DNA Ploidy by Image Analysis of Individual Foci of Prostate Cancer: A Preliminary Report


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ABSTRACT

The malignant potential of an individual focus of prostate cancer is difficult to determine. The established pathological features associated with malignant behavior include tumor volume, grade, and invasiveness (local extension or metastasis).

We used nuclear image analysis to determine the DNA ploidy value of each cancer in a series of 30 radical prostatectomy specimens from patients with early stage prostate cancer in order to further explore the malignant potential of each separate focus of cancer. The volume, grade, invasiveness (extracapsular extension or seminal vesicle invasion), and zone of origin of each of the 63 separate cancers were determined. The DNA ploidy histogram of 200 cancer cells was compared with 50 normal epithelial nuclei on the same Feulgen-stained tissue sections. Sixty % of the cancers were diploid, and 40% were nondiploid. Ploidy correlated with volume and grade. All cancers <0.02 cm³ were diploid; 26% of foci 0.02 to 2.0 cm³ and 82% of foci >2.0 cm³ were nondiploid. There were 16 cancers of transition zone origin ranging in size from 0.02 to 12.1 cm³ and only one (7.3 cm³) was nondiploid. There were 47 cancers of peripheral zone origin (range, 0.01 to 18.98) and 24 (51%) were nondiploid. Eight of the 24 nondiploid cancers were small (<1.0 cm³), and two were only 0.03 cm³.

We conclude that some very small prostate cancers are nondiploid and that progression of prostate cancer is not a function of volume alone, whereby tumors only acquire full malignant potential at large volumes. Cancers of peripheral zone origin acquire a nondiploid cell population at a smaller volume than do cancers of transition zone origin, further supporting a fundamental difference between cancers arising in these zones.

INTRODUCTION

One of the difficult problems in the management of patients with prostate cancer is the dearth of objective information about the natural history and malignant potential of small foci of prostate cancer. It is well established that the prevalence of prostate cancer at autopsy far exceeds that of the clinically observed cancer (1–7). The prevalence at autopsy increases with age and by the eighth decade ranges from 40% to 66% (5, 8). We have estimated that the lifetime risk of developing histologically recognizable cancer in the prostate is 42% for a 50-year-old American man (8), while the lifetime risk of being diagnosed with clinical prostate cancer is 9.5% and of dying of prostate cancer is 2.9% (9). Because of the discrepancy between autopsy and clinical patterns, it has been proposed that two different types of prostate cancer exist: a latent type (well-differentiated and focal) found in autopsy specimens and in tissue removed by TURP for relief of obstructive urinary symptoms and a more aggressive, clinically detectable type situated mainly in the posterior part of the gland (5, 10, 11).

The development of the concept of the zonal anatomy of the prostate by McNeal (12, 13) allows the assignment of a zone of origin to individual prostate cancer foci. Cancer foci detected incidentally in tissue removed by TURP (Stage A) are predominantly of TZ origin, while clinically palpable (Stage B) cancers are predominantly of PZ origin (14). McNeal identified 68% of small prostate cancers as originating in the PZ, 24% in the TZ, and 8% in the CZ (14). In a subsequent study of unsuspected prostate cancers in cystoprostatectomy specimens, McNeal’s group noted that the principal feature distinguishing these cancers from the majority of clinically diagnosed cancers was their small volume. These unsuspected or incidental cancers were considered biological precursors of clinical tumors (15). McNeal also reported a strong correlation between tumor volume and loss of differentiation and rarely found evidence of invasiveness or metastasis in cancers smaller than 1 cm³ (16). From these observations, it follows that small incidental cancers, clinically detected cancers, and metastatic cancers can be considered a continuum. All cancers, once initiated, are committed to a predictable development in which malignant potential is volume dependent. Thus, the largest cancer is denoted as the “index” cancer, and smaller foci in the same gland are “incidental” in importance (16).

The concept of a multistep process in tumorogenesis was proposed by Foulds (17) and is supported by recent molecular evidence (18, 19). Although the specific molecular events in prostate carcinogenesis have not yet been identified, the epidemiological features of latent and clinical prostate cancer support a multistep process (20). The uniformly high prevalence rate of latent cancer worldwide contrasts markedly with the widely varying clinical incidence and mortality rate from prostate cancer in different countries (7, 20–22). Thus, the genetic events leading to a histologically recognizable but latent prostate cancer are relatively common, but the further steps necessary for progression to a clinical cancer with full malignant potential are uncommon and not inevitable (20). Tumor growth alone is not sufficient to explain progression from latent to clinical cancer. Progression may result in the evolution of different populations of cells within the tumor (heterogeneity), including the emergence of a nondiploid cell population or other markers of malignancy.

