Expression of Nuclear Antigen Ki-67 in Prostate Cancer Needle Biopsy and Radical Prostatectomy Specimens

Neil R. Mucci, Mark A. Rubin, Myla S. Strawderman, James E. Montie, David C. Smith, Kenneth J. Pienta

A good biomarker is defined as any test (molecular, morphometric, etc.) that adds independent information to standard prognostic indicators (e.g., pathologic evaluation or clinical stage), that can be reliably reproduced in the laboratory setting, and that aids in choosing treatment options (1). The primary objective of this study was to determine if the proliferation marker Ki-67, a nuclear antigen and molecular marker known to be associated with the progression of cancer, had significantly different levels of expression in normal prostate tissue, carcinoma (PCA) and adenocarcinoma (HGPIN), and prostate adenocarcinoma (PCA) (2). The secondary objective was to determine the degree to which the Ki-67 biomarker expression in sextant needle biopsy specimens (i.e., sections that represent the standard sextant regions [right-apex, right-mid, right-base, left-apex, left-mid, and left-base]) accurately represents expression in wholemount radical prostatectomy (RP) specimens. If Ki-67 expression in corresponding tissue is the same in presurgical biopsy and RP specimens, then it should be possible to measure what happens to this biomarker in response to interventions in the neoadjuvant setting (i.e., treatments before surgery) and in chemoprevention trials intended for healthy men.

After Institutional Review Board approval, and acquiring written informed consent, 45 RP patients with no history of androgen-ablation therapy and for whom diagnostic sextant needle biopsy specimens (presurgical biopsy specimens) were available were prospectively selected in a consecutive manner. Following RP, sextant needle biopsies were repeated (postsurgical biopsy specimens), and the entire prostate was then cut into 3-mm-thick slices along the basal–apical axis. Tissues were fixed, embedded in paraffin, and sectioned at 4 μm in thickness. The study pathologist (M. A. Rubin) identified and graded (3) all foci of HGPIN and PCA in all specimens. The specimens then underwent immunohistochemical staining for Ki-67 nuclear antigen (2). Digital analysis for quantification of the proliferation index (i.e., the percent of cells undergoing division) was performed by use of a Cell Analysis System 200 workstation with the dedicated Qualitative Proliferation Index application (Bacus Laboratory, Elmhurst, IL) (4). In the case of multiple HGPIN or PCA lesions in a single specimen, multiple measurements were collected from all foci. Measurements were made along the full length of needle biopsy specimens. Measurements of Ki-67 expression were centered on normal tissue, HGPIN, or PCA, and the proportion of basal cells, stromal cells, and other nonepithelial cells present in the microscopic fields was minimized.

Data were analyzed by use of a generalized mixed model (SAS Software, v.6.12 Glimmix Macro; SAS Institute, Inc., Cary, NC) to allow for comparison of the proliferation indices of tissue biopsy and RP specimens (5). The results were analyzed by Poisson regression, since they did not follow a normal distribution and no transformation of the data was successful at approximating a normal distribution (6). A large number of samples had a low degree of expression, which resulted in a highly skewed distribution. Data from presurgical and postsurgical needle biopsy specimens were combined. Initially, the data from the RP specimens and the data from biopsy samples were fit to separate models. Differences between tissue types were assessed by use of the Student’s two-sided t test, with standard errors properly adjusted for the multiple measurements. Finally, all data were analyzed together in one model so that the effect of the source of tissue (biopsy versus RP) on the degree of Ki-67 expression could be evaluated.

The histopathologic characteristics of the RP specimens are reported in Table 1 and are representative of the current types of prostate cancers seen at our institution. The biopsy samples of 45 patients were analyzed for expression of Ki-67 nuclear antigen. Thirty-eight of 45 patients had matching postsurgical biopsy and RP histology specimens available for comparative analysis. Sample dropout was due to inadequate staining of available material. The average proliferation indices as determined by Ki-67 staining for each tissue type were estimated from the model and are presented in Table 2. In both the RP and biopsy specimens, statistically significant increases in the level of expression of Ki-67 were seen from normal tissue to HGPIN to PCA. In the RP specimens, PCA tissue had a higher proliferation index than did HGPIN (P < .001), and HGPIN had a higher expression of Ki-67 than did normal tissue (P < .001).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean largest tumor diameter, cm</th>
<th>Mean gland weight, g</th>
<th>Multifocal tumor, %</th>
<th>Focal</th>
<th>Extensive</th>
<th>Surgical margin status, %</th>
<th>Seminal vesicle invasion, %</th>
<th>Mean Gleason score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.2</td>
<td>52.0</td>
<td>25.6</td>
<td>7.7</td>
<td>7.7</td>
<td>84.6</td>
<td>97.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Postsurgical pathology results for study case subjects*</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>*Number of subjects = 45.</td>
</tr>
</tbody>
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In the biopsy specimens, PCA tissue also had a higher expression of Ki-67 than did HGPIN (P<.001), and HGPIN had a higher expression of Ki-67 than did normal tissue (P<.001).

In the larger model that combined biopsy and RP data, no statistically significant effect or interaction between tissue type and source was detected, and no discernible difference in expression of Ki-67 was seen between the RP specimens and the biopsy samples. The variation in the levels of expression of Ki-67 in different tissue specimens and tissue types deserves further careful analysis.

Expression of Ki-67 was clearly different in normal, HGPIN, and PCA biopsy specimens. Multiple studies (7–14) have demonstrated that mean proliferation indices, as measured by Ki-67 staining, are associated with disease progression, stage, Gleason score, and mean pretreatment prostate-specific antigen level and show an increase from benign to malignant tissue. These investigations support our finding that the proliferation index may serve as a biomarker in future studies that compare biopsy specimens over time, biopsy and RP specimens (i.e., neoadjuvant studies), or multiple biopsy specimens in the setting of chemoprevention.

REFERENCES


NOTES

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