The Expression of Drug Resistance Gene Products during the Progression of Human Prostate Cancer

Gregory F. Sullivan, Peter S. Amenta, Jocelyn D. Villanueva, Carmelitta J. Alvarez, Jin-Ming Yang, and William N. Hait


ABSTRACT

Prostate cancer progresses from a localized disease to a widely disseminated malignancy. Each step along this progression pathway involves multiple genetic alterations that impart a survival advantage to the tumor cell over its normal counterparts and may confer resistance to therapy. Because metastatic prostate cancer is one of the most therapy-resistant human neoplasms, we studied the expression of certain molecular determinants of drug resistance in the context of tumor progression. Paraffin-embedded formalin-fixed resected prostates were chosen based on Gleason grade and surgical stage. Immunohistochemistry was used to detect the expression of multidrug resistance protein (MRP), topoisomerase IIα, p53, glutathione S-transferase π, Bcl-2, and P-glycoprotein in these specimens. We found that all of the proteins were expressed in resected prostate except for P-glycoprotein. The expression of MRP, topoisomerase IIα, p53, and Bcl-2 increased with the Gleason grade. In addition, the expression of MRP, topoisomerase IIα, and p53 increased with the surgical stage. In contrast, the glutathione S-transferase π and Bcl-2 expression decreased with the increasing surgical stage. Stage was the strongest indicator of protein expression. These results suggest that drug resistance gene products are expressed in prostate cancer at the time of surgical resection. Thus, although the emergence of the “pan-resistance” phenotype in prostate cancer may partly be a function of the selection pressure exerted by therapeutic interventions, certain determinants of chemoresistance may be caused by genetic changes accompanying tumorigenesis.

INTRODUCTION

Metastatic prostate cancer is one of the most therapy-resistant human neoplasms (1, 2). Despite the wide use of androgen ablation, median survival from the initiation of therapy is only 2.5–3 years. Furthermore, patients who relapse after treatment with androgen ablation develop disease that is both hormone-refractory and resistant to chemotherapy (1); after recurrence, the median survival is <1 year (2).

To date, an effective combination of chemotherapeutic drugs has not been identified for the treatment of prostate cancer at any stage during its progression (1, 2). In fact, the reliability of the transient remission after androgen deprivation has effectively precluded the use of chemotherapy before the progression of the disease to the androgen-independent stage. This, at least in part, has strengthened the concept that the “pan-resistant” phenotype is associated with androgen independence. Cellular viability factors that provide a selective advantage for cancer cells may in themselves contribute to the pan-resistant state (3). For example, the expression of anti-apoptotic molecules, such as Bcl-2, has recently been shown to be associated with androgen independence (4).

Little is known about the genetic determinants of drug resistance in prostate cancer. Biological studies have revealed that individual prostatic cancers are composed of multiple clones that are phenotypically heterogeneous even before therapeutic intervention (5). Thus, both the pan-resistant phenotype and the existence of heterogeneous regions in individual diseased prostates suggest that a variety of drug resistance genes may be expressed early in the progression of this disease.

Although the expression of p53, GST-π, and Bcl-2 has been characterized in human prostate cancer, the expression of these and other genes in relation to each other has not been examined (6–8). Molecules such as MRP, topoisomerase IIα, and P-gp have not been studied in human prostate tissue to date. Accordingly, the question of whether the expression of drug resistance genes is a characteristic unique to advanced prostate cancer or occurs early in the course of the disease remains unanswered.

Radical prostatectomy is frequently used to treat organ-confined prostate cancer, which is often detected through measurement of prostate-specific antigen (9). As a result, archival resected prostates are available for examination of the gene expression early in the natural history of the disease. Therefore, to determine whether the mechanisms responsible for resistance are present early in the progression of prostate cancer, we measured the expression of gene products that may be associated with drug resistance in archival material.

