β_{1C} Integrin in Epithelial Cells Correlates with a Nonproliferative Phenotype

Forced Expression of β_{1C} Inhibits Prostate Epithelial Cell Proliferation

Mara Fornaro,* Michela Manzotti,*
Giovanni Tallini,* Amy E. Slear,* Silvano Bosari,[†]
Erkki Ruoslahti,[‡] and Lucia R. Languino*

From the Department of Pathology,* Yale University School of Medicine, New Haven, Connecticut; Department of Pathology,[†] European Institute of Oncology and University of Milan School of Medicine, Milan, Italy; and Cancer Research Center,[‡] The Burnham Institute, La Jolla, California

The expression of the β_{1C} integrin, an alternatively spliced variant of the β_1 subunit, was investigated in human adult and fetal tissues. In the adult, β_{1C} immunoreactivity was found in nonproliferative, differentiated simple, and/or pseudostratified epithelia in prostate glands and liver bile ducts. In contrast, β_{1C} was undetectable in stratified squamous epithelium of the epidermis and/or in hepatocytes. Luminal prostate epithelial cells expressed β_{1C} *in vivo* and *in vitro*, but no β_{1C} was seen in basal cells, which are proliferating cells. Fetal prostate expressed β_{1C} in differentiated glands that had a defined lumen, but not in budding glands, indicating that β_{1C} is a marker of prostate epithelium differentiation. The β_{1C} and the common β_{1A} variants are differentially distributed: β_{1A} was found in luminal and basal epithelial as well as in stromal cells in the prostate. In the liver, β_{1C} and β_{1A} were coexpressed in biliary epithelium, whereas vascular cells expressed only β_{1A} . Because we found β_{1C} in nonproliferative and differentiated epithelium, we investigated whether β_{1C} could have a causal role in inhibiting epithelial cell proliferation. The results showed that exogenous expression of a β_{1C} , but not of a β_{1A} , cytoplasmic domain chimeric construct, completely inhibited thymidine incorporation in response to serum by prostate cancer epithelial cells. Consistent with these *in vitro* results, β_{1C} appeared to be downregulated in prostate glands that exhibit regenerative features in benign hyperplastic epithelium. These data show that the presence of β_{1C} integrins in epithelial cells correlates with a nonproliferative, differentiated phenotype and is growth inhibitory to prostate epithelial cells *in vitro*. These

findings indicate a novel pathophysiological role for this integrin variant in epithelial cell proliferation. (*Am J Pathol* 1998, 153:1079–1087)

Cell interactions with extracellular matrix proteins control proliferation, differentiation, and survival,^{1–3} as well as tumor growth, angiogenesis, and metastasis.⁴ These interactions are predominantly mediated by integrins, cell adhesion receptors composed of an α and a β subunit.^{5–7} In addition to mediating physical interactions, integrins signal intracellularly through their cytoplasmic domains.⁸ The cytoplasmic domains also control integrin affinity for ligands and subcellular localization.^{8–10}

The cytoplasmic domain of the β_1 subunit, in its canonical form (β_{1A}), is highly conserved from fungi and invertebrates to vertebrates.¹¹ Experimental modifications of the β_1 cytoplasmic domain have been shown to affect cell proliferation,^{12,13} development,¹⁴ migration,^{15,16} integrin localization,^{17,18} and mitogen-activated protein kinase activation^{19,20} and phosphorylation of focal adhesion kinase or paxillin.^{15,21–23} Alternative splicing events, by creating variant cytodomains, generate functionally distinct integrin complexes.⁸ Alternatively spliced forms of the β (β_1 , β_3 , β_4 , and β_5) and α (α_3 , α_6 , and α_7) integrin cytoplasmic domains have been identified,^{8,24} thus adding further complexity to the regulatory pathways mediated by integrins.

The integrin β_1 variants are known as: the canonical β_{1A} , β_{1B} , β_{1C} , β_{1C-2} , and β_{1D} ; they differ in the COOH-terminal sequences.⁸ The β_{1C} integrin (formerly β_{1S})²⁵ is

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MF and MM contributed equally to this report.

MM's present address: Department of Pathology, European Institute of Oncology, Milan, Italy.

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Address reprint requests to Dr. Lucia R. Languino, Department of Pathology, Yale University School of Medicine, P.O. Box 208023, 310 Cedar Street, New Haven, CT 06520. E-mail: lucia.languino@yale.edu.

