Regulation of mRNA and Protein Levels of $\beta 1$ Integrin Variants in Human Prostate Carcinoma

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Alterations of integrin expression levels in cancer cells correlate with changes in invasiveness, tumor progression, and metastatic potential. The $\beta 1C$ integrin, an alternatively spliced form of the human $\beta 1$ integrin, has been shown to inhibit prostate cell proliferation. Furthermore, $\beta 1C$ protein levels were found to be abundant in normal prostate glandular epithelium and down-regulated in prostatic adenocarcinoma. To gain further insights into the molecular mechanisms underlying abnormal cancer cell proliferation, we have studied $\beta 1C$ and $\beta 1$ integrin expression at both mRNA and protein levels by Northern and immunoblotting analysis using freshly isolated neoplastic and normal human prostate tissue specimens. Steady-state mRNA levels were evaluated in 38 specimens: 33 prostatic adenocarcinomas exhibiting different Gleason's grade and five normal tissue specimens that did not show any histological manifestation of benign prostatic hypertrophy. Our results demonstrate that $\beta 1C$ mRNA is expressed in normal prostate and is significantly down-regulated in neoplastic prostate specimens. In addition, using a probe that hybridizes with all $\beta 1$ variants, mRNA levels of $\beta 1$ are found reduced in neoplastic *versus* normal prostate tissues. We demonstrate that $\beta 1C$ mRNA down-regulation does not correlate with either tumor grade or differentiation according to Gleason's grade and TNM system evaluation, and that $\beta 1C$ mRNA levels are not affected by hormonal therapy. In parallel, $\beta 1C$ protein levels were analyzed. As expected, $\beta 1C$ is found to be expressed in normal prostate and dramatically reduced in neoplastic prostate tissues; in contrast, using an antibody to $\beta 1$ that recognizes all $\beta 1$ variants, the levels of $\beta 1$ are comparable in normal and neoplastic prostate, thus indicating a selective down-regulation of the $\beta 1C$ protein in prostate carcinoma.

These results demonstrate for the first time that $\beta 1C$ and $\beta 1$ mRNA expression is down-regulated in prostate carcinoma, whereas only $\beta 1C$ protein levels are reduced. Our data highlight a selective pressure to reduce the expression levels of $\beta 1C$, a very efficient inhibitor of cell proliferation, in prostate malignant transformation. (Am J Pathol 2000, 157:1727-1734)

Prostatic carcinoma is the most common type of cancer found in men in the western world and its distant metastases become a life-threatening event in tumor bearing patients. Although the biology of prostate cancer and metastases is poorly understood, it is becoming clear that major determinants of the malignant or hyperplastic phenotype are adhesion molecules, various growth factors, and their receptors, whose inappropriate expression or loss disrupts normal regulation of cell proliferation and differentiation.¹

Integrins are a superfamily of cell surface adhesion receptors that play a critical role in tumor progression and metastases as well as in a number of physiological processes such as inflammation, cell adhesion, migration, proliferation, survival, and differentiation.²⁻⁵ Integrins are receptors for extracellular matrix proteins such as fibronectin, vitronectin, collagen, and laminin.⁶ In addition to mediate cell adhesion to the extracellular matrix, integrins also transduce biochemical signals into the cell thus regulating cell proliferation and differentiation.^{7,8}

Integrins are transmembrane glycoproteins composed of α and β subunits that associate to form a heterodimer; 16 α subunits and eight β subunits, that associate to form at least 22 different receptors, have been discovered to date.^{7,8} Each subunit has a large extracellular domain, a single transmembrane domain and a short cytoplasmic domain.⁹ The role of the integrin cytoplasmic domain in modulating integrin functions and signaling events is well established.^{10,11}

Alternatively spliced variants of the integrin cytoplasmic domain have been described for some of the α and

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Table 1. Clinicopathological Features of 33 Patients with Prostate Carcinoma