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3 The abbreviations used are: GST, glutathione S-transferase; MRP, multidrug resistance protein; P-gp, P-glycoprotein; PEPT, paraffin-embedded formalin-fixed tissue; BGE, benign glandular elements.
Fig. 1  See following page for parts I–N and legend.
Fig. 1 Immunohistochemical detection of drug resistance gene products in representative low- and high-grade/stage prostate tissue. The tissue was PEFT; protein expression was detected using an avidin-biotin-complex immunohistochemical technique as described in “Materials and Methods.” A, H&E-stained prostate cancer (Gleason 3 and stage B; ×41.25); B, H&E-stained prostate cancer (Gleason 9 and stage D; ×41.25); C, MRP is identified in benign glandular epithelium of a prostate gland (Gleason 3 and surgical stage B; arrow, focal staining in adjacent adenocarcinoma); D, MRP is identified in prostate cancer (Gleason 7 and stage D; ×41.25); E, topoisomerase IIα is detected in the cells of a prostate gland (Gleason 4 and stage B; ×41.25); F, topoisomerase IIα in prostate cancer (Gleason 7 and stage C; ×41.25); G, p53 in prostate cancer (Gleason 4 and stage C; ×82.5); H, p53 in prostate cancer (Gleason 8 and stage D; ×41.25); I, GST-π in the benign glandular epithelium of a prostate gland (Gleason 3 and stage B; ×41.25); J, GST-π is absent from prostate cancer (Gleason 7 and stage D; ×41.25); K, Bcl-2 in the benign glandular epithelium of a prostate gland (Gleason 5 and stage B; ×82.5); L, Bcl-2 in prostate cancer (Gleason 9 and stage D; ×165); M, P-gp is absent from low-grade/stage prostate cancer (Gleason 4 and stage B; ×41.25); N, P-gp is absent from high-grade/stage prostate cancer (Gleason 7 and stage D; ×41.25).

MATERIALS AND METHODS

Tissue Specimens
PEFT prostate sections from 95 patients were obtained from the Robert Wood Johnson University Hospital archives with their corresponding pathology reports. The specimens were chosen to include a wide range of Gleason grades and surgical stages. All of the tissues were from prostatectomies; none were exposed to prior therapy of any form. Paraffin-embedded tissues
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(a) three categories: tam evenly distributed sample sizes, specimens were grouped in section by H&E staining, and these slides were compared with 10). Grading was carried out by means of the Gleason system. (b) Classification system described by Jewett with minor modifications had been routinely fixed in 10% neutral buffered formalin for 12-24 h.

Staging and Grading

Pathological staging was performed according to the classification system described by Jewett with minor modifications (10). Grading was carried out by means of the Gleason system. Grades were assigned by one pathologist and reviewed by at least two of us (P. S. A., J. D. V., and C. J. A.). Benign glandular elements were distinguished from cancer in each tissue section by H&E staining, and these slides were compared with the immunohistochemical staining of adjacent sections. To obtain evenly distributed sample sizes, specimens were grouped in three categories: (a) Gleason scores of 2-4; (b) Gleason scores of 5-6; and (c) Gleason scores of 7-10.

Antibodies

The dilution used for each antibody was determined by titration on archival paraffin-embedded formalin-fixed colon cancer (p53), tonsil (topoisomerase Ila and Bcl-2), and liver (MRP, GST-π, and P-gp) tissues. These tissues were also used for positive and negative controls each time a group of slides was stained. For negative controls, mouse ascites fluid and normal rabbit serum was substituted for monoclonal and polyclonal antibodies, respectively.

MRP. MRP1, a monoclonal rat multidrug resistance-associated protein antibody (clone MRP1, Signet Laboratories, Dedham, MA), which was generated by using a bacterial fusion protein of MRP that contained a segment of 168 amino acids in the amino-proximal half of the protein, was used to detect MRP. MRP1 reacts with the external epitope of MRP (11, 12). This antibody does not cross-react with P-gp (11, 12). A dilution of 1:4 in 1% BSA/PBS was used for MRP1.

