Identify of Numerical Chromosomal Changes Detected by Interphase Fluorescence In Situ Hybridization in High-Grade Prostate Intraepithelial Neoplasia as a Predictor of Carcinoma

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Context.—High-grade prostate intraepithelial neoplasia (HPIN) is the most likely precursor of prostate cancer. The condition of many patients with a diagnosis of HPIN in prostate needle core biopsy could, if left untreated, progress to invasive cancer. Currently there is no available clinical, immunohistochemical, or morphologic criteria that are predictive of this progression.

Objective.—To determine whether chromosomal instability in these precursor lesions could increase their predictive value for cancer detection.

Design.—Dual-color interphase fluorescence in situ hybridization analysis was performed on archived prostate needle core biopsies from 54 patients with initial diagnosis of isolated HPIN and follow-up of 3 years or more. We used commercially available centromere probes for chromosomes 4, 7, 8, and 10. We had interpretable results in 44 patients as follows: (1) group A: 24 HPIN patients with persistent HPIN and/or benign lesions in the follow-up biopsies, and (2) group B: 20 HPIN patients with progression to prostate carcinoma.

Results.—Twenty-five percent of the patients in group B displayed numeric chromosomal aberrations. Only 8.3% of the patients from group A had chromosomal abnormalities (P = .1). The observed overall chromosomal changes in HPIN were higher than those in normal or hyperplastic epithelium, with a statistically significant difference (P < .05). All aberrations were detected in the form of chromosomal gain. Overall, the commonest aberration was gain of chromosome 8, followed by gains of chromosomes 7 and 10.

Conclusion.—These results indicated that although no single numeric chromosomal abnormality could be assigned as a predictor of HPIN progression to carcinoma, the overall level of numeric chromosomal abnormalities shows a trend of elevation in HPIN patients whose condition subsequently progressed to carcinoma.

Arch Pathol Lab Med. 2002;126:165±169

Prostatic intraepithelial neoplasia (PIN) is characterized by intraluminal proliferation of epithelial cells and can be divided into high-grade (HPIN) and low-grade lesions. HPIN is the earliest accepted stage in prostatic carcinogenesis. HPIN is regarded as the most likely precursor for prostate carcinoma.1 The greatest value of PIN is its high predictive value as a marker for prostate carcinoma. This is particularly true for HPIN. If this lesion is identified, close surveillance and follow-up biopsy are indicated.2 In many patients, HPIN is diagnosed by a prostate needle core biopsy, and if left untreated, such patients’ conditions could progress to invasive carcinoma. HPIN, when diagnosed in a needle biopsy, is a powerful predictor of carcinoma in subsequent needle biopsies. No clinical or pathologic parameter has been found to be helpful for distinguishing patients who have carcinoma on the next biopsy from those who do not.

The term chromosomal instability refers to a high frequency of chromosomal loss and gain. It is a type of genomic instability that has been introduced recently to the field of human cancer biology.3,4 Recently chromosomal instability has been described in many human dysplastic lesions, and it has been proposed as a marker of progression to cancer and considered as a primary event in neoplastic transformation.5-15 Whether chromosomal instability in HPIN can provide additional predictive information for early cancer progression is still unknown. The objective of this project was to examine if chromosomal instability can increase the predictive value of HPIN diagnosis by using interphase fluorescence in situ hybridization (FISH) applied on prostate biopsies with a diagnosis of HPIN only.

MATERIALS AND METHODS

Patient Accrual

Between 1995 and 1997 in the record of The University Health Network, we had identified 123 patients with HPIN as the primary diagnosis in prostate needle core biopsies, and we compared them with 100 patients with HPIN associated with invasive cancer simultaneously. HPIN associated with cancer was designated as group 1, and isolated HPIN was designated as group 2. Group 2 was subdivided as follows:

Accepted for publication September 28, 2001.