Patients	Age (years)	Gleason's grade	Stage	Hormonal therapy (months)
N1	60		Control	
N2	62		Control	
N3	69		Control	
N4	72		Control	
N5	65		Control	
K1	57	≥7	T3N0M0	—
K2	63	≥7	T3N1M0	—
K3	77	≥7	T2N0M0	—
K4	70	<7	T2N0M0	3
K5	67	≥7	T2N0M0	—
K6	63	≥7	T3N0M0	—
K7	70	≥7	T3N0M0	6
K8	59	≥7	T3N1M0	—
K9	66	≥7	T3N1M0	—
K10	57	≥7	T3N1M0	—
K11	67	≥7	T2N0M0	6
K12	67	≥7	T3N0M0	—
K13	76	<7	T2N0M0	3
K14	76	≥7	T3N0M0	1
K15	59	≥7	T3N0M0	—
K16	61	≥7	T3N0M0	—
K17	64	<7	T2N0M0	—
K18	69	≥7	T2N0M0	6
K19	72	≥7	T3N1M0	—
K20	64	<7	T3N0M0	—
K21	59	≥7	T3N0M0	—
K22	60	≥7	T2N0M0	6
K23	69	≥7	T2N0M0	—
K24	63	≥7	T3N1M0	—
K25	62	≥7	T4N1M0	—
K26	66	≥7	T2N0M0	6
K27	76	≥7	T3N0M0	—
K28	70	≥7	T3N0M0	—
K29	66	≥7	T3N0M0	—
K30	75	≥7	T3N0M0	—
K31	67	≥7	T4N0M0	—
K32	67	<7	T2N0M0	3
K33	68	≥7	T3N0M0	—

K, prostate carcinoma; N, normal prostate; —, no hormonal therapy.

β subunits.¹¹ Alternative splicing events between exon 6 and exon 7 of the $\beta 1$ integrin subunit generate four different isoforms.¹¹ A $\beta 1$ isoform, $\beta 1C$, was found to be expressed in normal prostate epithelial cells.^{11,12} Its cytoplasmic domain consists of 26 amino acids encoded by exon 6, and 48 amino acids derived from an additional exon, exon C and part of exon 7, in the $\beta 1$ integrin gene. It has been demonstrated that $\beta 1C$ expression inhibits cell proliferation and causes growth arrest in the late G₁ phase of the cell cycle.^{13–15} Recent studies have also demonstrated that $\beta 1C$ causes up-regulation of the cyclin kinase inhibitor p27^{Kip1} protein levels in prostate cells shedding new lights into the molecular mechanisms underlying prostate cancer progression.¹⁶

Although several groups have analyzed integrin expression in prostate cancer *in vitro* or *in vivo*^{17–20} at the protein levels, very few studies have described integrin mRNA expression in prostate malignant transformation either *in vitro* or *in vivo*.^{21,22} We have studied $\beta 1C$ and $\beta 1$ expression at both mRNA and protein levels by Northern and immunoblotting analysis using specimens from 33 patients affected by prostatic carcinoma. Our results demonstrate for the first time that $\beta 1C$ and $\beta 1$ mRNA

expression is down-regulated in prostate carcinoma, whereas only $\beta 1C$ protein levels are reduced.

Materials and Methods

Tissue Specimens

This study was performed using 38 prostate specimens (Table 1) obtained from either patients who underwent radical cystoprostatectomy for bladder carcinoma noninvolving the prostate (five specimens) or patients with prostate cancer (33 specimens) hospitalized at the Department of Urology of the University of Bari, School of Medicine, in the years 1998 to 1999. Informed consent was obtained from all patients. Soon after surgical removal of the prostate, a sample was taken from all specimens, snap-frozen, and cryopreserved in liquid nitrogen for RNA extraction and immunoblotting analysis. The remaining tissue samples were fixed in 10% neutral-buffered formalin for 12 to 24 hours, embedded in paraffin, and stained with hematoxylin and eosin (H&E). H&E-stained sections were reviewed, and the tumor grade,

according to Gleason's criteria²³ and the stage of tumor, according to TNM system,²⁴ were estimated in each tumor sample.

A sample of normal human liver, obtained during cholecystectomy, was also used to generate mRNA.