Figure 1. The β_{1C} integrin is expressed in luminal epithelium of adult prostate but not in the epidermis. The immunohistochemical analysis was performed on adult prostate (A through D) and on adult skin tissue sections (E and F) using 1.85 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} (A and E); 1.85 $\mu\text{g}/\text{ml}$ of nonimmune rabbit IgG (B and F); 5 $\mu\text{g}/\text{ml}$ AE1/AE3, monoclonal antibody to type I and type II cytokeratins (C); or 0.2 $\mu\text{g}/\text{ml}$ 34 β E12, monoclonal antibody to cytokeratins 1, 5, 10, and 14 (D). Magnification, $\times 200$. Note that β_{1C} is undetectable in the basal layer of the adult prostate gland (A).

generated by the presence of an unspliced intervening 116-bp sequence (exon C), which causes a frame shift in the 3' end of the β_1 subunit and codes for a unique 48-amino acid COOH-terminal sequence. Unlike β_{1A} , β_{1C} inhibits cell proliferation and is downregulated in cancer cells.^{12,13,26}

We show here that β_{1C} is expressed in a subset of epithelial cells exhibiting a nonproliferating and differentiated phenotype, and that it is lost in regenerative areas of prostate glands. We also provide evidence suggestive of a causal role for the β_{1C} cytodomain in suppressing epithelial cell proliferation.

Materials and Methods

Cells and Reagents

The PC3 human prostatic carcinoma cell line was obtained from American Type Culture Collection (Manassas, VA). PC3 cells were maintained in RPMI (Life Technologies Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gemini Bioproducts Inc., Calabasas, CA), 2 mmol/L glutamine (Gemini Bioproducts Inc.), 100 $\mu\text{g}/\text{ml}$ streptomycin-100 U/ml pen-

icillin (Gemini Bioproducts Inc.), 0.1 mmol/L nonessential amino acids (Life Technologies), and 1 mmol/L sodium pyruvate (Life Technologies). Human prostate luminal epithelial cells, BPH-1,²⁷ were maintained in RPMI supplemented with 2.5% FCS, 2 mmol/L glutamine, and 100 $\mu\text{g}/\text{ml}$ streptomycin-100 U/ml penicillin.

Rabbit antibodies specific for the β_{1C} or the β_{1A} subunit cytoplasmic domains were generated and affinity purified as previously described.²⁶ The following antibodies were used: fluorescein isothiocyanate-conjugated rat monoclonal antibody to mouse CD4, clone YTS 191.1 (Caltag Laboratories, San Francisco, CA); mouse monoclonal antibody to the human β_1 integrin extracellular domain, mAb 13 (Beckton Dickinson, San Jose, CA) and kindly provided by Dr. K. M. Yamada (National Institutes of Health, Bethesda, MD); mouse monoclonal antibodies to basal cytokeratins 1, 5, 10, and 14, clone 34 β E12 (Enzo Diagnostic, Farmingdale, NY) or to type I and type II cytokeratins, clone AE1/AE3 (Boehringer Mannheim, Indianapolis, IN); and mouse monoclonal antibody to cytokeratins 8 and 18, clone CAM 5.2 (Becton Dickinson). Fluorescein isothiocyanate-rat immunoglobulin G (IgG) were purchased from Caltag.

Figure 2. *In vivo* and *in vitro* expression of β_{1C} in prostate cells. A through D, a representative case of adult normal prostate tissue is shown. β_{1C} immunostaining (A) was specifically inhibited by antibody absorption on the β_{1C} 785 to 808 peptide (10 $\mu\text{g}/\text{ml}$) used as antigen (B) but not on an irrelevant peptide (C). The affinity-purified antibody to β_{1C} (A through C) or nonimmune rabbit IgG (D) were used at a final concentration of 5 $\mu\text{g}/\text{ml}$. E: newborn (1-month-old) prostate tissue detergent extract was electrophoresed on 7.5% SDS-polyacrylamide gel under reducing conditions, transferred to nitrocellulose membranes, and immunostained using 5 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} (a- β_{1C} , lane 1); 5 $\mu\text{g}/\text{ml}$ nonimmune rabbit IgG (nonimmune IgG, lane 2); or 2.5 $\mu\text{g}/\text{ml}$ mAb 13, monoclonal antibody to β_1 (a- β_1 mAb, lane 3). Proteins were visualized by enhanced chemiluminescence. Prestained marker proteins in kD are shown. The bracket (E) indicates the expected electrophoretic mobility of β_{1C} and β_{1A} . F: BPH-1 cells were surface iodinated and proteins immunoprecipitated using rabbit antisera either to β_{1C} (a- β_{1C} , lane 1) or β_{1A} (a- β_{1A} , lane 3), or normal rabbit serum (non immune, lane 2). The immunocomplexes were separated on a 7.5% SDS-polyacrylamide gel under reducing conditions and visualized by autoradiography. Arrowheads indicate β_{1C} and β_{1A} . Magnification, $\times 200$ (A through D).