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Arch Pathol Lab Med—Vol 126, February 2002
Prostate cancer. Paraffin pretreatment and FISH procedure previously been shown to be associated with numeric change in aneuploidy. The appropriate section was chosen. Directly labeled VYSIS® FISH was performed on 5-μm unstained tissue sections using adjacent hematoxylin-eosin-stained sections as guidance. Interphase FISH was performed on 5-μm unstained tissue sections using adjacent hematoxylin-eosin-stained sections as guidance. The appropriate section was chosen. Directly labeled VYSIS CEP probes (Vysis Inc., Downers Grove, Ill) for chromosomes 4, 7, 8, and 10 were used, because chromosomes 7, 8, and 10 have previously been shown to be associated with numeric change in prostate cancer.16-20 Paraffin pretreatment and FISH procedure were performed according to the manufacturer’s instructions. Dual-probe hybridization was performed. For each probe 100 nuclei were counted by each observer. Ten of the cases could not be interpreted either because of poor hybridization, fluorescence background, or the decrease in size and subsequent disappearance of the HPIN foci on the deeper sections. In 10 cases at least one observer was able to count only 75 to 99 nuclei.

Materials and Methods

All the hematoxylin-eosin slides of the cases diagnosed as HPIN were reviewed by 2 pathologists to confirm the diagnosis and to determine the adequacy of the specimen for FISH analysis. Only those with sufficient material were included in the study. Interphase FISH was performed on 5-μm unstained tissue sections using adjacent hematoxylin-eosin-stained sections as guidance. The appropriate section was chosen. Directly labeled VYSIS CEP probes (Vysis Inc., Downers Grove, Ill) for chromosomes 4, 7, 8, and 10 were used, because chromosomes 7, 8, and 10 have previously been shown to be associated with numeric change in prostate cancer.16-20 Paraffin pretreatment and FISH procedure were performed according to the manufacturer’s instructions. Dual-probe hybridization was performed. For each probe 100 nuclei were counted by each observer. Ten of the cases could not be interpreted either because of poor hybridization, fluorescence background, or the decrease in size and subsequent disappearance of the HPIN foci on the deeper sections. In 10 cases at least one observer was able to count only 75 to 99 nuclei.

Criteria of Scoring and Evaluation of Numeric Chromosomal Anomalies

In preliminary experiments the hybridization efficiency of every probe was tested on prostate tissues.Slides were evaluated according to the accepted criteria.16 Briefly, only sections with hybridization in at least 80% of cells were evaluated. For each probe 2 independent investigators counted the number of FISH signals in 100 nonoverlapped intact (spherical) interphase nuclei from foci of HPIN. The number of signals per nucleus was scored as 0, 1, 2, 3, 4, and more than 4 signals per nucleus. Nuclei from stromal element were not enumerated. FISH using a centromere probe for chromosome 4 was used as a negative control, because this chromosome is not commonly seen as aneuploid in prostate carcinoma or prostatic tissues. Normal and hyperplastic glandular epithelium present in the biopsies was counted as an internal control. Because of truncation of the nuclei, artificial loss of signals is expected; however, we applied very conservative criteria to detect any significant true numeric changes. For the control cases using the chromosome 8 centromere probe, the mean + 3 SD percentage of nuclei with 3 or more signals was 4.6%, and the mean + 3 SD percentage of nuclei with 0 or 1 signals was 44.5%. Our criteria to evaluate numeric chromosomal abnormality were as follows:

1. Chromosomal gains were diagnosed when more than 4.6% of the nuclei exhibited more than 2 signals.
2. Chromosomal loss was diagnosed when more than 44.6% of the nuclei exhibited a reduction of signal number.
3. Tetraploidy was assumed when all chromosomes investigated showed signal gains up to 4. These cutoff values were in keeping with those published elsewhere.17-20

Statistical Analysis

The Fisher exact test was used to examine the difference between group A and group B regarding the presence of numeric chromosomal changes. The same test was used to analyze the difference between group 1 and group 2 as well as between group A and group B regarding PSA level, DRE, and ultrasound examination. Student t test was used to compare the age between the different groups.