Both flow cytometry (23–27) and image analysis (28) have established the importance of DNA ploidy in assessing the biological activity of prostate cancer and the prognostic difference between diploid and nondiploid cancers. In this study we used nuclear image analysis to determine the DNA ploidy of each separate cancer present in radical prostatectomy specimens and compared these findings with the volume, grade, invasiveness (extracapsular extension and seminal vesicle invasion), and zone of origin of each focus. Our aims were to determine whether all small cancers were well differentiated and diploid, or whether some of these small cancers had already
acquired a nondiploid cell population. We were also interested in comparing the ploidy status of cancers in the transition and peripheral zones, as we had previously noted marked differences in the pathological features of cancers in these zones (29, 30).

MATERIALS AND METHODS

Patient Population. Among 176 consecutive patients treated with radical prostatectomy for localized prostate cancer from 1983 to 1988, there were 96 who met all the following criteria for detailed morphometric analysis of the radical prostatectomy specimen, as previously described (30). Each was clinical Stage A or B, with no evidence of metastases by bone scan, a normal serum enzymatic prostatic acid phosphatase level, and normal pelvic lymph nodes by frozen section examination. The prostatectomy specimen had been serially sectioned, and new whole mount sections could be obtained for Feulgen staining for DNA ploidy analysis. This report concerns the results of ploidy analysis in the first 30 randomly selected cases from this series of 96.

Clinical stage was assigned prospectively by modification of the Whitmore-Jewett (or American) staging system (31). Clinical Stage A (12 patients) indicates an incidental cancer found at the time of TURP for relief of bladder outlet obstruction due to presumed benign prostatic hyperplasia. Substage A1 (n = 3) includes those with cancer of low or moderate grade (Gleason sum ≤7) in ≤5% of the TURP specimen; Stage A2 (n = 9) includes those with cancer in >5% or high grade (Gleason sum ≥8). Stage B (18 patients) includes those with a palpable cancer confined to the prostate, either B1 (one lobe) or B2 (more than one lobe). The patients ranged in age from 48 to 73 (63 ± 6.5 (SD)) yr.

Morphometric Observations. The radical prostatectomy specimens were serially sectioned in the transverse plane at 5-mm intervals from the apex of the gland (Section P1) through to the tip of the seminal vesicles as previously described in detail (32). Whole organ tissue sections were mounted on giant glass slides and stained with hematoxylin and eosin. Each separate cancer was identified, and its location, volume, and grade were recorded (29). The Gleason grading system was used as recommended by the National Cancer Institute Organ Site Program (33). Primary and secondary grades 1 to 5 were assigned to each cancer, and these were added together to yield a sum (total, 2 to 10). Extension of cancer through the capsule or into the seminal vesicles was recorded. Each cancer was assigned to the transition zone or "peripheral zone," the latter indicating a cancer of either central zone or peripheral zone origin ("nontransition zone"), since cancers in these zones are difficult to distinguish (14, 30). The volume of the prostate gland and of each separate cancer was calculated from tumor diagrams (29, 30, 32). For Stage A patients the volume of cancer present in the TURP specimen was also included in the calculations.

DNA PLOIDY ANALYSIS. The blocks were recut at the original levels, and two 4-μm sections were obtained from each block. One of the 4-μm sections was stained with hematoxylin and eosin to confirm the site of the original cancer, and the second 4-μm section was stained for DNA ploidy analysis. Each separate focus of cancer was marked in permanent ink on the Feulgen-stained slide by one pathologist. The number of cancer foci available for ploidy analysis was less than the original cancer, and the second 4-μm section was stained for DNA ploidy analysis. Each separate focus of cancer was marked in permanent ink. Each separate cancer was identified, and its location, volume, and grade were recorded (29).