Tissue Specimens

Samples (autopsy or surgical specimens) were obtained from the files of the Department of Pathology at Yale New Haven Hospital. The following adult tissues were included in the study: benign prostate (62 samples, including 25 benign prostatic hyperplastic samples), normal liver (12 samples), normal gallbladder (4 samples), normal kidney (5 samples), and normal lung (1 sample). Developing tissues from spontaneous or therapeutic abortions corresponding to different gestational ages were also included: prostate (4 samples, 18 to 22 weeks; 9 samples, 23 weeks or later) and liver (3 samples, 16 to 19 weeks). Hematoxylin and eosin sections were analyzed from all samples to assess the integrity of the tissue selected for the evaluation of β_{1C} , β_{1A} , or cytokeratin immunoreactivity. All tissues were obtained under review board-approved protocols.

Immunohistochemistry

Immunostaining of β_{1C} , β_{1A} , and cytokeratins was performed essentially as described,²⁶ with minor modifications. For immunostaining of liver specimens, nonspecific binding of biotin/avidin was prevented using the Avidin/Biotin blocking kit from Vector Laboratories (Burlingame, CA), according to the manufacturer's instructions. For immunostaining of prostate specimens, endogenous peroxidase was quenched with 3% H_2O_2 for 5 minutes at room temperature, microwave treatment was avoided, blocking was achieved with either 50% goat or horse serum in Tris-buffered saline containing 0.2% bovine serum albumin for 20 minutes at room temperature; each incubation step was followed by three washes with Tris-buffered saline. The specificity of the affinity-purified an-

tibody to the β_{1C} 785 to 808 peptide¹² in immunohistochemical staining was confirmed as follows: before the immunostaining procedure, the affinity-purified antibody to β_{1C} was preincubated for 30 minutes at 4°C with 10 to 30 $\mu\text{g}/\text{ml}$ of either β_{1C} 785 to 808 peptide or a control fibrinogen-derived peptide.²⁸

Immunoblotting and Immunoprecipitation

Frozen prostate tissue, obtained from autopsy specimens, was homogenized using lysis buffer containing 100 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO), 5% sodium dodecyl sulfate (SDS; American Bioanalytical, Natick, MA), 1 mmol/L phenylmethylsulfonyl fluoride (Life Technologies), 10 $\mu\text{g}/\text{ml}$ leupeptin (Calbiochem, San Diego, CA), 1 mmol/L benzamide (Sigma), 1 $\mu\text{mol}/\text{L}$ D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (Boehringer Mannheim), 10 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor (Life Technologies), using an OMNI 2000 homogenizer (OMNI International Inc., Gainesville, VA). Insoluble material was removed by centrifugation at $14,000 \times g$ for 30 minutes at 4°C. The protein content in each lysate was quantitated using the BCA protein assay reagent (Pierce, Rockford, IL) according to the manufacturer's instructions. To detect β_{1C} integrin, 200 μg of detergent tissue extract was electrophoresed on a 7.5% SDS-polyacrylamide gel under reducing conditions, transferred to nitrocellulose (Schleicher & Schuell, Keene, NH), and immunostained as described,²⁶ using either 5 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} ; 5 $\mu\text{g}/\text{ml}$ nonimmune rabbit IgG; or 2.5 $\mu\text{g}/\text{ml}$ mAb 13, monoclonal antibody to β_1 integrin.