RNA Extraction and Northern Blot Analysis

Frozen tissue samples were pulverized to a fine powder and cellular RNA was extracted using the guanidinium isothiocyanate-cesium chloride procedure.²⁵ Total RNA (25 μ g) isolated from the tissues was electrophoresed through 1% denaturing agarose gel containing 660 mmol/L formaldehyde, and transferred²⁶ to a nylon membrane (Hybond N⁺; Amersham, Milan, Italy). The filters were subsequently prehybridized overnight at 42°C with a buffer consisting of 50% formamide, 5 $\times$ Denhardt's solution (1% Ficoll 400, 1% polyvinylpyrrolidone, 1% bovine serum albumin), 5 $\times$ sodium chloride/sodium phosphate/ethylenediaminetetraacetic acid (SSPE) (3 mol/L NaCl, 200 mmol/L Na₂H₂PO₄, pH 7.0, 19 mmol/L ethylenediaminetetraacetic acid), 0.5% sodium dodecyl sulfate (SDS) and 100 μ g/ml of sonicated salmon sperm DNA. The filters were then hybridized for 20 hours at 42°C by adding 3 $\times$ 10⁶ cpm of ³²P-labeled probe/ml to the prehybridization solution. The filters were washed once with 2 $\times$ SSPE, 0.1% SDS for 10 minutes at room temperature, then with 1 $\times$ SSPE, 0.1% SDS at 42°C, followed by several washes in 0.1 $\times$ SSPE, 0.1% SDS, at 65°C and finally exposed at -80°C overnight or longer to Kodak X-Omat AR 5 film (Kodak, Rochester, NY). Radiolabeled probes were generated using the Megaprime DNA labeling kit (Amersham), 5 μ l of α -³²P-dCTP (3,000 Ci/mmol, Amersham)²⁷ and 25 ng of double-stranded either 116-bp fragment specific for the β 1C integrin or a full-length human β 1 cDNA.¹² The specific 116-bp β 1C fragment (nucleotides 2435 to 2550)¹² was generated by polymerase chain reaction using pBluescript β 1C plasmid as template and the resulting fragment was subcloned in the pBluescript vector. mRNA levels were normalized using ribosomal 28S RNA, a constitutively expressed gene.²⁸ For this purpose, blots were stripped in 0.1% boiling SDS and reprobed with the radiolabeled ³²P-28S cDNA probe. Quantitative analysis was performed by densitometric scanning of the autoradiographs using a Bio-Rad GS-700 densitometer (Bio-Rad, Richmond, CA); multiple exposures of the same Northern blots in a linear range were performed. The ratio between the 4.2-kb long β 1C mRNA levels and the 28S rRNA levels was calculated for each sample to take into account differences in RNA loading. The average of either β 1C or β 1 mRNA expression levels in control normal prostate derived from five patients was set at 100 (arbitrary units). β 1C or β 1 mRNA levels in neoplastic prostate were calculated as percentage of normal prostate mRNA levels hybridized on the same filter. For each specimen, the mean value ($\pm$ SEM) of results obtained in at least three experiments was calculated.

Immunoblotting

Either normal or tumor frozen tissue specimens obtained from radical prostatectomy were homogenized in lysis buffer containing 0.1% SDS, 1% Nonidet P-40, 50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 200 mmol/L LiCl, 5 mmol/L ethylenediaminetetraacetic, 10% glycerol, 10 μ g/ml aprotinin, 120 μ g/ml leupeptin, 170 μ g/ml phenylmethylsulfonyl fluoride. The homogenate was sonicated for 20 seconds, then centrifuged for 30 minutes at 14,000 $\times$ g at 4°C. Two-mercaptoethanol (1%) was added to each lysate for 30 minutes at 4°C to further solubilize potentially cross-linked molecules and 150 μ g of tissue extracts were electrophoresed on 7.5% SDS-polyacrylamide gel electrophoresis under reducing conditions. Immunoblotting was performed as previously described²⁰ using either 5 μ g/ml rabbit polyclonal affinity-purified antibody to β 1C integrin or 1 μ g/ml mouse monoclonal antibody to β 1 integrin (Transduction Laboratories, Milan, Italy) or 10 μ g/ml of antibody to β -tubulin (Sigma, St. Louis, MO) for 16 hours at 4°C in Tris-buffered saline/Tween 20 (TBS-T) (20 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 0.2% Tween-20). The membrane was then washed three times in TBS-T and incubated with horseradish-peroxidase-conjugated goat affinity-purified antibody to either rabbit or mouse IgG (Amersham), in TBS-T for 1 hour at room temperature. After three washes in TBS-T, the proteins were visualized using the Amersham enhanced chemiluminescent system according to the manufacturer's instructions. Densitometric values for immunoreactive bands were quantified using a GS-700 Imaging Densitometer (Bio-Rad). β 1C and β 1 protein levels were calculated as percentage of control (normal prostate tissue) upon normalization using β -tubulin as control for protein loading.

