8974. [PubMed: 2247472]
38. Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA. Distinct functions for integrins alpha 3 beta 1 in focal adhesions and alpha 6 beta 4/bullous pemphigoid antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J Cell Biol* 1990;111(6 Pt 2):3141–3154. [PubMed: 2269668]
39. Jones JC, Kurpakus MA, Cooper HM, Quaranta V. A function for the integrin alpha 6 beta 4 in the hemidesmosome. *Cell Regul* 1991;2(6):427–438. [PubMed: 1883873]
40. Kurpakus MA, Quaranta V, Jones JC. Surface relocation of alpha 6 beta 4 integrins and assembly of hemidesmosomes in an in vitro model of wound healing. *J Cell Biol* 1991;115(6):1737–1750. [PubMed: 1757471]
41. Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, Falcioni R, Kennel SJ, Aplin JD, Baker J, Loizidou M. Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J Cell Biol* 1991;113(4):907–917. [PubMed: 2026654]
42. Spinardi L, Ren YL, Sanders R, Giancotti FG. The beta 4 subunit cytoplasmic domain mediates the interaction of alpha 6 beta 4 integrin with the cytoskeleton of hemidesmosomes. *Mol Biol Cell* 1993;4 (9):871–884. [PubMed: 8257791]
43. Spinardi L, Einheber S, Cullen T, Milner TA, Giancotti FG. A recombinant tail-less integrin beta 4 subunit disrupts hemidesmosomes, but does not suppress alpha 6 beta 4-mediated cell adhesion to laminins. *J Cell Biol* 1995;129(2):473–487. [PubMed: 7721947]
44. Rabinovitz I, Mercurio AM. The integrin alpha6beta4 functions in carcinoma cell migration on laminin-1 by mediating the formation and stabilization of actin-containing motility structures. *J Cell Biol* 1997;139(7):1873–1884. [PubMed: 9412479]
45. Tennenbaum T, Li L, Belanger AJ, De Luca LM, Yuspa SH. Selective changes in laminin adhesion and alpha 6 beta 4 integrin regulation are associated with the initial steps in keratinocyte maturation. *Cell Growth Differ* 1996;7(5):615–628. [PubMed: 8732671]
46. Rabinovitz I, Mercurio AM. The integrin alpha 6 beta 4 and the biology of carcinoma. *Biochem Cell Biol* 1996;74(6):811–821. [PubMed: 9164650]
47. Rossen K, Dahlstrom KK, Mercurio AM, Wewer UM. Expression of the alpha 6 beta 4 integrin by squamous cell carcinomas and basal cell carcinomas: possible relation to invasive potential? *Acta Derm Venereol* 1994;74(2):101–105. [PubMed: 7911612]
48. Liebert M, Washington R, Wedemeyer G, Carey TE, Grossman HB. Loss of co-localization of alpha 6 beta 4 integrin and collagen VII in bladder cancer. *Am J Pathol* 1994;144(4):787–795. [PubMed: 7512792]
49. Dalton SL, Scharf E, Briesewitz R, Marcantonio EE, Assoian RK. Cell adhesion to extracellular matrix regulates the life cycle of integrins. *Mol Biol Cell* 1995;6(12):1781–1791. [PubMed: 8590805]
50. Potts AJ, Croall DE, Hemler ME. Proteolytic cleavage of the integrin beta 4 subunit. *Exp Cell Res* 1994;212(1):2–9. [PubMed: 8174638]

51. von Bredow DC, Nagle RB, Bowden GT, Cress AE. Cleavage of beta 4 integrin by matrilysin. *Exp Cell Res* 1997;236(1):341–345. [PubMed: 9344615]
52. Knox JD, Wolf C, McDaniel K, Clark V, Lorient M, Bowden GT, Nagle RB. Matrilysin expression in human prostate carcinoma. *Mol Carcinog* 1996;15(1):57–63. [PubMed: 8561867]
53. Patton BL, Miner JH, Chiu AY, Sanes JR. Distribution and function of laminins in the neuromuscular system of developing, adult, and mutant mice. *J Cell Biol* 1997;139(6):1507–1521. [PubMed: 9396756]
54. Niessen CM, van der Raaij-Helmer MH, Hulsman EH, van der Neut R, Jonkman MF, Sonnenberg A. Deficiency of the integrin beta 4 subunit in junctional epidermolysis bullosa with pyloric atresia: consequences for hemidesmosome formation and adhesion properties [published erratum appears in *J Cell Sci* 1996 Aug;109(Pt 8):preceding table of contents]. *J Cell Sci* 1996;109(Pt 7):1695–1706. [PubMed: 8832392]
55. Garnick MB, Fair WR. Prostate cancer: emerging concepts. Part I [see comments]. *Ann Intern Med* 1996;125(2):118–125. [PubMed: 8678366]

Fig. 1.
Relative position of the epitopes on the β_4 integrin. The epitopes detected by the antibodies used in this study are positioned on either the extracellular or cytosolic portions of the β_4 integrin.

