Distinct Altered Patterns of p27KIP1 Gene Expression in Benign Prostatic Hyperplasia and Prostatic Carcinoma

Carlos Cordon-Cardo, Andrew Koff, Marija Drobnjak, Paola Capodieci, Iman Osman, S. Sean Millard, Paul B. Gaudin, Melissa Fazzari, Zuo-Feng Zhang, Joan Massague, Howard I. Scher*

Background: The p27KIP1 gene, whose protein product is a negative regulator of the cell cycle, is a potential tumor suppressor gene; however, no tumor-specific mutations of this gene have been found in humans. This study was undertaken to identify and to assess potential alterations of p27KIP1 gene expression in patients with benign prostatic hyperplasia (BPH) and patients with prostate cancer.

Methods: We analyzed 130 prostate carcinomas from primary and metastatic sites, as well as prostate samples from normal subjects and from patients with BPH. Immunohistochemistry and in situ hybridization were used to determine the levels of expression and the microanatomical localization of p27 protein and messenger RNA (mRNA), respectively. Immunoblotting and immunodepletion assays were performed on a subset of the prostate tumors. Associations between alterations in p27KIP1 expression and clinicopathologic variables were evaluated with a nonparametric test. The Kaplan–Meier method and the logrank test were used to compare disease-relapse-free survival. Prostate tissues of p27Kip1 null (i.e., knock-out) and wild-type mice were also evaluated.

Results: Normal human prostate tissue exhibited abundant amounts of p27 protein and high levels of p27KIP1 mRNA in both epithelial cells and stromal cells. However, p27 protein and p27KIP1 mRNA were almost undetectable in epithelial cells and stromal cells of BPH lesions. Furthermore, p27Kip1 null mice developed enlarged (hyperplastic) prostate glands. In contrast to BPH, prostate carcinomas were found to contain abundant p27KIP1 mRNA but either high or low to undetectable levels of p27 protein. Primary prostate carcinomas expressing lower levels of p27 protein appeared to be biologically more aggressive (two-sided \( P = .019 \) [Cox regression analysis]).

Conclusions/Implications: On the basis of these results, we infer that loss of p27KIP1 expression in the human prostate may be causally linked to BPH and that BPH is not a precursor to prostate cancer. [J Natl Cancer Inst 1998;90:1284–91]

Inactivation of the tumor suppressor genes p53 (also known as TP53) and RB (retinoblastoma) has been implicated in the development and progression of a number of different cancers (1,2). It has also been hypothesized that the loss of function of a new family of negative cell cycle regulators that act as cyclin-dependent kinase (CDK) inhibitors (termed CKIs) might also lead to tumor development (3). CKIs fall into two families, KIP and INK, on the basis of sequence homology (4). KIP family members include p21 (also known as WAF1, Cip1, or Sdi1) (5–7), p27KIP1 (8–10), and p57KIP2 (11,12). The INK group includes four members: p16INK4A/MTS1/CDKN2 (13), p15INK4B/MTS2 (14), p18INK4C (15), and p19INK4D (16). p27 is a negative regulator implicated in G1-phase arrest by transforming growth factor-β, cell–cell contact, agents that elevate adenosine 3′,5′-cyclic phosphate, and the growth-inhibitory drug rapamycin (17–21). p27 associates with multiple G1-phase CDKs in non-proliferating cells, nullifying their activity (4,8–10).

To assess its role as a potential tumor suppressor, we and others mapped the p27KIP1 gene to 12p12–p13.1 and observed no tumor-specific genomic mutations in a large group of primary...
Materials and Methods