BPH-1 cells were surface iodinated and proteins immunoprecipitated using rabbit antisera to β_{1C} or to β_{1A} or

Figure 3. Expression of β_{1C} in luminal epithelium of fetal prostate. The immunohistochemical analysis was performed on a 20-week-old fetus using 1.85 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} (A and C); 1.85 $\mu\text{g}/\text{ml}$ nonimmune rabbit IgG (B); or 0.2 $\mu\text{g}/\text{ml}$ 34 β E12, monoclonal antibody to cytokeratins 1, 5, 10, and 14 (D); or 5 $\mu\text{g}/\text{ml}$ AE1/AE3, monoclonal antibody to type I and type II cytokeratins (E). The β_{1C} immunostaining was specifically inhibited by antibody absorption on the β_{1C} 785 to 808 peptide (30 $\mu\text{g}/\text{ml}$) used as antigen (C). Magnification, $\times 100$. Note that β_{1C} expression is undetectable in the basal layer of the fetal prostate gland (A).

using normal rabbit serum, as previously described.¹² The immunocomplexes were separated on a 7.5% SDS-polyacrylamide gel under reducing conditions and visualized by autoradiography.

Inducible Expression of CD4- β_{1C} and CD4- β_{1A} Chimeric Constructs

*Hind*III DNA fragment, encoding the cytoplasmic domain of β_{1C} (nucleotides 2357 to 2613),²⁵ was isolated from the pBJ1- β_{1C} plasmid. The fragment was inserted into the *Hind*III site of the Ch2 chimera described by Lukashev et al²² which consists of the extracellular domain of murine CD4 joined to the transmembrane domain of the β_1 integrin. The resulting construct was designated Ch β_{1C} . Correct assembly of the construct was verified by nucleotide sequencing. The Ch1 chimeric construct, designated here Ch1 β_{1A} , containing the extracellular domain of murine CD4 and the transmembrane and cytoplasmic domains of the β_{1A} integrin, has been described by Lukashev et al.²² Each chimeric construct is expressed under the control of the mouse metallothionein I promoter, which can be induced by addition of ZnSO_4 to the growth medium. PC3 cells were electroporated using a Gene-pulser apparatus set at 250 V and 900 μF using either 10 or 30 μg of the Ch1 β_{1A} or the Ch β_{1C} chimera, respectively. Hygromycin-resistant cells were selected using growth medium containing 0.2 mg/ml hygromycin B (Boehringer Mannheim). Hygromycin-resistant colonies were pooled, and the resultant population was analyzed for cell surface expression of each chimeric construct by fluorescence-activated cell sorting using rat monoclonal antibody to mouse CD4. As negative control antibody, isotype-matched rat IgG was used. Stable transfectants were maintained in growth medium containing 0.2 mg/ml hygromycin B.

Cell Proliferation Assay

PC3 stable transfectants were starved for 24 hours in serum-free medium. Surface expression of chimeric constructs was induced by treating the cells with 75 $\mu\text{mol}/\text{L}$ ZnSO_4 (Sigma) in growth medium for 6 hours at 37°C. Cells were then detached with 0.05% trypsin/0.53 mmol/L ethylenediaminetetraacetic acid (Life Technologies), washed three times in serum-free medium, and analyzed by fluorescence-activated cell sorting using rat monoclonal antibody to mouse CD4 or isotype-matched rat IgG. Positive cells expressing comparable levels of the chimeric constructs were sorted using FACStar (Becton Dickinson). After sorting, the cell transfectants (15×10^3) were resuspended in serum-free medium and added to 96-well microtiter plates (ICN Biomedicals, Costa Mesa, CA) coated with 3 $\mu\text{g}/\text{ml}$ fibronectin to ensure comparable attachment. After 1 hour and 30 minutes at 37°C, cells were washed twice, incubated either in the absence or in the presence of 10% FCS for 18 hours at 37°C, and pulsed with 1 μCi [^3H]thymidine/well (5.0 Ci/mmol; Amersham Life Sciences, Arlington Heights, IL) during the last

Figure 4. Expression of β_{1C} in prostate budding glands. The immunohistochemical analysis was performed on a 20-week-old fetus using 1.85 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} (A); 1.85 $\mu\text{g}/\text{ml}$ of nonimmune rabbit IgG (B); or 1.25 $\mu\text{g}/\text{ml}$ CAM 5.2, monoclonal antibody to cytokeratins 8 and 18 (C). Magnification, $\times 200$. The expression of β_{1C} coincides with a differentiated phenotype of the prostate gland; note that β_{1C} is detectable only when a lumen is well defined in the gland.

