Immunophenotypic Characterization of 225 Prostate Adenocarcinomas With Intermediate or High Gleason Scores

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Abstract

This study provides detailed staining results for 225 prostate adenocarcinomas, including 150 Gleason score 8, 9, and 10 adenocarcinomas with cytokeratins (CKs) 7, 20, 5/6, and 17, prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), carcinoembryonic antigen (CEA), WT1, thyroid transcription factor-1 (TTF-1), and villin. CK7 was reactive in 112 adenocarcinomas (49.8%). The percentage of CK7-reactive adenocarcinomas and the percentage of CK7-stained cells increased in higher Gleason score adenocarcinomas; most reactive neoplasms had CK7 staining of fewer than 25% of cells. CK20 had similar results. The percentage of PSA- and PAP-reactive adenocarcinomas and the percentage of stained cells in reactive neoplasms decreased in higher Gleason score adenocarcinomas. CK5/6 and CK17, WT1, CA-125, TTF-1, and villin were nonreactive. The prostate can be the primary site of metastatic adenocarcinoma that is nonreactive for PAP and PSA and has CK7 or CK20 reactivity in fewer than 50% of the cells. The likelihood that a metastatic adenocarcinoma is from the prostate is low if reactivity with any of the cytokeratin antibodies, CEA, TTF-1, CA-125, WT1, or villin is extensive.

The prostate often is included in the list of possible primary sites of metastatic and poorly differentiated adenocarcinomas. Immunohistochemical analysis often can identify the primary site or shorten the list of possible primary sites. In addition to cytokeratin 7 and 20 antibodies, which are frequently on the roster of tumor-identification antibody panels, several recently introduced antibodies seem to be useful additions. There is limited or no staining experience with these antibodies, including cytokeratin 7 and 20 in intermediate- and high-grade prostate adenocarcinoma.

The goals of this study were to characterize the immunoreactivity and to provide detailed staining results in prostate adenocarcinomas with intermediate and high Gleason scores with the antibodies that are used commonly in tumor-identification antibody panels. These results provide a comparative framework for determining the likelihood of whether a metastatic or poorly differentiated adenocarcinoma is from the prostate.

Materials and Methods

Whole-mount slides of 225 radical prostatectomies with large volume adenocarcinomas with homogeneous Gleason scores were randomly selected based on their Gleason score from the files of William Beaumont Hospital, Royal Oak, MI, during the period January 1989 through December 2000. A region of abundant adenocarcinoma with homogeneous Gleason scores was circled on a whole-mount slide from each case, and regular-sized slide sections were cut from these areas for immunohistochemical staining. The minimum diameter of the circled regions was 1.8 cm. Twenty-five
adenocarcinomas (11.1%) were Gleason score 6, 50 (22.2%) were Gleason score 7, 54 (24.0%) were Gleason score 8, 58 (25.8%) were Gleason score 9, and 38 (16.9%) were Gleason score 10.

Fourteen adenocarcinomas had a significant large duct component, including the large ducts of the verumontanum. Four were Gleason score 7 (4 + 3), 7 were Gleason score 8, and 3 were Gleason score 9. All 14 neoplasms were peripheral zone–predominant adenocarcinomas.

Immunohistochemical Analysis

Consecutive 3-µm-thick sections were cut from the circled area of the whole mount block, and each section was placed on charged slides. Sections were deparaffinized, immersed in EDTA buffer (pH 7.0), and placed in a commercial vegetable steamer at 95°C for 30 minutes. They were transferred to a commercial immunohistochemical autostainer (DAKO, Carpinteria, CA), and the primary antibody was incubated over the sections for 20 minutes. The Envision-plus (DAKO) detection system was used. Table 1 lists the primary antibodies used in the study.

A positive control slide containing known cytokeratin-reactive nonprostatic tissues was included with each batch of simultaneously stained slides. All of the cases had at least 1 negative control slide that was run parallel with a test slide in which the primary antibody step was omitted. Cytokeratin AE1/AE3 served as a “fixation” control and was diffusely and strongly reactive in all 225 adenocarcinomas.