HL60 Cells

Human leukemia HL60 cells were grown in RPMI 1640 (Gibco, Life Technologies, Milan, Italy), with 50 μ g/ml gentamicin, 2 mmol/L glutamine, and 15% inactivated fetal calf serum, at 37°C in presence of 5% CO₂. Total RNA from differentiated cells was prepared 24 hours after incubation with 160 nmol/L TPA (or PMA phorbol-12-myristate-13-acetate; Sigma) as previously described.²⁹

Statistical Analysis

Data are reported as the mean $\pm$ SEM for the indicated experiments. Statistical analysis was performed using the Student's *t*-test. All experiments were repeated at least three times.

Results

β 1C mRNA Expression in Neoplastic and Normal Prostate Tissues

This study was performed using 38 prostate specimens obtained from patients with either prostate or bladder

Figure 1. $\beta 1C$ mRNA expression is down-regulated in human prostate neoplastic tissues. Total RNA was isolated from five normal and 13 neoplastic prostate specimens. $\beta 1C$ mRNA expression was evaluated by Northern blotting using the 116-bp $\beta 1C$ -specific probe shown in Figure 2. Twenty-five μ g total RNA were used for each sample. **Lane 1:** RNA from HL60 cells was used as positive control. **Lane 2:** RNA from human liver was used as negative control. **Lanes 3 to 7:** RNA from normal prostate tissues. **Lanes 8 to 20:** RNA from neoplastic prostate tissues. To normalize the amount of total RNA loaded for each sample, the blot was stripped and rehybridized using a 28S rRNA probe.

cancer. The patients were divided into two groups (Table 1). The first group included five patients (N1 to N5; age range, 60 to 72 years) with normal prostate who underwent radical cystoprostatectomy for bladder carcinoma noninvolving the prostate. The second group included 33 patients (K1 to K33; age range, 57 to 77 years) with histologically proven prostatic adenocarcinoma, who underwent radical prostatectomy. In the first group, normal prostatic tissue was histologically confirmed. In the second group, the grade of tumor differentiation and the stage of tumor were estimated in each tumor sample according to Gleason's criteria and to TNM system, respectively. Based on Gleason's criteria²³ the prostate specimens were divided in five moderately differentiated (Gleason's score <7) and 28 poorly differentiated (Gleason's score ≥ 7) prostate tumors; based on TNM system,²⁴ the specimens were divided in 11 T2N0M0 (stage II), 14 T3N0M0 (stage III), and six T3N1M0, one T4N0M0, and one T4N1M0 (stage IV) prostate tumors. A neo-adjuvant hormonal therapy consisting of the association of luteinizing hormone releasing hormone analogue (goserelin depot, 3.6 mg/q 28 days) and a nonsteroidal anti-androgen (bicalutamide, 50 mg/day) was administered to nine patients affected by prostate carcinoma (Table 1) before surgery to reduce prostate and tumor volume and to obtain downstaging of the tumor, as reported in preliminary clinical trials.^{30,31}

Expression of $\beta 1C$ and $\beta 1$ was examined at the RNA level in 38 prostate tissues using either a $\beta 1C$ -specific probe (Figure 2) or a $\beta 1$ full-length probe that hybridizes with all of the $\beta 1$ variants.¹¹ Steady-state levels of $\beta 1C$ (Figure 1 and not shown) and $\beta 1$ (Figure 3 and not shown) mRNA were evaluated by Northern blotting analysis of total RNA isolated from 33 neoplastic and five normal tissues (Table 1). Because of the low amount of RNA obtained from the tissue samples, total RNA rather than poly(A⁺) RNA was analyzed. A 4.2-kb transcript was detected in all samples (Figure 1). This band corresponds to the $\beta 1C$ mRNA because the probe used is specific for exon C, which is found only in $\beta 1C$ (Figure 2). Total RNA extracted from TPA-differentiated HL60 cells and from human liver, was used as positive and negative controls for $\beta 1C$ mRNA expression, respectively (Figure 1, lanes 1 and 2), because $\beta 1C$ is expressed in TPA-differentiated HL60 cells and is barely detectable in normal human liver.^{12,15} As expected, low levels of $\beta 1C$

Figure 2. $\beta 1C$ -specific probe. Schematic drawing showing $\beta 1C$ and $\beta 1A$ cytoplasmic domains.¹¹ The specific 116-bp $\beta 1C$ probe is shown. The 116-bp fragment used in Northern blotting analysis (see Figure 1) was generated by polymerase chain reaction using pBluescript- $\beta 1C$ plasmid as template. E, exon.