3 hours of the 18-hour culture. Thymidine incorporation was evaluated as described.¹² In each experiment, duplicate or triplicate observations were performed, and the values are reported as mean $\pm$ standard error (SE). In parallel, in each experiment, the number of attached cells was evaluated after fixing and staining the cells with 0.5%

crystal violet (Sigma), as described.²⁹ The results were evaluated using a 630-nm wavelength filter in a Titertek Multiskan Bichromatic enzyme-linked immunosorbent assay reader (ICN). Group differences were compared using one-way analysis of variance followed by Bonferroni *post hoc* contrast.

Figure 5. The β_{1C} integrin is expressed in adult and fetal liver bile ducts but not in hepatocytes. Immunoperoxidase staining of β_{1C} was performed on adult (A through F) and 19-week-old fetal (G and H) liver using 5 $\mu\text{g}/\text{ml}$ affinity-purified antibody to β_{1C} (A, C through E, G, and H) or 5 $\mu\text{g}/\text{ml}$ nonimmune rabbit IgG (B). The β_{1C} immunostaining (D) was specifically inhibited by antibody absorption on the β_{1C} 785 to 808 peptide (10 $\mu\text{g}/\text{ml}$) used as antigen (E), not on an irrelevant peptide (F). Magnification, $\times 100$ (A and B); $\times 200$ (C through F), and $\times 400$ (G and H). Note strong β_{1C} immunoreactivity in the bile ducts (A, D, F, G, and H) and not in hepatocytes (C and G).

Figure 6. Differential expression of β_{1C} and β_{1A} in adult liver. Immunoperoxidase staining of β_{1C} and β_{1A} in liver with mild parenchyma alterations (steatohepatitis) was performed using 5 μ g/ml affinity-purified antibodies to β_{1C} (A and B) or to β_{1A} (C and D). Nonimmune rabbit IgG (5 μ g/ml) was used as a negative control (E). Magnification, $\times 200$. Note, β_{1C} is selectively expressed in biliary epithelium (B), whereas β_{1A} is found in bile ducts (D), as well as in sinusoids (C). Arrows (A and C) point to sinusoids.

Results

β_{1C} Expression in Epithelial Cells in Adult and Fetal Tissues

Immunohistochemical analysis of adult and fetal human tissue specimens was performed using an affinity-purified antibody to β_{1C} ; β_{1C} was detected in a subset of epithelial cells. Specifically, simple or pseudostratified epithelia in prostate, liver, gallbladder, lung, and kidney were positive for β_{1C} (see Figures 1 through 6 and 8, and data not shown) in all the analyzed cases, whereas stratified squamous epithelium of the epidermis was negative (Figure 1E). In the prostate, β_{1C} was preferentially expressed in the luminal glandular epithelium (Figure 1A). In contrast, the basal epithelium, composed of proliferating cells³⁰ identified by an antibody to basal cell-specific cytokeratins 1, 5, 10, and 14 (Figure 1D), did not reveal detectable levels of β_{1C} (Figure 1A). Similarly, β_{1C} immunoreactivity was undetectable in stromal and endothelial cells in all the analyzed tissues (see Figures 1 through 6). The specificity of the immunoreactivity of the antibody to β_{1C} (Figure 2A) was confirmed by 1) inhibition by the β_{1C} peptide, 785 to 808, used as the antigen (Figure 2B), 2) immunoblotting of detergent lysate of prostate tissue (Figure 2E), and 3) immunoprecipitation of surface-iodinated BPH-1 luminal epithelial cells (Figure 2F). The immunoblotting and immunoprecipitation both revealed bands with an electrophoretic mobility similar to that of β_{1A} (Figure 2, E and F).¹²

Immunohistochemical analysis performed on second-trimester fetal tissues showed that, as in adult tissue, β_{1C} was confined to glandular epithelium in completely differentiated glands with a defined lumen (Figures 3A and 4A and not shown), whereas it was absent in budding glands (Figure 4A). Cytokeratin 8 and 18 staining was used for the identification of prostate glands³¹ that had not differentiated completely and lacked a lumen (Figure 4C). The basal epithelium, identified using an antibody to

basal cell-specific cytokeratins 1, 5, 10, and 14 (Figure 3D), did not contain detectable β_{1C} (Figure 3A). The specificity of β_{1C} immunoreactivity (Figure 3A) was confirmed by inhibitions with the β_{1C} peptide used as the antigen (Figure 3C). These staining results suggest that β_{1C} is a marker of prostate epithelial cell differentiation.

