Histologic Changes Associated With Neoadjuvant Chemotherapy Are Predictive of Nodal Metastases in Patients With High-Risk Prostate Cancer

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Abstract

Clinical trials are evaluating the effect of neoadjuvant chemotherapy on men with high-risk prostate cancer. Little is known about the clinical significance of postchemotherapy tumor histopathologic features. We assessed the prognostic and predictive value of histologic features (intraductal carcinoma, vacuolated cell morphologic features, inconspicuous glands, cribriform architecture, and inconspicuous cancer cells) observed in 50 high-risk prostate cancers treated with preprostatectomy docetaxel and mitoxantrone. At a median follow-up of 65 months, the overall relapse-free survival (RFS) rates at 2 and 5 years were 65% and 49%, respectively. In univariate analyses (using the Kaplan-Meier method and log-rank tests), intraductal \((P = .001)\) and cribriform \((P = .014)\) histologic features were associated with shorter RFS. In multivariate analyses, using the Cox proportional hazards regression, baseline prostate-specific antigen \((P = .004)\), lymph node metastases \((P < .001)\), and cribriform histologic features \((P = .007)\) were associated with shorter RFS. In multivariable logistic regression analysis, only intraductal pattern \((P = .007)\) predicted lymph node metastases. Intraductal and cribriform histologic features apparently predict postchemotherapy outcome.

Prostate cancer is the most common malignancy (excluding skin cancer) diagnosed in American men. In 2008, an estimated 186,320 men were diagnosed with prostate cancer, and 28,660 have died of their disease.1

The risk of relapse and death of disease after prostatectomy is significant in patients with prostate cancer who are at high risk of progression, ie, more than 60% of men who underwent prostatectomy for carcinoma with a Gleason score of 4 + 3 or more died of disease at 10 years’ follow-up in one series.2 Accordingly, there is a need to develop more effective treatment of prostate adenocarcinoma. Although the negative surgical margin rate was improved with neoadjuvant hormonal therapy,3-5 randomized clinical trials have failed to demonstrate a relapse-free survival (RFS) or overall survival (OS) benefit with this approach.3,6-8

Neoadjuvant cytotoxic chemotherapy, capable of targeting androgen-independent clones, is therefore under investigation in high-risk prostate cancer.9-13 While in most cases hormonal therapy has been combined with chemotherapy in neoadjuvant studies, several investigators have examined chemotherapy in isolation, permitting a more careful assessment of the impact of cytotoxic chemotherapy on untreated prostate cancer.10,11,14,15

Some types of systemic therapy result in characteristic histologic effects on benign and cancerous prostate tissue. For example, androgen-deprivation therapy has a predictable effect on nonneoplastic and malignant prostate glands. Nonneoplastic glands are characterized by reduced gland diameter, flattened epithelium with loss of micropapillary architecture, and prominence of the basal cell layer, which is hyperplastic. Carcinoma glands collapse and shrink. Cancer cells are characterized by pyknotic nuclei and voluminous vacuolated cytoplasm.16-22
Histologic changes in prostate tissue treated with chemotherapy have been previously described, although only in a limited manner. We report histologic patterns observed in radical prostatectomy specimens obtained from patients at high risk for prostate cancer progression who were treated with neoadjuvant docetaxel and mitoxantrone. Furthermore, we discuss the predictive power of 2 histologic patterns, intraductal carcinoma (IDC) and cribriform architecture, for nodal metastases and RFS.

Materials and Methods

Patients

Between January 2001 and November 2004, 57 patients with localized prostate cancer at high risk for progression were recruited for a phase II clinical trial of neoadjuvant chemotherapy. To be categorized as having high-risk disease, patients had to meet at least 1 of 3 criteria: tumor was stage cT2b or cT3a, serum prostate-specific antigen (PSA) level was 15 ng/mL (15 µg/L) or more, or a Gleason score of 4+3 or more. The design of the clinical trial has been previously reported. The study was approved by the institutional review

Selection of Radical Prostatectomy Slides for Histologic Evaluation

Samples from radical prostatectomy tissue from the first 50 patients were used to construct a tissue microarray (TMA) that was assembled to identify prognostic biomarkers. In selecting the tissue blocks from which to obtain cores of tissue for the TMA, the slides that had the greatest amount of prostate carcinoma were identified and reviewed by the pathologist (L.D.T.) for this study.