Clinical Stage and Zone of Origin. The distribution of cancer by zone of origin and ploidy value within each clinical stage is shown in Table 1, and an example of a map of a step section is shown in Fig. 1A. All 8 cancers in the 3 Stage A1 patients were diploid regardless of zone of origin (Fig. 1B). In Stage A2 (Fig. 1C), one of 6 TZ cancers and 2 of 12 PZ cancers were nondiploid; thus, 3 of 9 Stage A2 prostatectomy specimens contained a nondiploid cancer. In Stage B the TZ cancers present were generally small, well differentiated, and uniformly diploid. However, PZ cancers in Stage B were predominantly (22 of 30) nondiploid (Fig. 1D and E). Overall, all 12 Stage B1 and 4 of 6 Stage B2 patients had one or more nondiploid cancers in the removed prostate.

Grade. Table 2 shows the ploidy results for tumors within each grade (Gleason sum). All 6 cancers Gleason sum 4 or less

### RESULTS

Sixty-three separate cancers in 30 patients were available for DNA ploidy analysis (mean, 2.1 foci per prostate; range 1 to 5). Sixteen (25%) of these cancers were of TZ origin, and 47 (75%) were of PZ origin. This ratio was consistent through the range of tumor volumes studied. The mean tumor volume was 1.94 cm³ (range, 0.01 to 18.94 cm³). Of the 63 separate cancers, 38 (60%) were diploid, and 25 (40%) were nondiploid. The mean DNA index of diploid tumors was 0.94 (range, 0.8 to 1.12) with a standard deviation of 0.09. The coefficient of variation of diploid peaks was 12.6 ± 3.5% (mean ± SD) and of nondiploid peaks was 11.8 ± 3.5%. The coefficient of variation of control nuclei, taken on the same section as tumor tissue sections, DNA analysis was also performed on 50 nuclei within areas of normal epithelium on the same section. Results were calculated as the DNA index, whereby the mean peak value of the tumor nuclei is divided by the mean of the internal control nuclei. The diploid range was defined as a peak value of DNA index 0.8 to 1.2 (2 N peak). A tetraploid peak ranged from 1.8 to 2.2 (4 N peak) (35). Tumors were classified as diploid if the main peak was in the range of 0.8 to 1.2 and tetraploid if the main peak was in the range of 1.8 to 2.2. All patients were diploid regardless of zone of origin (Fig. 1B). In Stage B the TZ cancers present were generally small, well differentiated, and uniformly diploid. However, PZ cancers in Stage B were predominantly (22 of 30) nondiploid (Fig. 1D and E). Overall, all 12 Stage B1 and 4 of 6 Stage B2 patients had one or more nondiploid cancers in the removed prostate.

Grade. Table 2 shows the ploidy results for tumors within each grade (Gleason sum). All 6 cancers Gleason sum 4 or less

### Table 1 Ploidy value (diploid or nondiploid) of individual cancers in each zone (TZ or PZ) listed by stage

<table>
<thead>
<tr>
<th>Clinical stage</th>
<th>TZ</th>
<th>PZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cancers</td>
<td>Diploid</td>
</tr>
<tr>
<td>A1 (3)*</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>A2 (9)</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>B1 (12)</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>B2 (6)</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Total (30)</td>
<td>63</td>
<td>15</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of patients.

\[4085\]

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DNA PLOIDY BY IMAGE ANALYSIS OF INDIVIDUAL FOCI OF PROSTATE CANCER

Fig. 1. A, Diagrammatic key to tumor maps; tumor maps of step-sectioned radical prostatectomy specimens illustrating the location, zone of origin, and grade of each separate cancer (color coded). Transverse sections at 5-mm intervals are labeled sequentially (P2, P3, etc.) from apex to seminal vesicles. B, In this and diagrams C–E, PZ, cancer of peripheral zone origin; TZ, transition zone origin; PIN, prostatic intraepithelial neoplasia. Numbers refer to the Gleason primary and secondary grade. DNA ploidy histograms are shown for selected cancers and for controls (areas of normal epithelium on the same section as the sampled cancer). B, Stage A1 (see text for definitions of stages). A TZ cancer (green) and control area of normal epithelium were analyzed on Section P2, and a PZ cancer (red) and control on P6. Both cancers were diploid (DNA index = 0.9). C, Stage A2. The TZ cancer (green) and a PZ cancer on Section P6 were both diploid (DNA index = 1.0). On Section P8, one PZ cancer (brown) was diploid, while a separate PZ cancer (red) was aneuploid (DNA index = 1.6). The area of prostatic tissue anterior to the TURP defect was lost in processing (shaded area). D, Stage B1. The index tumor (red), a grade 3 + 4 PZ cancer, extended through the prostatic capsule. Section P5 was analyzed, and the tumor was aneuploid (DNA index = 1.5) with a small corresponding G2-M peak. A second PZ cancer (yellow) on the opposite side (P7) was diploid (DNA index = 0.87). E, Stage B2. A large Grade 2 or 3 TZ cancer (green) on Section P6 was diploid (DNA index = 1.1), while a smaller but higher grade PZ cancer (red) on Section P5 was aneuploid (DNA index = 1.7) and extended through the prostatic capsule.
were diploid, and the majority (67%) of Gleason sum 5 to 6 were diploid. However, 73% of the cancers with a Gleason sum of 7 were nondiploid, and a Gleason grade of 4, either as the primary or secondary pattern, frequently (11 of 15) indicated the presence of a nondiploid cell population. The overall correlation between ploidy and grade was modest ($r = 0.43$) but statistically significant ($P = 0.02$).