In the adult liver, β_{1C} immunoreactivity was restricted to biliary epithelium (Figure 5, A, D, and F), which stained at the apical, basal, and lateral cell surfaces; no β_{1C} was found in hepatocytes (Figure 5C). A similar staining pattern showing expression in bile ducts, but not in hepatocytes, was observed in 16, 17, and 19 week-gestation fetal livers (Figure 5, G and H, and not shown). The specificity of the staining was confirmed by inhibition with the β_{1C} peptide (Figure 5E). The control of β_{1C} expression appears to be independent of β_{1A} , as the two variants are differentially regulated in a cell type-specific manner. In contrast to β_{1C} , which has a distribution restricted to biliary epithelium (Figure 6, A and B), the β_{1A} variant was found in bile duct epithelium (Figure 6D); hepatic vasculature (Figure 6C and not shown); and, weakly, in hepatocytes, as shown previously.³²

β_{1C} Inhibits Epithelial Cell Proliferation

Because β_{1C} has a powerful growth-inhibitory activity in fibroblasts^{12,13} and because, as shown above, it is predominantly found in nonproliferative epithelium, we explored the possibility that β_{1C} would have a causal role in inhibiting epithelial cell proliferation. Chimeric constructs containing either β_{1C} (Ch β_{1C} ; Figure 7, A and B) or β_{1A} (Ch β_{1A} ; Figure 7, C and D) cytoplasmic tails under the control of an inducible promoter were stably expressed in PC3 prostate cancer cells. Serum-starved stable transfectants were incubated with ZnSO₄ (Figure 7, A and C) to induce surface expression of the chimeric proteins and sorted by fluorescence-activated cell sorting to isolate positive cells expressing comparable levels of Ch β_{1C} or

Figure 7. Inhibition of prostate cell growth in response to β_{1C} expression. PC3 cells were stably transfected using chimeric constructs containing the cytoplasmic domain of either β_{1C} (Ch β_{1C} , A and B) or β_{1A} (Ch1 β_{1A} , C and D). Serum-starved stable transfectants were incubated either in the absence (C and D) or in the presence of 75 μ M ZnSO₄ (A and B) for 6 hours at 37°C in growth medium to induce surface expression of the chimeric proteins (A and C). Stable cell transfectants were then stained with fluorescein isothiocyanate-conjugated rat monoclonal antibody to mouse CD4 (solid line) or isotype-matched fluorescein isothiocyanate-conjugated rat IgG (dotted line) and sorted by fluorescence-activated cell sorting to isolate positive cells expressing comparable levels of Ch β_{1C} or Ch1 β_{1A} . The Ch β_{1C} and the Ch1 β_{1A} sorted cells were compared in proliferation assays (E) on 96-well plates coated with 3 μ g/ml fibronectin. Attached cells were incubated for 15 hours at 37°C either in the absence or in the presence of 10% FCS and then for an additional 3 hours at 37°C with 1 μ Ci/well [³H]thymidine to measure their proliferative response to FCS. Results are mean $\pm$ SE values of duplicate determinations. Group differences were compared using one-way analysis of variance followed by Bonferroni *post hoc* contrast. The differences in proliferation between Ch β_{1C} and Ch1 β_{1A} transfectants in the presence of 10% FCS and between nonstimulated and 10% FCS-stimulated Ch1 β_{1A} transfectants are statistically significant ($P < 0.05$). A representative experiment of three is shown.

Ch1 β_{1A} . The sorted cells were compared in proliferation assays on fibronectin (Figure 7E). Under the experimental conditions used, adhesion to fibronectin was not affected by the expression of either Ch β_{1C} or Ch1 β_{1A} (not shown). However, the expression of β_{1C} completely inhibited thymidine incorporation in response to serum in the cells, whereas β_{1A} had no effect.

The *in vitro* data showing strong inhibition of prostate epithelial cell proliferation by β_{1C} were further supported by immunohistochemical analysis of regenerative areas of hyperplastic prostate tissues. Luminal expression of β_{1C} (Figure 8A), which was observed in normal and hyperplastic prostatic glandular epithelia of all the analyzed cases, was selectively downregulated in regenerative areas displaying hyperchromatic nuclei and cellular crowding (Figure 8, C and D). β_{1A} was always detected in both the basal and luminal layers (Figure 8B, and not shown).