Selection of Core Biopsy Slides for Histologic Evaluation

Pretreatment core biopsy tissue slides were retrieved for 22 of the 50 patients whose postneoadjuvant therapy cancers were evaluated. Because the pretreatment biopsy specimens were not systematically collected as part of the study, the remaining 28 could not be retrieved for analysis.

Histologic Evaluation of Radical Prostatectomy Specimens

Sections that contained the largest fraction of tumor in each case were reviewed to select blocks for constructing TMAs. Based on the observation that many of these tumors exhibited histologic patterns that are infrequent to rare in prostate cancers that had not been exposed to neoadjuvant chemotherapy, all sections of each radical prostatectomy sample were systematically rereviewed to tabulate the relative frequency of each of these patterns. The following patterns were tabulated: (1) intraductal growth pattern (IDC), (2) prominent vacuolization of tumor cells, (3) inconspicuous appearance of collapsed tumor glands, (4) cribriform architecture, and (5) inconspicuous appearance of cancer cells (cancer cells that if viewed at relatively low magnification, eg, ×40 final magnification, might be easily overlooked).

We also assessed the sections for features that characterize benign and malignant prostate consequent to other modalities of nonsurgical therapy, such as androgen-deprivation therapy. These features included atrophy and shrinkage of nonneoplastic and/or cancer glands; basal cell hyperplasia and prominence and squamous metaplasia of benign glands; and stromal changes, eg, stromal cell atypia, focal necrosis, foci of hypocellular scarring, vascular ectasia, and prominent stromal inflammation. Finally, 2 tumors had features that are quite rare in conventional prostate adenocarcinomas. The majority of carcinoma cells in one cancer were large and had abundant eosinophilic cytoplasm with markedly pleomorphic nuclei. Most cells in a second tumor had a nested, carcinoid-like growth pattern. The fact that there were only single examples of each of these histologic patterns precluded assessing the predictive power of these patterns.

Based on prior publications, we used the following criteria for IDC in the present study: (1) 2-fold expansion of prostate gland lumina; (2) neoplastic cells span, in a solid or cribriform architecture, the lumen of glands in which a basal cell layer is retained; and (3) nuclear atypia. This histologic lesion is different from its near namesake—prostatic duct adenocarcinoma—that is invasive and has a tubulovillous architecture resembling endometrioid adenocarcinoma of the gynecologic tract. None of the cases in this series was a prostatic duct adenocarcinoma. Descriptively, the distinction between intraductal and ductal adenocarcinoma is based on, first, the fact that ductal adenocarcinoma most frequently is in a central location and has a tubulovillous pattern, although one of the patterns of ductal carcinoma is that of a solid intraductal-type growth pattern, and, second, that foci of ductal adenocarcinoma having a cribriform pattern are a lot larger than any of the foci of IDC seen in our cases.

Histologic Evaluation of Core Biopsy Sections

Because virtually all of the histologic patterns that we found distinctive of prostate cancer treated with neoadjuvant chemotherapy occur in other settings—treatment-naïve and following other neoadjuvant regimens—we sought to tabulate the frequency of each of the 6 patterns in the pretreatment biopsy specimens. The retrieved slides were anonymized so that the pathology review was blinded as to the patient outcome and the pathology of the prostatectomy sample. Histologic criteria applied to reviewing the Gleason grade of...
cancer in the neoadjuvant biopsy specimens were those of the 2005 International Society of Urological Pathology Consensus Conference on Gleason Grading.30

Results

Patient Characteristics and Sample Collection

The characteristics of the 50 patients whose tissue was evaluated are outlined in Table 1. The median serum PSA level was 12.0 ng/mL (12 μg/L; range, 1.4-58.6 ng/mL [1.4-58.6 μg/L]). Of the 50 patients, 44 (88%) had Gleason scores of 7, 8, or 9 in their biopsy specimens. Five patients had Gleason score 6 cancers, and 1 patient had a Gleason score 10 cancer. The clinical stage of most patients was at least T2a, reflecting the prespecified entry criteria for the trial.

Histologic Patterns Identified in Radical Prostatectomy Slides

Most of the carcinomas in the prostatectomy specimens had at least one of the distinctive histologic patterns, which, in decreasing frequency of occurrence, were: (1) relatively inconspicuous, collapsed glands (23 [46%]) Image 1A; (2) small, inconspicuous tumor cells (14 [28%]) Image 1B; (3) prominently vacuolated tumor cell cytoplasm (13 [26%]) Image 1C; (4) intraductal growth pattern (10 [20%]) (Images 1B and 1C); (5) cribriform architecture (7 [14%]) Image 1D; (6) tumor-associated, basophilic, mucin-like material (2 cases) (not illustrated); (7) nested growth pattern (1 case) (not illustrated); and (8) individual, large, pleomorphic, eosinophilic tumor cells (1 case) Image 1E. Tumors in 36 (72%) of the 50 patients exhibited at least one pattern, while 17 (34%) had at least 2 patterns Table 2 and Table 3.