Tumor Volume. Regardless of zone of origin, Table 3 shows that all cancers less than 0.02 cm$^3$ were diploid. While the majority of cancers between 0.02 and 2.0 cm$^3$ were diploid, 29% were nondiploid. Above 2 cm$^3$, 82% were nondiploid. There was a significant difference between the volume of diploid and nondiploid cancers ($P < 0.02$, unpaired $t$ test). When a diploid tumor and a nondiploid tumor were present in the same prostate (12 patients), the nondiploid tumor was larger in 8 patients (66%). The clinically detected cancer (TURP or digital rectal examination) or "index cancer" was diploid in 13 and nondiploid in 17 patients.

The median and range of volumes of the TZ cancers (0.15 cm$^3$; range, 0.01 to 12.1) and the PZ cancers (0.6 cm$^3$; range, 0.01 to 18.98) were similar ($P = 0.57$, unpaired $t$ test). The median volume of diploid TZ cancers was 0.15 cm$^3$ (range, 0.01 to 12.1), and there was only one nondiploid TZ cancer which was very large (7.3 cm$^3$). Diploid PZ cancers (median, 0.14 cm$^3$; range, 0.01 to 18.98) were similar in volume to diploid TZ cancers, but smaller than nondiploid PZ cancers (median, 2.35 cm$^3$; range, 0.03 to 11.06). While all small TZ cancers were diploid, 8 (29%) of PZ cancers less than 1 cm$^3$ were nondiploid (two of these were 0.03 cm$^3$). Despite their small size, these were moderately differentiated (4 were Gleason sum 5 and 4 Gleason sum 6), and 3 (38%) had complete extracapsular extension. Fig. 2 shows the log of tumor volume for diploid and nondiploid cancers within each zone. The different pattern of ploidy/volume relations is apparent in the TZ when compared with the PZ.

Extracapsular Extension and Seminal Vesicle Invasion. ECE of tumor arose from 19 separate cancers in 17 patients (3 Stage A and 13 Stage B). The zonal origin and DNA ploidy status of cancers grouped by extent of invasion are shown in Table 4. Of 16 TZ cancers, only 2 (13%) extended outside the prostate gland itself compared with 23 (43%) of 53 PZ cancers. Of 38 diploid cancers, 34 (89%) were confined to the prostate. Of 31 nondiploid cancers, 10 (68%) invaded outside the prostate. The difference between diploid and nondiploid cancers in the frequency of ECE was highly significant ($P = 0.0001$, unpaired $t$ test). Only 4 diploid cancers completely penetrated the capsule, while the other 15 cancers with ECE were nondiploid.

Seminal vesicle invasion was present in 6 patients, each of whom also had ECE. In all cases the SVI originated from a nondiploid peripheral zone cancer (Table 4).

**DISCUSSION**

There are few objective markers of the malignant potential of an individual focus of prostate cancer. The features that have an established correlation with the frequency of metastases and with prognosis include the volume and grade of the tumor and the presence of extracapsular extension, seminal vesicle invasion, and lymph node metastases (16, 31, 38). However, volume, extracapsular extension, and seminal vesicle invasion are difficult to determine clinically, and grade does not adequately predict risk of progression because of clustering in the moderately differentiated range. As efforts to detect early prostate cancer increase, the problem of characterization of individual cancer foci in TURP specimens or in transrectal biopsy specimens will become more important.