These results suggest a unique role for β_{1C} in controlling epithelial cell proliferation under normal and pathological conditions.

Discussion

The results described here show that the β_{1C} integrin, an alternatively spliced variant of the β_1 subunit,²⁵ is expressed *in vivo* in a cell type-specific manner, predominantly in nonproliferating differentiated epithelial cells, and that is an early marker of differentiation of prostate epithelium. We also show that β_{1C} inhibits *in vitro* growth of malignant epithelial cells from the prostate.

Our *in vivo* study shows a selective expression of β_{1C} in a subset of epithelial cells that does overlap with the expression of β_{1A} . This analysis of β_{1C} distribution was made possible by the availability of highly specific antibodies to the β_{1C} cytoplasmic domain and emphasizes the importance of using antibodies specific to the different variants when analyzing β_1 distribution. The β_{1C} distribution is unique among the β_1 variants. The β_{1B} isoform has been found to be restricted to skin and liver tissues,³³ whereas the β_{1D} subunit is expressed in striated muscles, where it replaces β_{1A} ^{19,34} in a developmentally regulated manner.³⁵

Functional differences have been described for the β_1 variants. β_{1D} is functionally similar to β_{1A} , in that both are localized at focal contacts and can stimulate focal adhesion kinase activation,^{19,34} whereas β_{1B} inhibits it.¹⁵ This suggests that modulation of splicing patterns of β_1 mRNA may provide an accessory mechanism to regulate signaling pathways initiated by integrins. A recent study shows that the ability of β_{1C} to inhibit cell proliferation is shared by β_{1D} ³⁶; however, the downstream signaling pathways by these variants are unknown and may be different.

The mechanisms that regulate epithelial cell proliferation and differentiation in developing embryonal and fetal tissue are largely unknown. In general terms during organogenesis, a proliferative phase is followed by one in which the cells progressively differentiate and acquire the phenotype required for their highly specialized functions.³⁷ The results of our study indicate that β_{1C} is expressed at the transition between these phases and suggest that its expression might be an important mechanism by which cellular proliferation is inhibited in differentiating fetal epithelia in specific organs.

Altered expression of integrins in epithelial cells has been shown to generate pathological phenotypes.³² Abnormal expression of β_1 in the suprabasal epidermal layers of the skin causes epidermal hyperproliferation, as shown in studies performed using transgenic mice, thus confirming that incorrect integrin distribution can be a trigger for increased *in vivo* growth rate.^{38,39} In normal skin, β_1 integrins are found only in the basal layer of the epidermis, whereas during wound healing and psoriasis, which are associated with hyperproliferation, they also appear in the suprabasal layers of the skin.⁴⁰ Integrin expression affects the growth of tumor cells *in vitro* and tumor growth and metastasis *in vivo*.^{41–50} On the basis of

Figure 8. β_{1C} is down regulated in regenerative areas of prostate glands. Prostate tissue sections from a hyperplastic specimen were analyzed by immunohistochemistry using 3.75 $\mu\text{g/ml}$ affinity-purified antibody to β_{1C} (A, C, and D); 2 $\mu\text{g/ml}$ affinity-purified antibody to β_{1A} (B); of 3.75 $\mu\text{g/ml}$ nonimmune rabbit IgG (E). Magnification, $\times 200$ (A and B) and $\times 400$ (C through E) Regenerative areas displaying hyperchromatic nuclei and cellular crowding. (arrows, C and D), downregulate β_{1C} , suggesting a selective loss of expression of β_{1C} in proliferative areas. The results also show that β_{1C} and β_{1A} are differentially expressed in prostate epithelial cells; specifically, basal cells fail to express β_{1C} (A) but strongly express β_{1A} (B).

the different effects of β_{1C} and β_{1A} on cell proliferation, we would predict that replacement of the β_{1C} with β_{1A} could also lead to abnormal proliferation in epithelium.

The expression of β_{1C} completely inhibited thymidine incorporation of prostate cancer epithelial cells in response to serum. Consistent with these *in vitro* results, β_{1C} appeared to be downregulated in prostate glands that exhibit regenerative features in benign hyperplastic epithelium. These observations, and previous findings showing that β_{1C} is downregulated in prostate cancer,²⁶ suggest that the loss of β_{1C} expression may contribute malignant progression in prostate cells.

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