RESULTS

Clinicopathologic Findings

The mean ages were 67 years and 64 years for groups 1 and 2, respectively (P < .05). The PSA levels were 9.9 ng/mL and 8 ng/mL for groups 1 and 2, respectively (P < .05). In group 1, the ultrasound and DRE examinations were positive in 50% and 44%, respectively, and in group 2, ultrasound and DRE were positive in 30% and 23%, respectively, which was statistically significant (P < .05 for DRE and P < .01 for ultrasound). The patients with isolated HPIN as the primary diagnosis (n = 123) had at least 1 follow-up biopsy. Prostate carcinoma was identified in 33 cases (27%) in the follow-up biopsies, with range of follow-up time between 2 and 36 months. The carcinoma was identified in the same side as the HPIN in 55% of the cases; in 33% of cases it was found in both sides; and in 12% of cases it was found in the opposite side. Gleason scores were predominantly 6 and 7. When we divided the patients into group A (did not progress to cancer) and group B (progressed to cancer), we found that the mean ages for groups A and B were 64 years and 62 years (P = .2) and that the mean PSA values were 8 ng/mL and 7.8 ng/mL (P = .4), respectively. Ultrasound was positive in 11 of 33 and in 18 of 90 in groups A and B, respectively (P = .1). Digital rectal examination was positive in 11 of 33 and in 26 of 90 in groups A and B, respectively (P = .5). Pathologic review showed similarity between the two groups, with the presence of cribriform, tufting, micropapillary, and flat microscopic subtypes (Figure 1, A through D) present in 7%, 55%, 50%, and 11%, respectively, in group A and 6%, 60%, 55%, and 12%, respectively, in group B. The most common morphologic HPIN subtypes were the tufting and the micropapillary patterns. No statistically significant difference was observed between group A (no subsequent carcinoma) and group B (developed carcinoma) in any of the clinical parameters examined including age, PSA level, DRE, rectal ultrasound, or morphologic subtypes.

Interphase FISH Findings

We had interpretable results in 44 cases (24 from group A and 20 from group B) for chromosomes 4, 7, 8, and 10. Our findings indicated the presence of different chromosomal anomalies in 5 of 20 (25%) cases of group B and in 2 of 24 (8.3%) cases of group A (P = .1). The results from the patient samples with chromosomal anomalies are shown in Table 2. All the chromosomal changes were in the form of a gain (Figures 2 and 3). No chromosomal losses were identified in any case. Gain of chromosome 8 was seen in 5 cases, gain of chromosome 7 in 3 cases, and gain of chromosome 10 in 2 cases. No numeric chromosomal changes were detected in chromosome 4. No nu-

Table 1. Age and Prostate-Specific Antigen (PSA) Levels in Patients Used for Fluorescence In Situ Hybridization Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age, y</th>
<th>Mean PSA, ng/mL</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>65.1</td>
<td>8.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>B</td>
<td>62.0</td>
<td>7.9</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Chromosomal Changes to Predict Carcinoma—Al-Maghrabi et al
meric chromosomal anomalies were noticed in the adjacent hyperplastic or normal prostate glandular epithelium, which was counted in 30 cases in the study group. We applied the same cytogenetic technique on 5-μm paraffin-embedded sections from transurethral resection of prostate (TURP) specimens from patients with nodular hyperplasia (benign prostatic hyperplasia) using probe for chromosome 8 and found that there were no chromosomal numeric anomalies in any of the 14 specimens that were examined. We found that a total of 7 of 44 (16%) cases of HPIN showed chromosomal anomalies in at least 1 chromosome. At the time of the diagnosis, 3 of the 7 patients were below the age 60 years (all of them had a cancer in the follow-up biopsies); only 2 of those 7 patients had abnormal ultrasound results, and none of them had abnormal DRE results. Six of 7 had PSA levels above 4 ng/mL.

**COMMENT**

The rationale for this study was to determine whether chromosomal aneusomies detected in HPIN foci could be predictive of subsequent carcinoma or could increase the predictive value of HPIN.