mRNA were found in normal liver compared with HL60 cells (Figure 1, lanes 1 and 2). Northern blotting analysis showed a significant decrease ($49 \pm 4\%$ decrease) of $\beta 1C$ mRNA levels in neoplastic tissues (Figure 1, lanes 8 to 20) compared with normal prostate tissues (Figure 1, lanes 3 to 7). Decreased $\beta 1C$ steady-state mRNA levels were detected in 94% of the prostatic carcinoma specimens compared with normal prostate samples and the differences were statistically significant ($P < 0.005$; Figure 4). In one instance (K16), $\beta 1C$ mRNA expression was found increased ($121 \pm 8\%$) compared with the levels in normal prostate tissues, whereas in a different specimen (K10), $\beta 1C$ mRNA levels were comparable ($100 \pm 4\%$) to normal prostate levels (Figure 4).

Using full-length $\beta 1$ cDNA, that hybridizes with all $\beta 1$ variants,¹⁴ as probe, we analyzed the 38 prostate tissue specimens described above. The results show that among the 33 prostatic adenocarcinoma, 29 showed reduced $\beta 1$ mRNA levels when compared with the five normal prostate tissues (Figure 3 and not shown).

The results show that $\beta 1C$ as well as $\beta 1$ mRNA levels are reduced in prostatic adenocarcinoma compared with normal prostate tissues.

Figure 3. β 1 mRNA expression is down-regulated in human neoplastic prostate tissues. Total RNA was isolated from five normal and 13 neoplastic prostate specimens. β 1 mRNA levels were evaluated by Northern blotting using a human full-length β 1 probe. **Lane 1:** RNA from HL60 cells. **Lane 2:** RNA from human liver. **Lanes 3 to 7:** RNA from normal prostate tissues. **Lanes 8 to 20:** RNA from neoplastic prostate tissues. To normalize the amount of total RNA loaded for each sample, the blot was stripped and rehybridized using a 28S rRNA probe.

β 1C mRNA Expression and Clinical Progression

To investigate whether β 1C expression is associated with tumor stage, correlation of β 1C mRNA levels with clinicopathological parameters (Gleason's grade and tumor stage) was evaluated. As shown in Figure 5A, β 1C mRNA expression was comparable in patients with Gleason's grade <7 ($57 \pm 7\%$, $n = 5$) and Gleason's grade ≥ 7 ($50 \pm 5\%$, $n = 28$). In parallel, the specimens were analyzed using the TNM system for tumor stage classification (Figure 5B). The differences in mRNA levels in specimens at different stages were not statistically significant ($P > 0.05$); in fact, as shown in Figure 5B, β 1C mRNA expression was comparable, although lower than normal prostate controls, in stage II, III, and IV tumors ($48 \pm 6\%$, $52 \pm 8\%$, and $56 \pm 7\%$, respectively). Moreover, we investigated whether a correlation between β 1C mRNA expression and hormonal therapy occurred. As shown in Figure 6, the differences between patients with 3- or 6-month hormonal treatment and patients who did not undergo hormonal therapy were not significant ($P > 0.05$ in both cases) although they were reduced *versus* normal prostate controls ($64 \pm 10\%$, $38 \pm 7\%$, $52 \pm 5\%$, respectively). In the only available case where 1-month hormonal therapy had been administered, there was a statistically significant increase ($156 \pm 4\%$) with respect to the patients that had not received any therapy; however, the results related to short-term (1 month) therapy need to be further investigated using a larger number of cases, when available.

In conclusion, down-regulation of β 1C mRNA levels did not correlate with either tumor grade, or tumor stage, or hormonal therapy.

β 1C Protein Expression in Neoplastic and Normal Prostate Tissues

Among the specimens showing down-regulation of β 1C mRNA, 13 were selected for immunoblotting analysis of β 1C and β 1 integrins. Figure 7, A and B, shows the results of our immunoblotting analysis. β 1C and β 1 were both expressed in normal (Figure 7A, lanes 1 and 2) and in tumor prostate tissue (Figure 7A, lanes 3 to 15) as described previously.^{16,20} The results in this set of specimens show a dramatic down-regulation of β 1C in lysates from neoplastic tissues compared with normal tissues: β 1C protein levels ranged from 8 to 28% (with an average value of $19 \pm 4\%$) of the levels found in normal tissues (Figure 7B). In contrast, β 1 protein levels were comparable in normal and prostatic adenocarcinoma tissues (Figure 7, A and B).