Patient Characteristics and Tissues

A cohort of 130 patients with prostatic carcinoma was evaluated; this cohort included 98 patients with primary tumors and 32 patients with metastatic disease (nine with metastases to lymph node and 23 with metastases to bone). All primary tumors (n = 98) represented consecutive cases of patients who underwent radical prostatectomy for prostatic cancer during the period from 1989 through 1991 at the Memorial Sloan-Kettering Cancer Center in New York City and were followed up at the center. All metastatic cases (n = 32) were selected on the basis of the availability of tissue in the tumor bank. All tissues were obtained from the Department of Pathology, Memorial Sloan-Kettering Cancer Center. Samples were formalin-fixed, paraffin-embedded tissue specimens. Normal prostatic tissues (n = 4) and 14 samples of BPH were also evaluated. In addition, BPH lesions were observed adjacent to the tumor in sections of 46 cases analyzed; these were also evaluated as part of the study. Ten pairs of frozen normal and tumor prostate tissues were used for antibody titration and comparative analyses of results from immunohistochemistry, immunoblotting, and immunodepletion assays (see below). Representative hematoxylin–eosin-stained sections were examined to evaluate the histopathologic characteristics of the lesions to be analyzed, including the ratio of normal to tumor content for microdissection techniques.

To evaluate prostatic tissue of p27 null mice, we used eight 7-month-old and six more than 12-month-old littermate pairs of wild-type and p27 knockout animals. Guidelines established by the National Cancer Institute (Bethesda, MD) for the proper and humane use of animals in research were followed. Tissues were dissected, weighed, and processed for histology by formalin fixation and paraffin embedding. Tissue sections were cut and stained with hematoxylin and eosin for histologic analysis. Sections were also used to assess the proliferative activity by counting nuclei in the acini that contained immunoreactivity for Ki-67 proliferative marker. Sections were also used to assess the proliferative activity by counting nuclei in the acini that contained immunoreactivity for Ki-67 proliferative marker. Sections were also used to assess the proliferative activity by counting nuclei in the acini that contained immunoreactivity for Ki-67 proliferative marker. Sections were also used to assess the proliferative activity by counting nuclei in the acini that contained immunoreactivity for Ki-67 proliferative marker.

Antibodies and Immunohistochemistry

The following well-characterized antibodies and corresponding formalin working dilutions were used in all samples analyzed: monoclonal antibody p27Kip1 (Ab-2; Oncogene Science, Inc., Boston, MA; 0.1 μg/mL, final concentration), anti-p27 affinity-purified rabbit antiserum (Memorial Sloan-Kettering Cancer Center; 1: 500 dilution), and anti-Ki-67 affinity-purified rabbit antiserum (Vector Laboratories, Inc., Burlingame, CA; 1: 10,000 dilution). Nonimmune rabbit serum and a mouse monoclonal antibody (MgS-Kpl) were used as the negative controls at similar working dilutions. Deparaffinized sections were treated with 3% H2O2 to block endogenous peroxidase activity. Sections were subsequently immersed in boiling 0.01% citric acid (pH 6.0) in a microwave oven for 15 minutes to enhance antigen retrieval, allowed to cool, and incubated with 10% normal horse serum or 10% normal goat serum to block nonspecific tissue immunoreactivities. Primary antibodies were then incubated overnight at 4 °C. Biotinylated horse anti-mouse immunoglobulin G (IgG) antibodies (Vector Laboratories, Inc.; 1: 500 dilution) or goat anti-rabbit IgG antibodies (Vector Laboratories, Inc.; 1: 800 dilution) were applied for 1 hour, followed by avidin–bixin peroxidase complexes that were applied for 30 minutes (Vector Laboratories, Inc.; 1: 25 dilution). Diaminobenzidine was used as the final chromogen, and hematoxylin was used as the nuclear counterstain. Nuclear immunoreactivities were classified on a continuous scale with values that ranged from undetectable levels or 0% to homogeneous staining or 100%. Tumors were grouped into two categories, defined as follows: Group A had neoplasms with 40% or more of tumor cells with nuclear staining, and group B had from 0% or undetectable staining to less than 40% of tumor cells with nuclear staining (see “Statistical Analyses” section).