In our study, we identified 123 patients with isolated HPIN as the primary diagnosis who had a subsequent follow-up biopsy. Prostate carcinoma was identified in 27% at the first follow-up, with a follow-up period ranging between 2 and 36 months. That figure is consistent with the incidence described in the literature (27% to 100%). Davidson et al. found that the likelihood of finding cancer was greater in patients with PIN undergoing more than 1 follow-up biopsy (44%) than in those with only 1 biopsy (32%). The carcinoma was identified in the same side as the HPIN in 55% of the cases, in both sides in 33% of cases, and in the opposite side in 12%. Gleason scores were predominantly 6 and 7. The mean ages were 64 years and 62 years and the mean PSA values were 8 ng/mL and 7.8 ng/mL, respectively, for the group that did not develop prostate carcinoma (group A) and for the group that did (group B). No statistically significant difference was identified between these 2 groups in any of the clinical parameters examined including age, PSA level, DRE, or rectal ultrasound, which is consistent with other authors. However, other investigators have indicated that age, PSA positivity, and DRE may be factors that increase the risk of subsequent cancer, suggesting that HPIN may not always be detectable by elevated serum PSA or by transrectal ultrasound or DRE.

The underlying mechanism of progression of PIN to prostate carcinoma is not clear. However, the progression might be caused by the onset of genomic instability, resulting in a clone that has the ability to invade. Chromosomal instability may be defined as an excess of chromosomal activity that is detected in HPIN foci.
Chromosomal instability is thought to arise as a result of aberrations in mitotic machinery of chromosome constitution, leading to excessive numeric chromosomal changes. Chromosomal instability is usually manifested in a form of numeric chromosomal changes of 1 or more chromosomes. In this study we found the presence of chromosomal aneusomies in 5 of 20 (25%) cases of men with HPIN who subsequently developed prostate carcinoma compared with 2 of 24 (8.3%) cases of the men with HPIN who did not develop prostate carcinoma ($P = .1$). This difference was not statistically significant; however, it is highly suggestive that patients with HPIN with chromosomal aneusomies have a higher chance of progression to invasive cancer. No numeric chromosomal anomalies have been noticed in the adjacent hyperplastic or normal prostate glandular epithelium present in the same biopsies.

The overall chromosomal changes in HPIN were higher than those in the normal or hyperplastic epithelium, with statistically significant difference ($P < .05$).

The advantage of interphase FISH when applied to histologic sections is that the tumor cells can be precisely evaluated, and normal and dysplastic foci can be evaluated for chromosomal instability even in small biopsies. FISH has been used in PIN to determine chromosomal anomalies in a few studies. However, in those studies it was performed on prostatectomy specimens, which already contain prostate carcinoma foci. Using centromere FISH probes, the most common numeric changes in PIN and prostate carcinoma include gain of chromosome 7, 8, and 10 and loss of chromosome Y. The overall frequency of numeric chromosomal anomalies in PIN and in prostate carcinoma is remarkably similar, which is in keeping with the hypothesis that PIN is a precursor of carcinoma. The
overall incidence of any numeric chromosomal anomaly was 7 of 44 (16%) in our study. The presence of any anomaly in PIN ranged in the literature between 12% and 62%. Our relatively lower incidence of chromosomal changes in HPIN foci can be explained by the smaller foci of tissue in biopsies, which might not be representative of the whole HPIN foci. In our study all the chromosomal changes were in the form of a gain with no chromosomal losses identified in any case. Gain of chromosome 8 was the most common finding in our study, which is also consistent with the previous studies. Gain of chromosome 7 has been shown to be frequent in prostate carcinoma and is associated with higher tumor grade, advanced stage, and early patient death of prostate carcinoma. However, this change could occur in an early stage of prostate carcinoma tumorogenesis.

Heterogeneity and multifocality are noteworthy features of prostate carcinoma and the associated prostatic tissues, which make it relatively difficult to study using traditional bulk nucleic acid extraction methods. It will be important for future studies addressing genetic differences in lesions such as HPIN and microfoci of prostate carcinoma to use laser capture methodologies to provide a more accurate molecular assessment of genetic change in prostate carcinoma and HPIN.

We thank Dr. Waleed A. Milaat from the Department of Community Medicine and Primary Health Care, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia. We also thank our lab members: Khiadoum Alromaia, Ben Beheshti, Zong Zhang, Bisera Vukovic, Paula Marrano, Jana Karaskova, Paul Park, Elena Kolometz, and Jane Bayani for their contribution to this work. Financial support for this project is funded by the US Army Medical Research and Material Command Prostate Cancer Research Program and the National Cancer Institute of Canada, with funds from the Canadian Cancer Society.

References