Other histologic features were so common—atrophy and shrinkage of nonneoplastic and/or cancer glands and basal cell hyperplasia—or so rare—squamous metaplasia of benign glands and stromal changes, eg, stromal cell atypia, focal necrosis, foci of hypocellular scarring, vascular ectasia, and prominent stromal inflammation—as to not warrant statistical analysis.

Histologic Patterns Identified in Core Biopsy Slides and Specimens

Of the 22 patients whose biopsy specimens we could evaluate, 3 specimens exhibited histologic patterns in the neoadjuvant therapy biopsy specimens that were similar to the histologic patterns seen in the corresponding radical prostatectomy specimens—cribriform architecture, vacuolated cytoplasm, and inconspicuous collapsed tumor glands. The results are summarized in Table 2. None of the preprostatectomy biopsy specimens contained IDC.

Correlation With Pathologic and Other End Points

Prognostic Significance

At a median follow-up time of 65.1 months, the overall estimated RFS at 2 years was 65.3% (95% confidence interval [CI], 52.0%-78.6%) and at 5 years was 48.8% (95% CI, 34.1%-63.5%). Of the 50 patients whose tissue was evaluated, 48 received at least 1 cycle of chemotherapy. These 48 patients were evaluated for clinical end points.

In univariate analyses, IDC (P = .001) and cribriform architecture (P = .014) were associated with shorter RFS. In univariate analyses stratified for lymph node status, IDC (P = .023) was associated with shorter RFS in lymph node–negative patients, whereas cribriform architecture (P = .036) was associated with shorter RFS in lymph node–positive patients. These 2 histologic patterns (IDC and cribriform architecture) were included in a multivariate analysis that included biopsy Gleason score, clinical T stage, serum PSA level, and lymph node status (N0 vs N1). In this model, cribriform architecture (hazard ratio [HR], 2.1; 95% CI, 1.2-3.7; P = .007), baseline PSA (HR, 1.05; 95% CI, 1.01-1.08; P = .004), and lymph node metastases (HR, 8.1;
Many histologic features of prostate cancer can serve as tissue biomarkers of tumor biology. For example, the microscopic architecture of primary prostate cancer (the Gleason score) provides a powerful predictor of prognosis and of the likelihood that primary therapy will be successful. Histologic features also reflect and are characteristic of different systemic therapies. Androgen-deprived prostate cancer is characterized by inconspicuous, shrunken glands and small inconspicuous tumor cells that have pyknotic, basophilic nuclei lacking observable nucleoli and having scant cytoplasm.\textsuperscript{16-20} Tumor cells subjected to high-dose radiation from brachytherapy are also typically inconspicuous. Also note (arrowhead) a duct peripherally lined by prominent basal cells and expanded by a population of dysplastic neoplastic cells having cribriform architecture. This pattern meets current histologic criteria for intraductal carcinoma (H&E, ×200). C, Vacuolated tumor cells. Sheets of carcinoma are composed of cells, many having prominent, large, clear cytoplasmic vacuoles. Also illustrated (arrow) is a focus of intraductal carcinoma (H&E, ×200).

Discussion

Many histologic features of prostate cancer can serve as tissue biomarkers of tumor biology. For example, the microscopic architecture of primary prostate cancer (the Gleason score) provides a powerful predictor of prognosis and of the likelihood that primary therapy will be successful. Histologic features also reflect and are characteristic of different systemic therapies. Androgen-deprived prostate cancer is characterized by inconspicuous, shrunken glands and small inconspicuous tumor cells that have pyknotic, often hyperchromatic, nuclei with inconspicuous nucleoli.\textsuperscript{16-20} Tumor cells subjected to high-dose radiation from brachytherapy are also typically inconspicuous and have abundant, vacuolated cytoplasm.\textsuperscript{31,32} Although histologic changes of prostate cancer in patients treated with neoadjuvant chemotherapy have been previously
described in a limited manner,23 this study has found that 2 of these features, IDC and cribriform architecture, have apparent power in predicting outcome after neoadjuvant chemotherapy. The cribriform architecture retains its prognostic value in the context of a comprehensive multivariate analysis.