In this study we used tissue sections for DNA ploidy analysis because we wanted to examine the pattern of ploidy in multiple tumors within each prostate. We also wanted to compare ploidy results with the other pathological features of prostate cancer. There are some difficulties with ploidy analysis of tissue sections. The most important consideration is section thickness,

<table>
<thead>
<tr>
<th>Grade (Gleason sum)</th>
<th>Diploid</th>
<th>Non-Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%</td>
</tr>
<tr>
<td>3-4</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>5-6</td>
<td>28</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 2 Correlation of grade with ploidy status ($r = 0.43$, $P = 0.02$)

The lowest grade was Gleason sum 3 and the highest was 7.

<table>
<thead>
<tr>
<th>Volume (cm$^3$)</th>
<th>Diploid</th>
<th>Non-Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%</td>
</tr>
<tr>
<td>$&lt;0.02$</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>0.02-2.0</td>
<td>27</td>
<td>71</td>
</tr>
<tr>
<td>$&gt;2.0$</td>
<td>3</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3 Tumor volume in diploid and nondiploid cancers

There was a statistically significant difference in tumor volume between diploid and nondiploid cancers ($P < 0.02$, unpaired $t$ test).

<table>
<thead>
<tr>
<th>Extent of invasion</th>
<th>No. of cancers</th>
<th>Diploid</th>
<th>Non-Diploid</th>
<th>PZ Diploid</th>
<th>Non-Diploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confined to prostate</td>
<td>44</td>
<td>14</td>
<td>32</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>Extracapsular extension</td>
<td>19</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 4 Number of separate cancer foci with complete ECE and seminal vesicle invasion for diploid and nondiploid cancers by zone of origin
and since the prostate nucleus measures 4 to 6 \( \mu m \) (39), we chose 4-\( \mu m \) sections to minimize nuclear overlap present in thicker sections. Previous studies have shown ploidy results from 4-\( \mu m \) sections and from smeared of prostate cancer cells to correlate reasonably well (37). Nevertheless, examination of tissue sections may underestimate the tetraploid cell population, and baseline shifts have been reported (37, 39). With touch imprints, the coefficient of variation is smaller than with tissue sections (35). To minimize these errors we performed internal control analyses on each section examined so that variations in nuclear thickness and stain quality would be the same for control and tumor nuclei. Any error introduced by the use of tissue sections would tend to underestimate the extent of nondiploidy present, yet our results showed nondiploid cell populations present more frequently in early stage tumors than previous studies using flow cytometry (23–25, 27, 28, 40, 41).

Our findings of ploidy changes in very small cancer foci (particularly in PZ cancers) are strongly supported by a recent study by Montironi et al. (42), which demonstrated ploidy changes in areas of high-grade prostatic intraepithelial neoplasia using nuclear image analysis.

In this study we assessed DNA ploidy in individual cancer foci in patients with early prostate cancer and correlated these findings with the established pathological parameters of aggressiveness of prostate cancer. The biological activity of prostate cancer has been related to tumor volume, and volume does not correlate with grade and invasiveness (15, 16). However, the correlation between increasing volume and Gleason grade is only modest (30, 43). For example, Partin et al. (43) found a correlation coefficient of only 0.38 between volume and grade and noted obvious exceptions. We found a significant association of tumor volume with ploidy status (Table 3), but there were frequent exceptions. While large volume cancers were predominantly nondiploid, and all very small cancers were diploid, nondiploid cell populations were observed in many cancers 1 cm\(^3\) in volume (Fig. 2). Of course, we could not determine when in the course of progression these cancers acquired a nondiploid cell population, since each specimen examined represented a single point in time in a continuous disease process. It is unlikely that the relation of ploidy to early malignant events in prostate cancer will be elucidated in the investigation of human tissues, because of the difficulties of repeatedly sampling the same cancer over time in a solid organ.

We have undertaken a separate time course study of DNA ploidy in an in vivo mouse prostate cancer model (44) to examine the relation of ploidy to cancer initiation and progression.