The percentage of moderately or strongly reactive invasive adenocarcinoma cells in each case was quantified and tabulated as follows: 0%, fewer than 5%, 5% to 25%, 26% to 50%, 51% to 75%, and more than 75%. Reactivity of fewer than 5% of the adenocarcinoma cells was rare, individual stained cells, constituting no more than 1 cell in a medium magnification field using the 10× objective. Reactivity in the 5% to 25% group was either an individual cell pattern of several cells per 10× field or widely dispersed small cell groups of reactive cells with a density of no more than 1 per 10× field. Reactivity of 26% to 50% had the appearance that a minority of cells were reactive at low magnification. At higher magnification, the pattern of reactivity was patchy or homogeneous. Patchy reactivity was extensive reactivity in a region of the slide while other regions were nonreactive. The homogeneous pattern was small groups of usually fewer than 5 reactive cells interspersed among nonreactive cells. Reactivity of 51% to 75% was similar to the 25% to 50% group except that reactive cells were clearly predominant. The group with more than 75% reactivity had the appearance of unquestionable reactivity in the overwhelming majority of cells. Borderline cases between the 26% to 50% and the 51% to 75% reactivity groups were classified on the low-power magnification appearance of whether reactive or nonreactive cells constituted the major population. Borderline cases between the 51% to 75% and more than 75% reactivity groups were classified based on the low-power magnification appearance of whether the reactive cells constituted a slight or dominant majority of cells.

Results

Cytokeratin 7 was reactive in 112 (49.8%) of the 225 adenocarcinomas Table 2 and Image 1. The proportion of reactive cases increased with increasing Gleason score of the adenocarcinomas, from 8 (32%) of 25 neoplasms with a Gleason score of 6 to 28 (74%) of the adenocarcinomas with a Gleason score of 10. The percentage of stained cells in cytokeratin 7–reactive neoplasms also increased with the Gleason score of the adenocarcinomas. The predominant staining pattern in reactive cases was rare individual cells in adenocarcinomas with lower Gleason scores and widely dispersed clusters of several reactive cells in adenocarcinomas with higher Gleason scores. Reactive cells had homogeneous, brown, finely granular cytoplasmic staining. None

<table>
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<th>Antibody</th>
<th>Company</th>
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<td>Cytokeratin 5/6</td>
<td>Boehringer Mannheim, Indianapolis, IN</td>
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<td>Cytokeratin 17</td>
<td>DAKO</td>
<td>E3</td>
<td>1:200</td>
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<td>DAKO</td>
<td>K, 20.8</td>
<td>1:500</td>
</tr>
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<td>WT1</td>
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of the reactive neoplasms in the study had cytokeratin staining in more than 50% of the cells.

Cytokeratin 20 reactivity was present in 141 adenocarcinomas (62.7%; Table 2) \textbf{Image 2}. The proportion of reactive cases increased with the Gleason score of the adenocarcinomas, and there also was an increase in the percentage of cytokeratin 20–reactive cells with increasing Gleason scores of the adenocarcinomas. The predominant staining pattern was rare individual cells in adenocarcinomas with lower Gleason scores and widely dispersed clusters of several reactive cells in adenocarcinomas with higher Gleason scores. Groups of reactive cells that constituted a significant minority were present in 10 (26%) of 38 adenocarcinomas with a Gleason score of 10.

No adenocarcinoma had cytokeratin 20 reactivity in more than 50% of the neoplastic cells. Reactive cells had homogeneous, brown, finely granular cytoplasmic staining.

All adenocarcinomas with cytokeratin 20 reactivity in 26% to 50% of the neoplastic cells also had cytokeratin 7 reactivity in 5% to 25% or 26% to 50% of the cells. Of the 76 cytokeratin 20–reactive adenocarcinomas with staining in fewer than 5% of the cells, 11 (14%) had no cytokeratin 7 reactivity.

All adenocarcinomas with Gleason scores of 6 or 7 were nonreactive for carcinoembryonic antigen (CEA) \textbf{Table 3}. 