Topoisomerase Ila. Mouse monoclonal topoisomerase Ila antibody Ab-2 (clone JH2.7, Lab Vision/NeoMarkers, Fremont, CA), which was generated using a fragment of recombinant human topoisomerase Ila protein, was used to detect topoisomerase Ila. Ab-2 recognizes an epitope that maps between amino acid residues 854-1447 and does not cross-react with topoisomerases IIβ or topoisomerase I (13, 14). Ab-2 was used at a dilution of 1:50 in 1% BSA/PBS.

p53. Monoclonal antihuman p53 antibody clone DO-7 (Dako Corporation, Carpinteria, CA) recognizes an epitope in the NH₂ terminus of the human p53 protein. The epitope for this antibody is known to reside between amino acids 19 and 26 (15-18). Anti-p53 antibody was diluted 1:10 in 1% BSA/PBS.

GST-π. Rabbit polyclonal antibody against GST-π (Signet Laboratories), which was generated using GST-π purified from human chronic lymphoblastic leukemia cells, was used to detect GST-π (19-21). Anti-GST-π was used diluted 1:40 in 1% BSA/PBS.

Bcl-2. Monoclonal mouse anti-Bcl-2 antibody (clone Bcl-2-100, Zymed Laboratories, South San Francisco, CA), which reacts with amino acids 41-54 of the Bcl-2 protein, was used to detect Bcl-2 (22). The dilution of anti-Bcl-2 antibody was 1:100 in 1% BSA/PBS.

P-gp. JSB-1 murine monoclonal antibody (Signet Laboratories), which was generated using the multidrug-resistant Chinese hamster ovary cell line (CHC5), was used to detect P-gp. JSB-1 identifies an independent, strongly conserved cytoplasmic epitope present on mammalian P-gp (23-25). A dilution of 1:4 in 1% BSA/PBS was used for JSB-1.

Immunohistochemistry

PEPT sections 4 μm in size were placed on positively charged glass slides and allowed to dry. After the slides were incubated at 60°C for 30 min, they were deparaffinized in xylens for 30 min and rehydrated through graded alcohols and distilled water into PBS (pH 7.2). After each of the subsequent steps, two 5-min washes in chilled PBS were carried out. Endogenous peroxidase activity was blocked by incubation in the dark in 3% hydrogen peroxide in methanol (MRP, Bcl-2, and P-gp) for 20 min. Free aldehyde groups were reduced by incubation in sodium borohydride at 4°C for 20 min in the GST-π protocol. Antigen retrieval was performed by boiling citrate buffer (pH 6.0), for 10 min (p53) and in 95°C citrate buffer for 45 min (MRP, topoisomerase Ila, Bcl-2, P-gp). Nonspecific protein interactions were blocked by incubation with 5% BSA for 30 min at 25°C in a humidified chamber. Primary antibodies were incubated at the optimal dilutions in a humidified chamber for the following time periods: (a) MRP, Bcl-2, and P-gp, 12-24 h at 4°C; (b) p53 and GST-π, 30 min at 37°C; and (c) topoisomerase Ila, 1 h at 25°C. This was followed by a 1-h incubation in a humidified chamber at 25°C with rabbit antimouse biotinylated immunoglobulins (Dako Corporation) diluted 1:400 in 1% BSA (topoisomerase Ila, p53, Bcl-2, and P-gp), rabbit antirat biotinylated immunoglobulins (Dako Corporation) diluted 1:600 in 1% BSA (MRP), or swine antirabbit biotinylated immunoglobulins (Dako Corporation) diluted 1:600 in 1% BSA (GST-π). Next, slides were incubated for 1 h at 25°C with streptavidin conjugated horseradish peroxidase (Dako Corporation) diluted 1:600 in 1% BSA. 3,3’-diaminobenzidine tetrahydrochloride tablets (Dako Corporation) were used as the chromagen according to the manufacturer’s instructions. Slides were then dehydrated through graded alcohols to xylens and mounted with coverslips.