Probes and In Situ Hybridization

Digoxigenin-labeled probes were used for in situ hybridization, and 1 μg of recombinant plasmid pCR3.1 (Invitrogen Corp., San Diego, CA), containing the full-length human p27Kip1 gene (a gift from Dr. M. Pagano, New York University School of Medicine), was linearized by BanHI and XbaI to generate antisense and sense transcripts. RNA probes were generated by use of T7 and SP6 polymerase for 2 hours at 37 °C in 1x transcription buffer (Boehringer Mannheim Biochemicals, Indianapolis, IN), 20 U of ribonuclease inhibitor, adenosine 5-triphosphate (10 mmol/L), guanosine 5-triphosphate (10 mmol/L), cytidine 5-triphosphate (10 mmol/L), uridine 5-triphosphate (UTP) (6.5 mmol/L), and digoxigenin-labeled UTP (3.5 mmol/L). Deparaffinized tissue sections were rinsed in water and phosphate-buffered saline (PBS) for 10 minutes. The slides were digested with proteinase K (50 μg/mL) for 18 minutes at 37 °C in PBS and post-fixed in 2× SSC for 45 °C in a freshly prepared solution of 4% paraformaldehyde in PBS for 5 minutes. Prehybridization was done for 30 minutes at 45 °C in 50% formamide and 2x standard saline citrate (SSC). The hybridization buffer contained 50% (vol/vol) deionized formamide, 10% dextran sulfate (50% stock solution), 2x SSC (20x stock solution), 1% sodium dodecyl sulfate (10% stock solution), and herring sperm DNA (0.25 mg/mL, from a stock of 10 mg/mL). Hybridization was performed overnight at 45 °C by applying digoxigenin-labeled RNA probe (10 pmol/L, in 50 μL of hybridization buffer per section) under a coverslip. The coverslips were removed, and the slides were washed in prewarmed 2x SSC for 20 minutes at 60 °C twice, followed by washes in prewarmed 0.5x SSC and 0.01x SSC at 60 °C for 20 minutes, respectively. After these washes, the slides were incubated in normal sheep serum diluted in buffer at pH 7.5 and successively in the same buffer with antibody anti-digoxigenin conjugated to alkaline phosphatase (Boehringer Mannheim Biochemicals) at a dilution of 1: 1500 for 1 hour at room temperature. The visualization was accomplished by use of nitroblue tetrazolium 5-bromo-4-chloro-3-indoly phosphate. The slides were counterstained with methyl green and mounted.

Immunoblotting and Immunodepletion Assays

Proteins were extracted from three OCT (i.e., optimal cutting temperature) compound-embedded prostatic carcinomas and resolved on polyacrylamide gels for immunoblotting with p27-specific antibodies. Extracts obtained from tumors with undetectable p27 and homogeneously expressing p27 were subjected to sequential depletion with antibodies specific to p27 or a nonspecific rabbit anti-mouse isotype. After depletion, the proteins in the supernatants were resolved, and the presence of p27 was determined by immunoblotting. Aliquots of these supernatants were boiled briefly; after clarification, the soluble fraction was incubated with different amounts of recombinant cyclin E/CDK2, and the degree of inhibition of cyclin E/CDK2 activity on histone H1 substrate was measured (17).

Statistical Analyses

The statistical analyses of the data from the 98 patients with primary prostate cancer (patients with metastatic disease were excluded) were conducted as follows. The response variable time to prostate-specific antigen (PSA) relapse was defined as the time from radical prostatectomy to the time of the first detectable (nonzero) PSA measurement. Three consecutive increases in PSA levels were required to confirm PSA relapse; however, the time of relapse was taken as the time of the first detectable PSA measurement. Patients who did not achieve a nonmeasurable PSA level after radical prostatectomy were excluded from the analysis. Patients who were still alive at the time of the analysis without relapse were censored at the date of last follow-up. The baseline variables examined were PSA (units) at time of diagnosis, Gleason score (29) (divided into the following three mutually exclusive categories: ≥7, <7, or hormone sensitive with a Gleason score that was not assessable), T stage of disease (≥3 or <3) (30), and percent p27 expression.