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Gleason Score & No. of Neoplasms & 0% & <5% & 5%-25% & 26%-50% & 51%-75% & >75% \\
\hline
Cytokeratin 7 & 6 & 25 & 17 (68) & 8 (32) & 0 (0) & 0 (0) & 0 (0) \\
 & 7 & 50 & 31 (62) & 18 (36) & 1 (2) & 0 (0) & 0 (0) \\
 & 8 & 54 & 34 (63) & 17 (31) & 3 (6) & 0 (0) & 0 (0) \\
 & 9 & 58 & 21 (36) & 15 (26) & 16 (28) & 6 (10) & 0 (0) \\
 & 10 & 38 & 10 (26) & 11 (29) & 12 (32) & 5 (13) & 0 (0) \\
Total & 225 & 113 (50.2) & 69 (30.7) & 32 (14.2) & 11 (4.9) & 0 (0) & 0 (0) \\
Cytokeratin 20 & 6 & 25 & 18 (72) & 5 (20) & 2 (8) & 0 (0) & 0 (0) \\
 & 7 & 50 & 19 (38) & 26 (52) & 4 (8) & 1 (2) & 0 (0) \\
 & 8 & 54 & 23 (43) & 22 (41) & 7 (13) & 2 (4) & 0 (0) \\
 & 9 & 58 & 17 (29) & 14 (24) & 18 (31) & 9 (16) & 0 (0) \\
 & 10 & 38 & 7 (18) & 9 (24) & 12 (32) & 10 (26) & 0 (0) \\
Total & 225 & 84 (37.3) & 76 (33.8) & 43 (19.1) & 22 (9.8) & 0 (0) & 0 (0) \\
\hline
\end{tabular}
\caption{Cytokeratin 7 and 20 Immunoreactivity}
\end{table}

* Data are given as number (percentage).

\textbf{Image 11} Gleason score 8 prostate adenocarcinoma with cytokeratin 7 reactivity in 5% to 25% of neoplastic cells. Individual cells are reactive for cytokeratin 7 (hematoxylin counterstain, ×160).

\textbf{Image 21} Gleason score 7 prostate adenocarcinoma with cytokeratin 20 reactivity in 5% to 25% of the neoplastic cells (hematoxylin counterstain, ×64).
Fewer than 5% of the adenocarcinomas with a Gleason score of 8 to 10 had CEA reactivity that was always in fewer than 25% of the neoplastic cells.

Prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) reactivity decreased in adenocarcinomas with higher Gleason scores (Table 3). All 25 adenocarcinomas with a Gleason score of 6 were reactive for PSA and PAP; 24 (96%) had reactivity in more than 50% of the neoplastic cells. Reactivity to both antibodies decreased precipitously in adenocarcinomas with a Gleason score of 8 or greater. These antibodies were either nonreactive or reactive in fewer than 5% of the neoplastic cells in 15 (28%) and 10 (19%) of 54 adenocarcinomas with a Gleason score of 8, respectively. Similar distributions were observed in adenocarcinomas with Gleason scores of 9 or 10. All adenocarcinomas that expressed cytokeratin 20 in 26% to 50% of the neoplastic cells had no PSA reactivity or PSA reactivity in fewer than 5% of the neoplastic cells. All 6 neoplasms that were nonreactive for PAP also were nonreactive for PSA. There were no neoplasms that were reactive for PSA and nonreactive for CEA.

Two adenocarcinomas with abundant, large duct growth had extensive cytokeratin 20 reactivity of the neoplastic cells in the ejaculatory and seminal vesicle ducts of the verumontanum Image 3 and Image 4. There was an abrupt transition to reactivity in fewer than 25% of the neoplastic cells within the ducts at the point where the ducts left the verumontanum and became surrounded by the central zone tissue in both neoplasms. The transition points were 0.47 and 0.55 cm, respectively, from the urothelium-covered apex of the verumontanum. Cytokeratin 20 reactivity in the large ducts, small acini, and complex cribriform structures of adenocarcinoma cells within the peripheral zone was present in fewer than 5% of the neoplastic cells in both neoplasms. Cytokeratin 7 had a similar pattern of reactivity, but the intensity of staining was weak to moderate. These 2 adenocarcinomas had Gleason scores of 7 and 8, respectively. The cytokeratin 7– and cytokeratin 20–reactive duct segments were weakly reactive for PSA and PAP and nonreactive for CEA. The morphologic features of the cytokeratin 7– and cytokeratin 20–reactive large duct adenocarcinoma were identical to those of the nonreactive large duct adenocarcinoma in the peripheral zone. The urothelium was normal in both patients, and neither had transitional cell carcinoma. The other 12 adenocarcinomas with prominent large duct extension had similar patterns of reactivity for cytokeratins 7 and 20 in all areas of the prostate.