Scoring and Interpretation

We analyzed all of the specimens for the presence of each antigen. If any of the BGE or malignant elements had detectable

<table>
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<tr>
<th>Table I: MRP expression in human prostate cancer</th>
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<tr>
<td>No. of positive specimens</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>BGE</td>
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<tr>
<td>Stage B</td>
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<td>Stage C</td>
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<td>Stage D</td>
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<td>Stages B-D</td>
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<tr>
<td>Gleason 2-4</td>
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<td>Gleason 5-6</td>
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<td>Gleason 7-10</td>
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* Numbers in parentheses, total number of specimens examined.
staining, the specimen was scored as positive. We next evaluated the percentage of epithelial cells within each specimen that stained with each antibody. A prevalence of 1+ refers to any degree of staining up to 25% of the total specimen; 2+ refers to 25–75% staining of the total specimen; and 3+ refers to 76–100% staining of the total specimen.

A variety of technical issues prevented us from obtaining a complete evaluation for every specimen. Characteristics such as fixation of the surgical samples interfered with certain antibody reactions. Thus, the actual sample size for each drug resistance gene that was examined in the context of the Gleason score and the surgical stage was not identical.

Statistical Analysis
The results obtained by immunohistochemistry were analyzed statistically by the Wald χ² (26) and odds ratio methods (27).

RESULTS
To determine whether or not the expression of drug resistance genes changed with the progression of prostate cancer, we studied specimens that were obtained at the time of radical prostatectomy and were classified according to stage and Gleason grade. The left panels of Fig. 1 (A, C, E, G, I, K, and M) show representative examples of low-grade/stage specimens; e.g., Fig. 1A is a low-grade/stage H&E-stained specimen. The right panels of Fig. 1 (B, D, F, H, J, L, and N) show representative examples of high-grade/stage specimens; e.g., Fig. 1B is a high-grade/stage H&E-stained specimen.

MRP. MRP staining was positive in BGE (Fig. 1C) and focal in adjacent adenocarcinoma (Fig. 1C, arrow). In contrast, MRP staining covered the entire sheet of malignant epithelium in a high-grade/stage specimen (Fig. 1D). MRP was present in 37% of the BGE and in 83% of malignant epithelium in the prostate specimens examined (Table 1); its expression increased with advancing stage to 63% of B specimens, 92% of C specimens, and 75% of D specimens (Fig. 2). The prevalence of staining for MRP within each specimen also increased with advancing stage. Whereas less than 5% of BGE showed 2+ and 3+ staining, more than 50% of stage B, C, and D specimens stained 2+ or 3+ (Fig. 3).

MRP expression also increased with the advancing of the Gleason score from 67% in low-grade lesions to greater than 80% in moderate- and high-grade tumors (Fig. 4). These data are summarized in Table 1. The prevalence of staining for MRP within each specimen also increased with the Gleason score (Fig. 5). Whereas less than 10% of the low-grade lesions showed 3+ staining, more than 20% stained 3+ in moderate- and high-grade lesions.

PEFT liver sections were used as a positive control (data not shown). As reported previously, MRP is expressed in hepatocytes, and the ductal, stromal, and vascular components of the portal triad of the liver are negative (28). At higher magnification, a granular deposition of the antibody in the cytoplasm was apparent, whereas the nucleus was nonreactive.

Topoisomerase IIα. Topoisomerase IIα staining was present in the nucleus in low- and high-grade/stage tumors (Fig. 1, E and F, respectively). Topoisomerase IIα was present in 23% of BGE and in 88% of malignant epithelium in the prostate specimens examined (Table 2); its expression increased with advancing stage from 75% of stage B specimens to >90% of stage C and D specimens (Fig. 2). The prevalence of staining for topoisomerase IIα within each specimen also increased with advancing stage (Fig. 3). Whereas less than 25% of the specimens showed a 1+ staining in BGE, >90% of stage C and D specimens stained 1+ or 2+.