None of the histologic features that we observed is unique to prostate cancer treated with mitoxantrone and docetaxel. Single, small, inconspicuous tumor cells are seen in androgen-deprivation and radiation therapy and are a feature of one manifestation of Gleason pattern 5 primary prostate cancer. Collapsed, also relatively inconspicuous glands are also seen in androgen-deprived and irradiated prostate cancer. Although prominent, vacuolated cytoplasm is a feature of brachytherapy, the nature of vacuolization is subtly different from that seen in androgen-deprived prostate tissue. The vacuoles seen in the cancers in this study are round and clear. In contrast, the vacuoles in irradiated prostate cancer are irregular and often have a finely vesicular appearance.

Cribriform architecture, more frequent in our treated cancers, is architecturally indistinguishable from one variant (the cribriform variant) of Gleason pattern 4 carcinoma, although cytologically, the tumor cells are distinctive, having small pyknotic nuclei and scant, clear cytoplasm. The association of cribriform architecture with a more malignant course of disease is of interest because it suggests that the cribriform variant of Gleason pattern 4 biologically differs from the collapsed gland variant of pattern 4. However, this hypothesis cannot be addressed in the present study because the sample was small, particularly the number of preneoadjuvant therapy

![Image](cont)

**Image 1** (cont) **D**, Cribriform architecture. Foci of cancer (arrows) have a cribriform architecture. This pattern, which is indistinguishable from one form of Gleason pattern 4 prostate carcinoma, was exhibited by many of the cancers in the postchemotherapy manifestation (H&E, x100). **E**, Pleomorphic tumor cells. One case was characterized by individual, large, pleomorphic, tumor cells with abundant cytoplasm (arrow) (H&E, x200).

**Table 2**

**Table 3**

**Frequency of Specific Histologic Patterns in Tissue Specimens**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Radical Prostatectomy Specimens (n = 50)</th>
<th>Biopsy Specimens (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraductal carcinoma</td>
<td>10 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cribriform architecture</td>
<td>7 (14)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Prominent vacuolated cytoplasm</td>
<td>13 (26)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Inconspicuous, collapsed glands</td>
<td>23 (46)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Small, inconspicuous tumor cells</td>
<td>14 (28)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Any</td>
<td>34 (68)</td>
<td>8 (36)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>16 (32)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

**Histologic Features of Cancer in Prechemotherapy and Postchemotherapy Tissue Specimens From 8 Cases With at Least One of the Chemotherapy-Associated Patterns in the Prechemotherapy Biopsy Specimen**

<table>
<thead>
<tr>
<th>Case ID</th>
<th>Pattern(s) Identified in Biopsy Slides</th>
<th>Pattern(s) Identified in Prostatectomy Slides</th>
</tr>
</thead>
<tbody>
<tr>
<td>6082-01</td>
<td>Cribriform</td>
<td>None</td>
</tr>
<tr>
<td>6082-03</td>
<td>Vacuolated</td>
<td>None</td>
</tr>
<tr>
<td>6082-05</td>
<td>Cribriform; inconspicuous glands</td>
<td>Cribriform; intraductal</td>
</tr>
<tr>
<td>6082-19</td>
<td>Cribriform</td>
<td>Inconspicuous glands</td>
</tr>
<tr>
<td>6082-20</td>
<td>Inconspicuous glands</td>
<td>Inconspicuous glands; cribriform</td>
</tr>
<tr>
<td>6082-35</td>
<td>Cribriform</td>
<td>Inconspicuous glands</td>
</tr>
<tr>
<td>6082-36</td>
<td>Inconspicuous glands</td>
<td>None</td>
</tr>
<tr>
<td>6082-37</td>
<td>Inconspicuous glands</td>
<td>Inconspicuous glands</td>
</tr>
</tbody>
</table>
samples that had the cribriform variant of Gleason pattern 4 carcinoma.

Tumor-associated, basophilic, mucin-like material, seen in 2 cases, is an infrequent feature that characterizes a minority of primary, untreated prostate cancers. The nested growth pattern that we found in 1 case, though nonspecific, is one manifestation of neuroendocrine differentiation by tumors in many organ sites. This pattern is characterized by small, round aggregates of tumor cells. Finally, in 1 case, many tumor cells were large with abundant eosinophilic cytoplasm and pleomorphic nuclei. This histologic set of features has been previously reported as a rare histologic variant of prostate carcinoma that is associated with a poor outcome. Of note, although none of the 6 cases in the previous report were associated with neoadjuvant chemotherapy, 2 patients had received other modalities of neoadjuvant therapy, radiation and androgen deprivation, respectively.