A preponderance of nondiploid cancers among the larger tumor foci in the present series may reflect genetic instability associated with a faster growth rate than that of diploid cancers. If so, the finding of a nondiploid cell population in a small cancer may signify a more aggressive malignant potential. We were surprised to find nondiploid cancers as small as 0.03 cm\(^3\) in this small series of patients. Of 24 cancers less than 1 cm\(^3\) in volume, 8 were nondiploid. These 8 cancers, despite their small size, had a median Gleason sum of 6, and 3 showed ECE. These cancers had acquired some characteristics of aggressive malignant behavior even at small volume. In 3 of these cases (all Stage A), the largest cancer was diploid, yet there was a smaller, separate, accessory cancer that was nondiploid. These findings do not support the previously proposed view that malignant potential is acquired in a predictable, progressor manner closely related to prostate cancer volume (14, 16).

Previous studies have reported that well-differentiated tumors tend to be diploid, poorly differentiated tumors tend to be aneuploid, and about half the moderately differentiated tumors are diploid and half aneuploid (23, 40, 41). Our data support these findings (Table 2), although our series consists almost entirely of early stage prostate cancer of low and intermediate grade. The Gleason grading system for prostate cancer, however, is based entirely on glandular appearance (38). While useful for predicting the prognosis of patients with scores at the extremes (2 to 4 and 8 to 10), it is of limited usefulness in individual patients with a score of 5 to 7 who comprise the majority of patients with prostate cancer (43). The routine analysis of DNA ploidy in addition to the histological grade may allow a more accurate prediction of prognosis of patients with prostate cancer (27), particularly those with Stage A disease (29).

We have previously described the pathological features which distinguish TZ from PZ cancers and their distribution in Stages A and B adenocarcinoma of the prostate (29, 30). Volume for volume, peripheral zone cancers appear to be more biologically aggressive (higher grade, more invasive) than transition zone cancers. Data from this study support this concept. The relation between ploidy and tumor volume was substantially different in TZ and PZ cancers (Fig. 2). While TZ cancers have the potential to become aneuploid, they appear to do so only at much larger volume than PZ cancers. Other recent studies support a difference at the molecular level between Stage A (predominantly TZ cancers) and Stage B (PZ cancers) in the expression of p21, a protein coded by the ras gene (45).

Although our patient population was too small for definitive analysis by substages, we did find that all Stage A1 cancer foci were diploid, as expected (Table 1). In Stage A1, we found 3 nondiploid cancers (15% of the cancers and 33% of the patients): one large TZ cancer (7.3 cm\(^3\)) and 2 small PZ cancers (0.03 and 0.2 cm\(^3\)) (Fig. 1C). Since a TURP selectively samples the TZ, most Stage A prostate cancers are TZ cancers (14). Yet some 90% of patients with Stage A prostate cancer will have a cancer of PZ origin (29). In some of these patients the PZ cancer, though smaller, exhibited more aggressive features than did the TZ cancer in the same gland, such as higher grade or extension through the capsule. It appears, therefore, that in some patients with Stage A prostate cancer prognosis may depend more on the features of the undetected cancer in the PZ than the index cancer in the TZ. The presence of small nondiploid PZ cancers in 2 of the 12 Stage A patients in the series supports this concept. In Stage B patients, there was almost always a nondiploid cancer, usually the largest cancer present (Fig. 1D). The nondiploid cancer was invariably a PZ cancer, and TZ cancers in Stage B patients were usually small, well-differentiated, and diploid (Fig. 1D). Even when the TZ cancer was larger than the PZ cancer, it was diploid (Fig. 1E).

While none of the Stage A patients in the present series had lymph node metastases, deposits were found on permanent section in the lymph nodes of two Stage B patients. In one of these patients, the primary cancer was tetraploid and associated with ECE and SVI, while in the other patient, the primary cancer was aneuploid and associated with ECE. Previous reports have suggested lymph node metastases from primary cancers which were diploid by flow cytometry (46). Most human solid tumors have a diploid cell component (47), and in the majority of nondiploid cancers in this study, a diploid component was also present (Fig. 1, B to E). It is possible that a single biopsy core from a cancer focus or a section containing a...
relatively small cancer focus could cause a significant small nondiploid cell population to be missed on flow cytometry.

We conclude that some very small prostate cancers are nondiploid and that progression of prostate cancer is not a function of volume alone, whereby tumors only acquire full malignant potential at large volumes. Cancers originating in the peripheral zone acquire a nondiploid cell population at a much smaller volume than do cancers of transition zone origin, further supporting our earlier observations of fundamental differences between cancers arising in these zones.

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