Topoisomerase IIα expression also increased with the advancing of the Gleason score from 75% in low-grade lesions to 81% in moderate-grade and 100% in high-grade tumors (Fig. 4). These data are summarized in Table 2. The prevalence of staining for topoisomerase IIα within each specimen also increased with the Gleason score (Fig. 5). Whereas less than 80%
of BGE showed 1+ staining, 100% of stage D lesions stained 1+ or 2+.

PEFT tonsil sections were used as a positive control (data not shown). As reported in the Ab-2 data sheet, topoisomerase IIα was present in the nuclei of lymphocytes within the germinal center of the human tonsil (“Materials and Methods”). Here, except for rare lymphocytes, both the crypt epithelium and the adjacent mantle zone were nonreactive with the antibody.

**p53.** p53 staining was present in the nucleus in low- and high-grade/stage tumors (Fig. 1, G and H, respectively). p53 was not present in either BGE or stage B cancers but was seen in 15% of stage C specimens and 46% of stage D specimens (Table 3 and Fig. 2). The prevalence of staining for p53, when present, was always 1+ (Fig. 3).

p53 expression also increased with the advancing of the Gleason score from 13% in low-grade lesions to 14% in moderate-grade and 21% in high-grade tumors (Fig. 4). These data are summarized in Table 3. The prevalence of staining for p53 (1+) within each specimen did not change significantly with the Gleason score (Fig. 5).

PEFT colon cancer sections were used as a positive control (data not shown). As reported previously, p53 was present in the nuclei of colonic adenocarcinoma glandular epithelial cells (29).

**GST-π.** The expression of GST-π was limited to the basal cells of BGE and was largely absent from malignant epithelium (Fig. 1, I and J). GST-π was expressed in 93% of BGE and in <1% of malignant epithelium (Fig. 2 and Table 4). The prevalence of GST-π staining was also high in BGE. More than 80% of the BGE stained >2+ (<10% had no staining). GST-π expression was seen in only one prostate cancer specimen (Fig. 5 and Table 4).

PEFT liver sections were used as a positive control (data not shown). As reported previously, GST-π was expressed in the bile duct epithelium of the normal liver but not in the adjacent hepatocytes (30).

**Bcl-2.** Bcl-2 staining was present in the basal cells of BGE and stained malignant epithelium more diffusely (Fig. 1, K and L). Bcl-2 was expressed in 100% of BGE and in 87% of the malignant elements in the prostate specimens examined (Table 5). Its expression was high and remained elevated with advancing stage, staining 83% of stage B, 89% of stage C, and 88% of stage D specimens (Fig. 2). The prevalence of staining for Bcl-2
within each specimen also decreased with advancing stage (Fig. 3). More than 70% of BGE stained 3+; whereas less than 10% of stage B and stage C specimens stained 3+ and no stage D specimens stained 3+.

Bcl-2 expression increased with the advancing of the Gleason score from 57% in low-grade lesions to 92% in moderate- and high-grade tumors (Fig. 4). These data are summarized in Table 5. The prevalence of staining for Bcl-2 within each specimen also increased with the Gleason score (Fig. 5). Whereas >40% of low-grade lesions showed no staining, >90% of moderate- and high-grade lesions showed 1+ to 3+ staining.

PEFT tonsil sections were used as a positive control (data not shown). As reported in the Bcl-2 antibody data sheet, Bcl-2 was expressed in lymphocytes of the mantle zone in the normal human tonsil, whereas the epithelium and the germinal center were relatively nonreactive with the antibody (“Materials and Methods”). At a higher magnification, it was apparent that the positive lymphocytes display cytoplasmic staining.