The Cox proportional hazards model was used to examine the multivariate relationship between PSA-relapse-free time from prostatectomy and the baseline variables listed above. The data were consistent with the assumptions of the Cox proportional hazards model. The final model was determined by use of the “all subsets” procedure in the SAS PHREG program with the score criterion (31).
Multiple cut points for p27 were analyzed to examine possible categories (p27 low and p27 high levels of expression). Kaplan–Meier estimates of relapse-free survival stratified by p27 classification were also examined. The LIFETEST procedure in the SAS program package was used to generate the Kaplan–Meier estimates and the resulting survival curves (31,32).

The association between p27 value and clinicopathologic variables was evaluated with Wilcoxon’s two-sample test (33) or with the Kruskal–Wallis test, when appropriate (34). A permutation test was used to assess the differences between the p27-deficient mice and the wild-type mice with respect to the Ki-67 proliferative index in acini of prostatic glands (35). All P values are two-sided; values are considered statistically significant for P<.05.

**Results**

To determine whether loss of p27 expression was a common feature in prostate cancer, we analyzed 130 prostate carcinomas from primary and metastatic sites, representing different hormone sensitivities. Included were 98 primary tumors (56 hormone-naive and 42 hormone-sensitive tumors), some with associated prostatic intraepithelial neoplastic (PIN) lesions, and 32 metastatic carcinomas from lymph node tumors (n = 9) and bone metastases (n = 23). Thirteen of these metastatic lesions were from patients not receiving hormone therapy, and the remaining 19 metastases were obtained from patients who had undergone hormone treatment. PIN lesions that displayed a cribriform or pseudopapillary pattern (n = 11) expressed high levels of p27 protein (Fig. 1, A) and were associated with p27-positive invasive prostatic carcinomas. In contrast, PIN lesions that displayed a flat growth pattern (n = 9) had low to undetectable p27 levels (Fig. 1, B) and were associated with p27-negative invasive tumors. Of the 98 invasive primary prostatic carcinomas studied, 35 (36%; group A) had an intense immunoreactive- p27 nuclear pattern in the malignant cells (data not shown). The remaining 63 cases (64%; group B) displayed altered patterns of expression: 24 had undetectable p27 levels (<5% of tumor cells with nuclear immunostaining; Fig. 1, C), and 39 had low levels of p27 expression (>5% and <40% of tumor cells with nuclear immunostaining; data not shown). Of the 32 metastatic lesions, seven (22%) showed intense p27 nuclear immunostaining in most tumor cells (Fig. 1, D). The remaining 25 metastatic lesions (78%) had either undetectable or low nuclear expression of p27 (<40% of tumor cells with immunostaining) (Fig. 1, E and F). All but one of the nine patients with hormone-independent bone lesions displayed altered p27 expression. Of these nine cases, four had undetectable p27 protein expression (Fig. 1, F), four had low levels of p27 expression ranging from 30% to 40% of lymphocytes (cells in the germinal center display low p27 levels), and one expressed high levels of p27 in a metastatic lymph node (data not shown).

**Fig. 1.** Histologic analysis, immunohistochemistry, and in situ hybridization of human primary and metastatic prostatic carcinomas. A–C) Primary prostatic carcinomas were processed as follows: A) Immunohistochemical staining of p27 in a prostatic intraepithelial neoplastic (PIN) lesion is shown; note the intense immunoreaction observed in the nuclei of the tumor cells growing into the lumen. B) Immunohistochemical staining of p27 in another PIN lesion shows dysplastic changes; note the intense immunostaining in the nuclei of normal epithelial cells and the low to undetectable staining of the tumor cells bisecting the gland and growing into the lumen. C) p27 protein is not detected in an invasive primary prostatic carcinoma; note the staining of a normal gland trapped in the tumor. D–F) Metastatic prostatic carcinomas were processed as follows: D) Immunohistochemical staining of p27 in a prostate carcinoma metastatic to lymph node is shown; note the intense nuclear staining of both tumor cells and lymphocytes (cells in the germinal center display low p27 levels). E) Immunohistochemical staining of p27 in another prostate carcinoma metastatic to lymph node is shown; note the intense immunostaining in the nuclei of lymphocytes and the undetectable levels of p27 staining in the tumor cells. F) Immunohistochemical staining of p27 in a prostate carcinoma metastatic to bone is shown; note the immunohistochemical staining in the nuclei of osteoblasts and the lack of staining of tumor cells. G–I) Primary invasive prostatic carcinomas was processed as follows: G) Low to undetectable immunohistochemical staining of p27 in the tumor cells is shown; note the staining of a normal gland trapped in the tumor. H) In situ hybridization on a section consecutive to the one illustrated in panel G showing high p27 mRNA messenger RNA levels even in p27-negative tumor cells when the antisense probe to p27 mRNA was used. I) Negative control, sense probe. Original magnification ×400 (panels A–I).
tumor cells with weak positive staining, and only one case displayed 80% positive tumor cells. No differences were observed when we compared the two anti-p27 antibodies used to stain all samples. It is interesting that high levels of p27KIP1 messenger RNA (mRNA), as determined by in situ hybridization to a p27KIP1 complementary DNA probe, were found in all tumors even when the lesions displayed undetectable levels of p27 protein (Fig. 1, G–I).