Positive antibody reactions using a cut-point threshold of 25% staining are listed in Table 4. Adenocarcinomas with a Gleason score of 6, 7, or 8 had similar percentages of cytokeratin 7– and cytokeratin 20–reactive duct segments and could be grouped together. None of the 129 adenocarcinomas with a Gleason score of 6, 7, or 8 were positive for cytokeratin 7, compared with 11 (11%) of 96 adenocarcinomas with a Gleason score of 9 or 10. Three (2.3%) of the adenocarcinomas with a Gleason score of 6, 7, or 8 were positive for cytokeratin 20, compared with 19 (20%) of the adenocarcinomas with a Gleason score of 9 or 10.
The percentage of PSA- and PAP-positive cases decreased substantially and remained low in adenocarcinomas with a Gleason score of 8 or higher, allowing Gleason scores of 8, 9, and 10 to be grouped together for descriptive purposes. Of 75 adenocarcinomas with a Gleason score of 6 or 7, 74 (99%) were positive for PSA, compared with 88 (58.7%) of 150 adenocarcinomas with a Gleason score of 8, 9, or 10. All 75 adenocarcinomas with a Gleason score of 6 or 7 were positive for PAP, compared with 101 (67.3%) of the adenocarcinomas with a Gleason score of 8, 9, or 10.

All 225 adenocarcinomas were nonreactive for cytokeratin 5/6, cytokeratin 17, WT1, CA-125, thyroid transcription factor-1 (TTF-1), and villin.

Discussion

The results of this study suggest that sparse cytokeratin 7 and 20 immunoreactivity should not be an unexpected finding in adenocarcinomas with intermediate Gleason scores and an expected result in adenocarcinomas with high Gleason scores. The extent of immunoreactivity usually should be as rare individual cells within otherwise nonreactive acini or sheets, but several cells within structures should not be unexpected, especially in the neoplasms with higher Gleason scores. Extensive reactivity with either antibody should not be encountered in a prostate adenocarcinoma. These results are similar to the 4 studies with similar

<table>
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<th>Table 4</th>
<th>Positive* Immunoreactions and Adenocarcinoma Gleason Score</th>
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<td>Gleason Score</td>
<td>No. of Neoplasms</td>
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<td>7</td>
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<td>10</td>
<td>38</td>
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<tr>
<td>Total</td>
<td>225</td>
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CEA, carcinoembryonic antigen; CK, cytokeratin; PAP, prostatic acid phosphatase; PSA, prostate-specific antigen.

* Reactivity in >25% of cells.
† Data are given as number (percentage).
methods. Cytokeratin 7 reactivity in more than 10% of the neoplastic cells was present in 0% to 22% of the adenocarcinomas in these studies, and cytokeratin 20 reactivity was present in 3% to 48% of the neoplasms.

The finding in the present study of increased cytokeratin 7 and 20 reactivity in adenocarcinomas with higher Gleason scores differs from the results of other studies in which the authors noted that reactivity of cytokeratins 7 and 20 were similar among adenocarcinomas of different Gleason scores. Possible reasons for this difference were the large number of neoplasms in the present study and the opportunity to detect small changes in the distribution among the Gleason scores provided by the finer, quartile grouping of stained cells. It is important to point out that the extent of increased reactivity observed in the present study was almost completely in the range of rare to fewer than 26% of the cells. All authors agree that extensive increases in cytokeratin 7 or 20 reactivity are not a feature of adenocarcinomas with higher Gleason scores.