IDC has historically been a poorly defined and controversial entity. The controversy regards whether it is a variant of prostatic intraepithelial neoplasia or whether it represents a growth pattern of prostate adenocarcinoma that is invasive of prostate ducts and glands. Attempting to clarify this issue, several authors have articulated histologic criteria that provide a reasonable basis for defining “intraductal carcinoma” as a distinct histopathologic entity. Previous studies have found a correlation of IDC with poor prognostic parameters (high Gleason score, elevated serum PSA level, high tumor volume, and extraprostatic extension of tumor) and increased frequency of biochemical evidence of recurrence. These correlations have led investigators to conclude that IDC generally represents a late event in the progression of prostate adenocarcinoma.

IDC is an infrequent feature of prostate cancer, being represented in fewer than 5% of primary prostate cancers. In general, prostate carcinomas that have an intraductal component are of higher grade and greater volume. This pattern is predictive of more aggressive disease. We assessed the association of each pattern with the likelihood of progression of cancer. Three patterns of histologic features were too rare to have statistical predictive power in our series of patients: basophilic, mucin-like material; nested growth pattern; and pleomorphic tumor cells. Hence, these patterns were omitted from further analysis. We found that the IDC pattern was an independent predictor of nodal metastases in our group of patients with prostate cancer.

To assess whether the patterns seen in the radical prostatectomy specimens were induced by chemotherapy or whether they were innate features that were expressed in the treatment-naive state of the tumor, we reviewed the corresponding pretreatment biopsy slides in a blinded manner. We found very little evidence of the intraductal, cribriform, inconspicuous cell and gland and vacuolated cell patterns in the slides from the prechemotherapy core biopsy specimens.

In fact, whereas 36 (72%) of 50 patients whose radical prostatectomy (postchemotherapy) slides contained these histologic changes, only 8 (36%) of 22 of the biopsy slides contained similar patterns. There are several possible explanations for the difference in the frequency with which these histologic patterns are seen in prostatectomy specimens compared with biopsy specimens.

Selection Bias

Because the slides we reviewed from the radical prostatectomy specimens were selected for having large areas of normal or malignant prostate tissue, it is possible that we introduced some selection bias. IDC has been previously shown to be found primarily within areas of invasive cancer and rarely in areas where carcinoma represents a minor fraction of prostate tissue, and, as mentioned, there is an association between cancer volume and the presence of IDC. Furthermore, biopsy specimens were examined from only a subset of patients for whom prostatectomy specimens were available for analysis.

Differences in the Amount of Tissue Examined

In general, more tissue was histologically evaluated in the radical prostatectomy slides than in the core biopsy slides. There is an inherent limitation of core biopsy specimens owing to the small proportion of each cancer that is sampled with core biopsies. Therefore, it is possible that the histologic patterns seen in the radical prostatectomy specimens were present but were not sampled in the pretreatment biopsies.

Treatment With Chemotherapy Selected for Survival of Specific Patterns

The IDC pattern has been previously associated with more aggressive prostate cancer. Therefore, it is conceivable that this pattern is more resistant to chemotherapy than the classically described Gleason patterns. Resistance to chemotherapy might have resulted in selection of this histologic pattern following treatment. The same consideration pertains to the cribriform architecture, which is one variant of Gleason pattern 4, since Gleason pattern 4 is a histologic marker of a cancer at higher risk of progression than are cancers that have a Gleason score no greater than 3.

Changes Were Induced by Chemotherapy

It is also possible that some or all of these patterns represent a change in the histologic features of the prostate cancer caused by one or both of the chemotherapeutic agents. This possibility is unlikely because the 2 patterns that have been previously described (IDC and cribriform) occur in untreated prostate cancers. The one pattern that is quite distinctive is the large, pleomorphic tumor cell pattern, which was seen in 1 case (Image 1E).
Host Predisposition to IDC

That some, but not all, patients who otherwise shared all identifiable demographic and pathologic features had distinctive postchemotherapy histologic patterns raises the question of host genome predisposition to these distinctive tumor architectures.

The association between cribriform architecture and risk of relapse, as well as of IDC with nodal status, should be evaluated in an independent set of prostate cancers, ideally in a group of patients with untreated prostate cancer. Were the predictive power of these patterns to be validated, studies of the molecular phenotype of this pattern would be warranted to try to identify therapeutic molecular targets.

References