Discussion

We show in this study, for the first time, that β 1C integrin mRNA expression is reduced in neoplastic prostate *versus* normal prostate tissues. Using the same tissue specimens, we also show down-regulation of mRNA levels of all β 1 variants. At the protein level, decreased expression of β 1C is observed in agreement with previous findings in prostatic adenocarcinoma as well as in other neoplasia.^{20,32,33} In contrast, the protein levels of all β 1 variants are comparable in normal and neoplastic prostate, thus indicating a selective down-regulation of the β 1C protein.

Alterations of integrin expression in prostate cancer have been previously described by several groups.¹⁷⁻²² In normal prostate, it has been shown that β 1 and β 4 are found in epithelial cells with redistribution to the whole surface (β 1) or loss of expression (β 4) associated with the malignant phenotype.¹¹ In this study, a selective down-regulation of β 1C at the protein level is shown. β 1C is one of the four known β 1 variants; among these, the

Figure 4. β 1C and β 1 mRNA expression in neoplastic prostate specimens. β 1C and β 1 mRNA expression was analyzed as described in Figures 1 and 3. The average of either β 1C or β 1 mRNA expression levels in five normal prostate tissue specimens (N) was set at 100. β 1C and β 1 mRNA levels in neoplastic prostate tissues (K1-K33) were calculated as percentage of N. Mean values $\pm$ SEM from at least three different experiments are shown.

Figure 5. $\beta 1C$ mRNA expression does not correlate with Gleason's grade or with tumor stage in prostate carcinoma. $\beta 1C$ mRNA expression was evaluated as described in Figures 1 and 4: the average of $\beta 1C$ mRNA expression levels in five normal prostate tissue specimens (N) was set at 100. $\beta 1C$ mRNA levels in neoplastic prostate tissues were calculated as percentage of N. Mean values $\pm$ SEM from at least three different experiments are shown. **A:** Correlation of $\beta 1C$ mRNA expression with Gleason's grade. The 33 patients, described in Table 1, were divided into two groups: the first one included five patients affected by prostate carcinoma with Gleason's grade <7; the second one included 28 patients affected by prostate carcinoma with Gleason's grade ≥ 7 . **B:** Correlation of $\beta 1C$ mRNA expression with tumor stage. The 33 patients were divided into three groups: the first group included 11 patients affected by stage II prostate carcinoma; the second one included 14 patients affected by stage III prostate carcinoma, and the third one included eight patients affected by stage IV prostate carcinoma. N, normal prostate tissue specimens.

$\beta 1B$ and $\beta 1D$ variants are unlikely to be found in prostate cancer tissue, because $\beta 1B$ is restricted to skin and liver tissues, whereas $\beta 1D$ is found only in striated muscle cells.¹¹ The two remaining $\beta 1$ integrin subunits, $\beta 1C$ and $\beta 1A$, are pathophysiologically important for cancer growth because they differentially affect cell proliferation; $\beta 1C$ inhibits proliferation, whereas $\beta 1A$ promotes it. Thus, it is conceivable that a strong pressure in prostate cancer to maintain selectively reduced mRNA and protein levels of $\beta 1C$ would occur. In contrast, although reduced at the mRNA level, $\beta 1$ protein levels, that are likely to reflect $\beta 1A$ levels, are maintained constant in normal and neoplastic prostate tissues by compensatory mechanisms that remain to be identified.

Figure 6. Hormonal therapy does not affect $\beta 1C$ mRNA expression. $\beta 1C$ mRNA expression was evaluated as described in Figures 1 and 4: the average of $\beta 1C$ mRNA expression levels in five normal prostate tissue specimens (N) was set at 100. $\beta 1C$ mRNA levels in neoplastic prostate tissues were calculated as percentage of N. Mean values $\pm$ SEM from at least three different experiments are shown. The 33 patients, described in Table 1, were divided into four groups: the first group included one patient who had undergone a 1-month therapy (1 month); the second group included three patients who had undergone 3-month therapy (3 months); the third group included five patients who had undergone 6-month therapy (6 months). The fourth group included 24 patients who had not undergone any treatment (no therapy). N, normal prostate tissue specimens.