P-gp. P-gp was undetectable by immunohistochemistry in any of the resected specimens (Fig. 1, M and N). PEFT liver sections were used as a positive control (data not shown). As reported previously, P-gp was found to be present in the bile canaliculi of the liver in a periporal distribution (31). The structures of the portal triad were nonreactive except for the luminal aspect of the bile duct. At higher magnification, it was apparent that P-gp expression was restricted to a canalicular distribution and the hepatocytes were otherwise nonreactive with the antibody.

DISCUSSION
This study examines the expression of drug resistance gene products in prostate cancer as a function of disease progression. Our results indicate that several of these proteins are expressed early in the course of the disease. For example, MRP, topoisomerase IIα, p53, GST-π, and Bcl-2 are expressed at the time of surgical resection. For alterations in protein expression, the stage of disease is a stronger indicator than the grade (Tables 1–5).

MRP is a M, 190,000 membrane protein, which is a member of the superfamily of ATP-binding cassette transporters and shares 15% amino acid homology with P-gp (32). Like P-gp, MRP renders cells resistant to naturally occurring anticancer drugs such as anthracyclines, Vinca alkaloids, and epipodophyllotoxins (32). A unique feature of MRP is its ability to recognize and transport reduced-glutathione conjugates (33). However, the precise mechanism by which MRP confers drug resistance is unknown. The overexpression of MRP has been reported in several human cancers, including cancers of the lung, esophagus, breast, and ovary and leukemias (34). The status of MRP in human prostate cancer tissue has not been described previously.

MRP was expressed in both normal glands (37%) and prostatic adenocarcinoma (84%; Fig. 2 and Table 1). In BGE, most MRP expression localized to the basal cells of the normal prostatic glandular epithelium (Fig. 1C). In prostate cancer, MRP was seen diffusely throughout the epithelial tissue (Fig. 1D). The advancing stage, rather than the Gleason score, was the strongest predictor of MRP expression (P = 0.0001; odds ratio, 3.15; Table 1).

Topoisomerase IIα is one of two known isozymes of topoisomerase II (35). Topoisomerase IIα controls the topology of DNA by cleaving one DNA strand, passing the other DNA strand through the transient gap, and rejoining the two strands (36). Alterations in topoisomerase IIα is responsible for so-called “atypical” multidrug resistance, in which cross-resistance to several classes of drugs occurs without changes in drug accumulation (34). The expression of topoisomerase IIα has been found to be correlated with cell proliferation (35) and was increased in both transformed cells and drug resistant cells (37). Changes in the expression of topoisomerase IIα have not been studied in human prostate cancer in vivo.

Topoisomerase IIα was present in 23% of BGE and in 88%
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Fig. 5 Influence of Gleason score on the prevalence of immunohistochemical staining in each specimen: A, MRP; B, topoisomerase IIα; C, p53; D, GST-π, and E, Bcl-2. The prevalence of immunohistochemical staining was scored as 1+ (1–25%), 2+ (26–75%), or 3+ (76–100%), depending on the percentage of the specimen that stained positive as described in “Materials and Methods.”

Table 2 Topoisomerase IIα expression in human prostate cancer

<table>
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<tr>
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<th>No. of positive specimens</th>
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<th>n</th>
<th>Odds ratio</th>
<th>P</th>
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<tbody>
<tr>
<td>BGE</td>
<td>17 (75)*</td>
<td>23</td>
<td>23</td>
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</tr>
<tr>
<td>Stage B</td>
<td>15 (20)</td>
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<tr>
<td>Stage C</td>
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<td>93</td>
<td>149</td>
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<tr>
<td>Stage D</td>
<td>11 (12)</td>
<td>92</td>
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<tr>
<td>Stages B–D</td>
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<td>88</td>
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<tr>
<td>Gleason 2–4</td>
<td>12 (16)</td>
<td>75</td>
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<tr>
<td>Gleason 5–6</td>
<td>25 (31)</td>
<td>81</td>
<td>76</td>
<td>1.54</td>
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<tr>
<td>Gleason 7–10</td>
<td>29 (29)</td>
<td>100</td>
<td></td>
<td></td>
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</tbody>
</table>

* Numbers in parentheses, total number of specimens examined.