Two adenocarcinomas with prominent large duct adenocarcinoma components had unique patterns of cytokeratin 7 and 20 immunoreactivity within each neoplasm. More than 50% of the neoplastic cells within the large ducts of the periurethral region and verumontanum were reactive for cytokeratin 20, whereas fewer than 5% of the large duct and small acini cells in the peripheral and central zones had reactivity. Extensive cytokeratin 7 and 20 immunoreactivity is a characteristic of transitional cell carcinoma. Immunophenotypic transformation of prostate adenocarcinoma toward urothelial carcinoma and of urothelial carcinoma toward prostate adenocarcinoma when in the vicinity of the nonprimary organ has been described. One study described 5 poorly differentiated carcinomas of the bladder neck with a mixed prostate and urothelial carcinoma immunophenotype. Stromal induction has been suggested as the mechanism of action. This opinion is supported by a study in which the cytoplasmic constituents and differentiation pathway of prostate epithelial cell cultures were modulated by altering the growth factors within the cell media. These 2 cases provide a provocative case for influences of microenvironment, even within the anatomic confines of the prostate.

PSA and PAP immunoreactivity decreased in adenocarcinomas with higher Gleason scores in the present study. All adenocarcinomas with a Gleason score of 6 or 7 were reactive for PSA, whereas 13% of the adenocarcinomas with a Gleason score of 10 were nonreactive for PSA. None of the adenocarcinomas in the present study had morphologic features of neuroendocrine differentiation. These results are similar to those of 2 studies in which 6 (67%) of 9 and 3 (21%) of 14 adenocarcinomas with Gleason architectural pattern 5 adenocarcinoma component had no PSA immunoreactivity.

The potential lack of PSA and PAP immunoreactivity in adenocarcinomas with high Gleason scores is well known, and previous authors provided many cautionary comments that lack of PSA and PAP immunoreactivity in a neoplasm should not exclude the possibility of prostate adenocarcinoma. The results of the present study clearly reinforce these comments. No reactivity or rare, single or occasional small clusters of PSA- or PAP-reactive cells should be considered typical of adenocarcinomas with Gleason scores of 8, 9, and 10. It should be kept in mind that the differences between rare single PAP- or PSA-reactive cells and no reactivity may be influenced substantially by the amount of tissue evaluated. Extensive reactivity with both antibodies is the overwhelmingly expected finding in adenocarcinomas with a Gleason score of 6 or 7. No PSA or PAP reactivity or the rare single reactive cells in the small acini of adenocarcinomas with a Gleason score of 6 or 7 (Gleason architectural pattern 3) would be extremely unlikely.

CEA reactivity was present in 26 (11.6%) of the adenocarcinomas studied, always in fewer than 26% of the neoplastic cells. These results are similar to those of other studies, in which monoclonal CEA antibody reactivity was present in 0% to 30% of adenocarcinomas studied. Regardless of the Gleason score, no reactivity or rare, single stained cells is the typical, expected pattern for prostate adenocarcinoma.

The present study adds supportive data to the collective experience of reported immunoreactivity with several antibodies. All 225 adenocarcinomas in the present study were nonreactive for cytokeratin 17. One study reported a high-grade prostate adenocarcinoma with scattered positive cells. All 225 neoplasms also were nonreactive for TTF-1, cytokeratin 5/6, and WT1 in the present study, results identical to the collective results of 35 TTF-1–stained prostate adenocarcinomas, 8 cytokeratin 5/6–stained neoplasms, and 10 WT1-stained neoplasms reported by other authors. The present study serves as background data when one is considering whether a neoplasm is a metastasis from a prostate adenocarcinoma with a high Gleason score. Prostate adenocarcinoma should be a diagnostic consideration if the adenocarcinoma is reactive for cytokeratin 7 or 20 in fewer than 50% of the cells, is nonreactive for PSA or PAP, or has rare CEA-reactive cells. The likelihood that a metastasis is prostate adenocarcinoma is low if cytokeratin 7, cytokeratin 20, or CEA reactivity is extensive or the metastasis has small acini (Gleason architectural pattern 3) that are nonreactive for PSA or PAP.

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