The results reflect a specific down-regulation of $\beta 1C$ in prostate epithelial cells because this molecule is expressed in a cell-type-specific manner and is not found in the stroma.²⁰ This sophisticated regulation of the $\beta 1C$ variant in prostate cancer suggests a tight control at the transcriptional and/or translational level of its expression to prevent inhibition of prostate epithelial cell proliferation. It is conceivable that either a transcriptional or a posttranscriptional regulation of $\beta 1C$ expression might be responsible for the decreased mRNA levels, whereas at

Figure 7. $\beta 1C$ and $\beta 1$ protein expression in prostatic adenocarcinoma. **A:** Either normal (lanes 1 and 2) or tumor (lanes 3–15) tissue detergent extracts were electrophoresed on 7.5% SDS-polyacrylamide gel electrophoresis under reducing conditions, and immunostained using antibody either to $\beta 1C$ integrin or to $\beta 1$ integrin as indicated in Materials and Methods. **B:** The mean values $\pm$ SEM of either $\beta 1C$ or $\beta 1$ levels in neoplastic tissues, normalized using β -tubulin, were calculated as percentage of the levels detected in two normal prostate tissues (N). At least four separate measurements for each specimen were performed.

the protein level, translation activities or protein degradation that are expected to be increased in cancer cells, might be accounted responsible for the decreased β 1C protein levels. *In vitro*, regulation of integrin mRNA and protein expression has been shown to be modulated by the protein kinase C activator PMA. PMA was shown to stimulate adhesion of tumor cells to fibronectin and fibrinogen by modulating α IIb β 3 expression in human prostatic adenocarcinoma cells.²¹ Moreover, PMA determined changes of α V mRNA expression in leukemia cells³⁴ and caused increase of α 2 integrin cell surface expression in tumor progression by enhancing α 2 integrin transcription.³⁵ It is conceivable that protein kinase C might act also as a modulator of β 1C integrin expression *in vitro* and *in vivo*, and this remains to be investigated. Androgen-deprivation therapy did not seem to interfere with β 1C integrin expression, thus indicating that androgen-mediated mechanisms act through pathways that do not involve β 1C. Since, in addition to androgens, mitogens regulate cell proliferation and integrin expression, they may be important autocrine-paracrine modulators of the neoplastic phenotype and of β 1C expression *in vivo*.^{14,36–40}

Because β 1C is a spliced variant of the β 1 integrin subfamily, down-regulation of its mRNA could have been a reflection of an altered splicing mechanism occurring in prostate cancer. Our data show that altered splicing mechanisms are unlikely to explain the reduced β 1C mRNA levels in prostate cancer because all β 1 integrin mRNAs were found down-regulated. In this regard, Tamura et al⁴¹ and Belkin et al⁴² have demonstrated that splicing mechanisms control specific integrin expression at different stages of differentiation; our results show that β 1C mRNA and protein levels are down-regulated in differentiated (low to intermediate Gleason's grade) as well as poorly differentiated (high Gleason's grade) prostate cancer and are not regulated in a differentiation-dependent manner.

Consistent with our recent experimental evidence ruling out a role for β 1C cytoplasmic domain in $\alpha\beta$ heterodimer assembly or in determining ligand specificity but affecting selectively intracellular signaling,⁴³ our findings suggest that the regulated expression of different integrin-variant cytoplasmic domains might provide a highly specialized mechanism to control cell proliferation and intracellular signaling pathways in normal and pathological conditions. Recent studies have demonstrated that the β 1C variant, using a unique signaling mechanism, selectively inhibits the mitogen-activated protein kinase pathway by preventing Ras activation without affecting either survival signals stimulated by integrins or cellular interactions with the extracellular matrix.⁴³ Specifically, in human prostate epithelial cells, β 1C is co-expressed with the cell cycle inhibitor p27^{kip1}, the loss of which correlates with poor prognosis in prostate cancer;¹⁶ furthermore, exogenous expression of β 1C *in vitro* inhibits prostate cell proliferation, and is accompanied by an increase in p27^{kip1}.¹⁶ These findings suggest a role for β 1 specific cytodomain sequences in maintaining an intracellular balance of proliferation and survival signals and point to β 1C as an upstream regulator of p27^{kip1}

expression and, therefore, as a potential target for tumor suppression in prostate cancer.

Efforts to confirm the prognostic value of β 1C are in progress. Future studies will indicate whether loss of β 1C expression in association with other traditional or novel markers has greater prognostic potential than each factor alone.

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