Table 3 p53 expression in human prostate cancer

<table>
<thead>
<tr>
<th></th>
<th>No. of positive specimens</th>
<th>Positive specimens (%)</th>
<th>n</th>
<th>Odds ratio</th>
<th>P</th>
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<tbody>
<tr>
<td>BGE</td>
<td>0 (74)*</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage B</td>
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<tr>
<td>Stage C</td>
<td>6 (39)</td>
<td>15</td>
<td>145</td>
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<tr>
<td>Stage D</td>
<td>6 (13)</td>
<td>46</td>
<td></td>
<td></td>
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<tr>
<td>Stages B–D</td>
<td>12 (71)</td>
<td>17</td>
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<tr>
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<td>2 (16)</td>
<td>13</td>
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<td>73</td>
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<td>Gleason 7–10</td>
<td>6 (29)</td>
<td>21</td>
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* Numbers in parentheses, total number of specimens examined.

of the malignant specimens (Table 2). Both nuclear and cytoplasmic staining occurred, although nuclear staining was more common (data not shown). The expression of topoisomerase IIα increased with both grade and stage (Figs. 2 and 4). Advancing stage was the stronger predictor of topoisomerase IIα expression ($P = 0.0001$; odds ratio, 5.98; Table 2).

Changes in the expression of topoisomerase IIα have been reported to alter sensitivity to chemotherapeutic drugs in prostate cancer cell lines. For example, selection of DU-145 cells for resistance to 9-nitrocamptothecin, a topoisomerase I poison, produced a cell line with altered topoisomerase IIα activity and increased sensitivity to etoposide-induced apoptosis (38).
Topoisomerase II drugs are used in the treatment of prostate cancer (39, 40). Pienta and Lehr (39) reported that etoposide plus estramustine inhibited the growth of several prostate cancer cell lines in vivo and in vitro. Our results suggest that overexpression of topoisomerase IIα early in the course of the disease may render patients more sensitive to topoisomerase II drugs. The fact that these drugs are not highly effective in the clinic may be due to the concomitant expression of other drug resistance mechanisms such as that of MRP (41, 42).

The p53 tumor suppressor gene is the most frequently altered gene in human cancer (43). Its activity is mediated through transcriptional regulation of a variety of genes that can be either transcriptionally activated or repressed by the wild-type protein (43, 44). The presence of wild-type p53 is also believed to be involved in both drug- and radiation-induced apoptosis (44, 45); mutations in p53 have been associated with resistance to some anticancer drugs (46) and sensitivity to others (47).

p53 was not detectable in normal prostate epithelium but was seen in 17% of all of the prostate cancer specimens examined. As shown in Table 3, p53 expression was associated significantly with increasing stage (P = 0.0002; odds ratio, 7.60); 15% of stage C and 46% of stage D specimens stained for p53 (Fig. 2). The prevalence of epithelial staining of specimens was in the 1+ range (Fig. 3 and 5). p53 expression was not associated significantly with increasing Gleason grade (Table 3). p53 expression in prostate cancer has been studied previously but not in the context of other markers of drug resistance (48–51). In fact, Stattin et al. (52) have shown that the predictive value of immunoreactive p53 in prostate cancer depends on its association with high-grade and advanced stage and does not by itself seem to be a useful prognostic marker.

Detection of p53 by immunohistochemistry is, in most cases, due to the presence of a p53 mutation (49, 53). This is believed to be caused by the long half-life of the mutant compared with that of the wild-type protein (54). However, it is also known that certain proteins such as mdm2 can stabilize wild-type p53 and produce false positive results (55). Immunohistochemical detection of p53 in prostate cancer has been shown by single-strand conformational polymorphism analysis to be due to mutation (49, 53).