Fig. 2.

Expression pattern of the $\beta 4$ integrin in normal, PIN, and carcinoma of the prostate. Dual immunofluorescence staining of prostate with 18A anti-cytokeratin (A,B,C) and 3E1 anti- $\beta 4$ integrin (D,E,F). **A** and **D** show staining of $\beta 4$ in the basal cells of normal prostate gland (n) and adjacent microvasculature. There is a similar expression pattern for $\beta 4$ in the residual basal cells in the PIN lesion (p) (**B** and **E**). **C** and **F** show the absence of basal cells in the invasive carcinoma (Ca) and the complete absence of $\beta 4$ integrin staining with 3E1 anti- $\beta 4$ antibody. Magnification, $\times 400$ for (A,D,C,F), $\times 647$ for (B,E).

Fig. 3.

Expression pattern of assembled laminin-5 in normal, PIN, and carcinoma of the prostate. Dual immunofluorescence staining of prostate with 18A anti-cytokeratin (A,B,C) and GB3 anti-laminin (D,E,F). In **A** and **D**, the laminin-5 is polarized around the basal aspect of the normal gland (n) with some staining on the lateral aspect of the cells. In **B** and **E**, there is a similar pattern of expression at the basal cells that are attenuated in the PIN lesion (p). In places where basal cells are lost, there is no laminin-5 staining (arrows in **E**). In **C** and **F** showing the invasive carcinoma (Ca), note the complete absence of basal cells and the corresponding lack of laminin-5 staining. The punctate staining in panels **D**, **E** and **F** are due to autofluorescence of cytoplasmic lipofuscin. Magnification, $\times 400$.

Fig. 4.

Expression of the $\alpha 6 \beta 4$ integrin in human prostate, breast and colon carcinoma. Dual immunofluorescence staining with 18A anti-cytokeratin (A, B, C) and 3E1 anti- $\beta 4$ integrin (D, E, F) of prostate (A, D), breast (B, E), and colon (C, F) carcinomas. In the prostate and breast tissues, normal (n) and malignant carcinoma (Ca) are present in adjacent areas within the same section. Normal colon is not shown. Note that the colonic carcinoma expresses $\beta 4$ integrin polarized to the epithelial/stromal interface (F), but that the invasive prostate carcinoma (D) and the breast carcinoma (E) do not express this epitope. In addition to the $\beta 4$ staining of the normal gland in D and E, cytokeratin negative vessels (V) reveal $\beta 4$ staining. Magnification, $\times 400$.

Fig. 5.

Expression of assembled laminin-5 in human prostate, breast, and colon carcinoma. Dual immunofluorescence staining with 18A anti-cyokeratin (A, B, C) and GB3 anti-laminin-5 (D, E, F) of prostate (A, D), breast (B, E), and colon (C, F) carcinomas. Normal tissue components (n) are seen in prostate and breast images. Malignant components (Ca) are present in all three tissue types. Prominent laminin-5 staining can be seen in normal prostate and breast. Note there is no polarization of the laminin-5 in prostate carcinoma (D). In breast carcinoma, there is some weak cytoplasmic staining of laminin-5, but no polarization (E). In contrast, in colon carcinoma there is strong positive polarized staining surrounding the malignant glands (F). Magnification, $\times 400$ for (A, D, B, E), and $\times 613$ for C and F.

Fig. 6.

Expression of $\beta 4$ integrin in prostate, breast, and colon carcinoma. Human prostate, breast, and colon tissues containing both normal and carcinomatous regions were examined by immunohistochemistry using 5 different anti- $\beta 4$ integrin antibodies. Cases were examined for total positive staining and total polarized staining. Immunofluorescence staining was semi-quantitatively evaluated by the following scale of intensity: (+2) very strong staining, (+1) positive staining, ($\pm$) weak staining, (–) no staining above background. Cases which scored a (+1) or a (+2) were scored positive, while cases scoring ($\pm$) or (–) were scored negative. The resulting data are tabulated for prostate (**A**), breast (**B**), and colon (**C**) carcinoma. Values are expressed as percent positive cases examined (prostate, $n = 11$; for breast $n = 5$; for colon $n = 6$) for the two categories.