Three prostatic carcinomas for which frozen samples were available were selected to conduct functional assays aimed at determining the functional activity of p27 protein as a CKI. We measured the heat-stable Cdk inhibitory activity, using as a substrate cyclin E/CDK2, which remains in extracts after depletion with p27-specific antibodies. Immunoblotting data paralleled those observed for immunohistochemistry patterns, in that signals were not detected on blots from tumors that were negative for p27, although strong bands were obtained for tumors that were positive for p27 (data not shown). Extracts obtained from a p27-negative tumor and a p27-positive tumor were subjected to sequential depletion with antibodies specific to p27 or a non-specific rabbit anti-mouse serum. After depletion and incubation with various amounts of recombinant cyclin E/CDK2, the degree of inhibition was measured by use of histone H1 as the substrate. Depletion with either rabbit anti-mouse serum or p27-specific antibodies did not affect the inhibitory activity of the p27-negative tumor; however, depletion of p27 from the positive tumor extract completely ablated the heat-stable inhibitor activity, indicating that p27 was functional as a CKI in these samples.

Thus, these data suggest that prostatic carcinomas develop along two different pathways: one involving the loss of p27 and the other using alternative processes that may circumvent the growth-suppressive effects of p27. To determine whether these distinct pathways of prostate tumorigenesis are associated with clinical parameters, as reported for other tumor types (25–28), we assessed associations of the percent expression of p27, clinical stage, total Gleason score, and hormonal status of the tumor. No association was observed between percent expression of p27 and Gleason score (≥7 versus <7), clinical stage (primary versus metastatic disease), or hormonal status. To assess disease aggressiveness, we evaluated the time to PSA failure, the most sensitive indicator of success or failure after radical prostatectomy, in patients treated for localized disease. We considered only patients who had an undetectable PSA level after surgery, an indication that the resection was complete. A multivariate Cox regression analysis was performed to assess the prognostic importance of p27 after adjustment for known predictors of PSA relapse. There were 37 cases in which a PSA relapse was identified, 56 cases that had no relapse at the time of analysis, and five cases that did not reach a nonmeasurable PSA level after radical prostatectomy; these last five cases were excluded. The variables examined were percent expression of p27, PSA level at diagnosis, T stage (≥3 or <3), and Gleason score (≥7, <7, or hormone sensitive with a Gleason score that was not assessable).

The final model was selected on the basis of examination of all possible subsets with respect to the score criterion. This final model contained PSA level at diagnosis, percent of p27 expression, a Gleason score of greater than or equal to 7, or a Gleason score of less than 7 (β = 0.0112, P = .019; β = −0.0162, P = .025; β = 1.408, P = .006; and β = 1.427, P = .005, respectively).