GST-π is a member of the GSTs family of drug-metabolizing and detoxification enzymes. These enzymes conjugate glutathione to both xenobiotics and endogenous metabolites (56). Overexpression of GST-π produces resistance to chemotherapeutic drugs metabolized by this enzyme (57). GST-π is also believed to have anticarcinogenic properties through inactivation of reactive electrophiles by conjugation to reduced glutathione (6).

GST-π is expressed in BGE and is almost entirely absent from malignant epithelium (Table 4). GST-π was found in the BGE and in the neoplastic elements of 1 of 74 resected prostates examined (Table 4). Our results are in agreement with other studies showing that GST-π expression is rare in prostate cancer (7, 58). The loss of basal epithelium during oncogenesis per se does not explain the loss of GST-π inasmuch as both MRP and Bcl-2 showed a similar distribution in the BGE. Because of its role as a potential anticarcinogen and drug metabolizing agent, its loss may be a significant event in the development and progression of prostate cancer.

Bcl-2 is overexpressed in B cell follicular lymphomas as a result of the (t(14;18) translocation, which brings the bcl-2 gene from chromosome 18 into the immunoglobulin heavy chain joining region on chromosome 14 (59). Overexpression of this gene renders cells less sensitive to apoptosis induced by radiation, chemotherapy, and androgen withdrawal (3, 60–62). Although it has been suggested that Bcl-2 is associated with the emergence of androgen independence, the present investigations indicate that it is present much earlier (4).

Bcl-2 is overexpressed in 100% of BGE and >85% of malignant epithelium (Table 2). The results are higher than reported previously (4, 63–65). This difference may be due to the sensitivity of our antigen retrieval technique, which maintains the citrate buffer at the constant temperature of 95°C to maximize the disassembly of the formalin scaffolding responsible for epitope masking while minimizing the antigen destruction associated with the standard microwave technique. Because the patients from whom the resected prostates were obtained for this study had not been subject to any therapeutic modulation, it is unlikely that the increased incidence of Bcl-2 staining is due to factors other than the antigen retrieval technique. In fact, using the antibody and method described in McDonnell et al. (4) (data not shown), our results were similar.

In summary, our studies indicate that in early prostate cancer, several genes are expressed that may account for the pan-resistant phenotype. We observed that MRP was expressed in 83% of the specimens examined. The high degree of MRP...
expression is consistent with the lack of effectiveness of many clinical trials involving MRP substrates, such as etoposide, doxorubicin, mitoxantrone, and vincristine (41, 42, 66). One possible mechanism that may sensitize cells to MRP substrates is the abrogation of a pathway by which drugs are metabolized by conjugation for export. Interestingly, GST-\(\gamma\), a molecule that conjugates glutathione to drugs, is not expressed during the progression of prostate cancer (Table 4). This loss appears to disable the pathway by which certain drugs such as etoposide are extruded via MRP as glucuronate conjugates. However, there are many other GSTs that are capable of conjugating glutathione to chemotherapeutic agents (67). Furthermore, Keppler et al. (67) recently demonstrated that the direct transport of unconjugated lipophilic cytotoxic drugs by MRP may be the predominant mechanism of resistance.

Individual mechanisms of drug resistance, such as MRP expression, may be circumvented by using drugs not recognized by transporters. However, the expression of other drug resistance genes, such as bcl-2 or p53, could abrogate the effect of such drugs by inhibiting a common final pathway of cell death. Whether assessing prostate cancer by stage or grade, Bcl-2, MRP, and topoisomerase II\(\alpha\) are present at significant levels throughout the progression of the disease (Figs. 2 and 4). From the data, a picture of the multiple mechanisms of drug resistance in human prostate cancer begins to emerge. The therapeutic concept of interfering with a single mechanism of drug resistance in this context should be reconsidered.

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The expression of drug resistance gene products during the progression of human prostate cancer.


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