The negative coefficient associated with the percent of p27 expression in the multivariate analysis indicates that lower p27 expression is associated with an earlier PSA relapse. On the basis of this association, the possible categorization of p27 into two mutually exclusive categories (low or high expression) was examined. Three cut points (expression in 20%, 40%, and 60% of tumor cells) were examined with respect to the logrank test statistics and associated P values. On the basis of this examination (with the use of the cut point with the minimum P value), separation into two groups (group A, high p27 expression, ≥40% of tumor cells positive; group B, low p27 expression, <40% of tumor cells positive) is illustrated in Fig. 2. The unadjusted P value for the logrank test was less than .01 (P = .0091). Because of the multiple cut points examined, a simple Bonferroni adjustment was made, and the adjusted P value was less than .05 (adjusted P = .03). Although this P value is statistically

![Fig. 2. Kaplan–Meier curves for recurrence-free survival stratified by p27 groups (group A or group B) of patients with primary prostate carcinoma (n = 98), as assessed by time to detectable prostate-specific antigen (PSA). Patients who had PSA relapse were classified as having treatment failures, and patients with PSA relapse or those who were still alive, had died from another disease, or were lost to follow-up during the study period were coded as censored. Time to relapse was defined as the time from date of surgery to the end point (relapse or censoring). Disease-relapse-free survivals were evaluated with the Kaplan–Meier method and the logrank test.](http://jnci.oxfordjournals.org/)

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significant, the use of the 40% cut point for p27 needs to be validated in future studies.

These data prompted us to extend the characterization of p27 expression to normal prostate and BPH. In the normal human prostate, abundant amounts of p27 protein were detected by immunohistochemistry in the ductal and acinar cells, mainly of the lumen, and in the stromal cells. Epithelial cells displayed a strong nuclear immunostaining signal (Fig. 3, A). Likewise, both epithelial and stromal cells expressed abundant p27_KIP1 transcripts (Fig. 3, B and C), as detected by in situ hybridization. Strikingly, in all BPH cases studied, p27 expression was low to undetectable in epithelial and stromal cells in the hyperplastic nodules. Immunohistochemical staining revealed a low to undetectable immunoreaction in both epithelial and fibromuscular cells in the hyperplastic nodules (Fig. 3, D). This observation contrasts with the strong p27 nuclear immunostaining phenotype observed in the normal prostate. Likewise, p27_KIP1 mRNA levels were low to undetectable in consecutive sections of BPH tissue by in situ hybridization (Fig. 3, E and F). In nine BPH tissue samples studied, we found areas of basal cell hyperplasia. These cellular elements also had low to undetectable amounts of both p27 protein and transcripts (data not shown). Nevertheless, in the nonhyperplastic regions of these same BPH samples, normal ductal and acinar epithelial cells and stromal elements showed high levels of p27 expression. These results indicate that, in the development of BPH, p27_KIP1 transcription may be down-regulated. This finding was quite unexpected because this gene product is generally regulated at post-transcriptional levels (36–38), although members of the nuclear hormone receptor superfamily have been reported to regulate p27_KIP1 mRNA levels (39).

Fig. 3. Histologic analysis, immunohistochemistry, and in situ hybridization of human normal prostate and benign prostatic hyperplasia (BPH). A–C) Consecutive sections of normal prostate tissue were processed as follows: A) Immunohistochemical staining of p27 is shown; intense immunoreaction is observed in the nuclei of epithelial cells in the luminal side of the acini, with decreased reaction in the nuclei of basal and stromal cells. B) In situ hybridization shows high messenger RNA (mRNA) levels of p27_KIP1 in both epithelial and stromal cells when the antisense probe was used. C) In situ hybridization with the sense probe to p27_KIP1 shows lack of signals in both epithelial and stromal cells. D–F) Consecutive tissue sections of a BPH nodule were processed as follows: D) Immunohistochemical staining of p27; note the lack or almost undetectable levels of immunoreaction observed in the nuclei of both epithelial and stromal cells in the luminal side of the acini, with decreased reaction in the nuclei of basal and stromal cells. E) In situ hybridization showing low to undetectable p27_KIP1 mRNA also in both epithelial and stromal cells when the antisense probe was used; note the strong signal of the cellular inflammatory infiltrates that serve as an internal positive control. F) In situ hybridization with the sense probe to p27_KIP1 shows lack of signals in epithelial and stromal cells, as well as cellular inflammatory elements. Original magnifications ×1000 (panels A–C) and ×400 (panels D–F).
components. A few fibroblasts and supportive connective tissue acini of epithelial cells surrounded by a stroma contain a prostate gland of a p27+/+ mouse shows well-defined processed as follows: A) Hematoxylin–eosin staining of a prostate gland of a p27+/+ mouse shows well-defined acini of epithelial cells surrounded by a stroma containing few fibroblasts and supportive connective tissue components. B) Hematoxylin–eosin staining of a prostate gland of a p27−/− mouse shows multiple and complex glands and hypercellular acini of epithelial cells surrounded by fibromuscular stromal cells in a connective tissue displaying abundant supportive components. C and D) Hematoxylin–eosin stainings of a prostate gland of a p27+/+ mouse (high-power details) illustrate the complexity of the glands and abundant fibromuscular stromal elements (C), as well as the hypercellularity of the acini (D). E and F) Tissue sections of normal prostate from a p27+/+ mouse (E) and a p27−/− mouse (F) stained with anti-Ki-67 antibodies are shown; note the lack of nuclear Ki-67 antigen immunoreaction in the prostatic acini of a p27 wild-type mouse (E) compared with the positive nuclear Ki-67 antigen staining in the acini of a p27-deficient mouse (F). The proliferative index was significantly higher in the prostatic acini of p27−/− mice than in wild-type mice (P = .005). Original magnifications ×200 (panels A and B) and ×400 (panels C–F).

= .005). The Ki-67 protein has been shown to be a reliable marker of proliferation in rodent cells (43), and it readily identified cycling cells in positive control lymph node sections from wild-type mice (data not shown).

Discussion

Our findings are reminiscent of BPH in humans and support the hypothesis that the loss of p27 expression in human prostate may be causally linked to BPH. It has been suggested that BPH and malignant prostate growth share a common origin because they commonly coexist and demonstrate androgen dependency (44–46). However, this relationship remains unclear because BPH tends to develop in the transition zone, whereas most carcinomas develop in the peripheral zone (47–50). Results from our study reveal that, unlike cells from the BPH lesions, prostastic carcinoma cells regulate p27 expression at the post-transcriptional level. Thus, these data support the postulate that BPH is not a premalignant lesion in prostate cancer development.

Coordinate inactivation of the pathways involving the p53 and RB genes appears to be an essential requirement for the genesis of most human cancers. However, mutations in p53 and/or RB are reported to be late and uncommon events in prostate tumor progression (51–56). Contrary to these results, data from this study indicate that inactivation of p27 is a frequent and early event in some prostate cancers. Thus, our working hypothesis is that p27 represents another pathway of tumor suppression in certain human tumors, prostate cancer being a paradigm in which this concept could be further tested.

In summary, data from this study suggest that p27Kip1 gene ablation in the mouse causes a pronounced prostatic hyperplasia and that the loss of p27 expression in human prostate may be causally linked to BPH. In addition, our data suggest that prostatic carcinoma develops along two different pathways: one involving the loss of p27 and the other using alternative processes that circumvent the growth-suppressive effects of p27. These phenotypes can be identified as early as the PIN stage. Moreover, primary prostatic carcinomas displaying lower levels of p27 appear to be biologically more aggressive, based on their association with time to PSA detection after radical prostatectomy, while controlling for other variables. The consistent alteration of p27 expression observed in all androgen-independent metastatic lesions suggests an association with tumor progression, which may be the result of the metastatic process itself. Alternatively, it may be postulated that tumors with high levels of p27 are more sensitive to androgen ablation, the primary treatment of metastatic disease. Finally, two dissimilar mechanisms appear to be involved in the loss of p27 expression in BPH versus a subset of prostatic carcinomas. p27Kip1 mRNA levels are extensively reduced in BPH, whereas p27 proteins are diminished to undetectable levels in some prostatic carcinomas despite detectable levels of p27Kip1 mRNA as the result of a post-transcriptional event. These results support the postulate that BPH is not a premalignant lesion in prostate cancer development.

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Notes

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