SOME SMALL PROSTATE CANCERS ARE NONDIPLOID BY NUCLER IMAGE ANALYSIS. CORRELATION OF DEOXYRIBONUCLEIC ACID PLOIDY STATUS AND PATHOLOGICAL FEATURES

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ABSTRACT

The biological behavior of a prostate cancer can be predicted to some degree by the volume and extent (stage) of the tumor, and its histological grade. The deoxyribonucleic acid (DNA) ploidy status has been reported by some to be another independent prognostic factor for localized prostate cancer. We determined the DNA ploidy value of each individual focus of cancer in radical prostatectomy specimens using nuclear image analysis (CAS 200 system4). Ploidy results were correlated with the volume, Gleason grade and zone of origin (transition zone or peripheral zone) of each tumor, and with the presence of extracapsular extension or seminal vesicle invasion.

There were 141 separate cancers in 68 patients (mean 2.1 per prostate): 9 clinical stage A1, 22 stage A2, 23 stage B1 and 14 stage B2. DNA ploidy correlated significantly (p < 0.0001) with volume, grade, extraprostatic spread and zone of origin. Remarkably, some small cancers (1 cc or less) were nondiploid (as small as 0.03 cc). Overall, 15% of cancers 0.1 to 0.6 cc and 31% of those 0.11 to 1.0 cc in volume were nondiploid. Of 101 cancers confined to the prostate 76% were diploid, compared to only 13% of those with extraprostatic spread. Most cancers of transition zone origin (86%) were diploid, compared to only 49% of peripheral zone cancers, and ploidy and volume relationships were significantly different for peripheral zone cancers compared to transition zone cancers. All small nondiploid cancers arose in the peripheral zone, while in the transition zone the smallest nondiploid cancer was 1.17 cc.

We conclude that prostate cancers that are nondiploid are highly likely to have adverse pathological features. Some small prostate cancers contain a nondiploid cell population and these cancers arise predominantly within the peripheral zone of the prostate. Ploidy and volume relationships provide further support for the hypothesis that there is a difference in malignant potential between cancers of peripheral zone and transition zone origin.

KEY WORDS: prostatic neoplasms, DNA ploidy, nuclear medicine

Prostate cancer is remarkable for the wide discrepancy between the high prevalence of histological changes recognizable as cancer within the prostate and the much lower prevalence of the clinical disease. For a 50-year-old man the estimated lifetime risk of histologically recognizable cancer developing in the prostate is approximately 42%, while the risk of the disease developing clinically is approximately 9.5% and the risk of dying of the disease is approximately 2.9%. 1,2 Thus, most pathological (autopsy) cancers do not progress to become clinically apparent within the normal life expectancy of the host and they have been termed latent or clinically unimportant cancers. 1,2 These latent cancers appear similar cytologically and architecturally to the clinical disease but are usually small, well differentiated and confined to the prostate, while clinically detected tumors are often much larger, moderately or poorly differentiated and invade across normal anatomical boundaries. 3,4,5

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One of the challenging questions in the biology of prostate cancer is the relationship between latent and clinical disease.1,2 Some studies suggest that all small prostate cancers are precursors of larger clinical tumors and that the relationship between the 2 is essentially a function of time.3,4 Others advocate a multistep process in the development of prostate cancer, wherein small, histologically recognizable tumors have proceeded through some but not all of the molecular events necessary for growth and spread as a clinical cancer and may never undergo those additional events.5,6 Under the former hypothesis the volume of a cancer is a key feature of the tumor and is the best predictor of the biological behavior of the disease. In the latter hypothesis, however, volume would be less indicative of malignant potential, with some small cancers already having undergone all the steps necessary for expression of aggressive malignant behavior while other, larger tumors might not have attained the capacity for rapid growth, invasion and metastasis. However, these hypotheses are not mutually exclusive. Although increasing volume generally is a feature of multistep progression, in some cases the sequential accumulation of multiple genetic alterations may precede an increase in volume.

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Fig. 1. Tumor maps of prostate cancers in radical prostatectomy specimens with serial transverse sections labeled P2, P3 and so forth from apex to base to seminal vesicles. DNA ploidy histogram of each cancer and of control normal epithelial cells on same section are shown, with cell count on vertical axis and DNA content on horizontal axis. Zone of origin (PZ—peripheral zone and TZ—transition zone), and Gleason primary and secondary grade are shown for each cancer. Numbers at top of each histogram indicate DNA index. PIN, prostatic intraepithelial neoplasia. A, tumor map from transverse step-sectioned stage A1 radical prostatectomy specimen shows transition zone cancer (green) and 2 peripheral zone cancers (red and yellow). All cancer foci were Gleason grade 2 plus 3 and diploid. Incidental or latent nature of cancer sometimes sampled at transurethral resection of prostate is apparent from this specimen. Cross-hatched areas represent tissue missing from that section. B, characteristic pattern of stage A2 prostate cancer illustrates multifocal nature of tumor. Nondiploid peripheral zone cancer (red) is present along with 3 diploid cancers of peripheral zone origin (turquoise, yellow and purple) and 1 of transition zone origin (green). Latter was sampled at transurethral resection of prostate (section P5) and is not representative of all of cancer in prostate. C, typical stage B1 tumor map shows 2 peripheral zone cancers, with larger palpable tumor (red) being nondiploid while smaller incidental cancer (yellow) was diploid. D, stage B2 tumor map reveals single large peripheral zone cancer (red) extending to both sides of prostate. Gleason sum was 3 plus 4 and tumor was tetraploid.
TABLE 1. DNA ploidy status of each of 68 radical prostatectomy specimens studied, listed by the number of separate cancers found per prostate

<table>
<thead>
<tr>
<th>No. Tumors/Prostate</th>
<th>No. Pts.</th>
<th>All Ploidy Status*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Diploid Mixed</td>
<td>Non</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ploidy Nonploidy</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>5 0 15</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>9 14 4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>5 12 1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0 2 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0 0 0</td>
<td></td>
</tr>
<tr>
<td>Total No. pts. (%)</td>
<td>68</td>
<td>19 (28) 29 (45) 20 (30)</td>
<td></td>
</tr>
</tbody>
</table>

Specimens of mixed ploidy had at least 1 diploid and 1 nonploidy cancer. Otherwise, all cancers in a specimen were uniformly diploid or nonploidy.

TABLE 2. DNA ploidy status of each of 141 individual cancers by grade (Gleason sum), tumor volume and pathological extent

<table>
<thead>
<tr>
<th>No. Individual Tumors</th>
<th>Tumors</th>
<th>Diploid</th>
<th>Nonploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>3 to 4</td>
<td>141</td>
<td>82 (58)</td>
<td>59 (42)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>10 (100)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>47 (84)</td>
<td>9 (16)</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>20 (49)</td>
<td>21 (51)</td>
</tr>
<tr>
<td>7 or more</td>
<td>34</td>
<td>5 (15)</td>
<td>29 (85)</td>
</tr>
<tr>
<td>Tumor vol. (cc):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01 or less</td>
<td>11</td>
<td>11 (100)</td>
<td>-</td>
</tr>
<tr>
<td>More than 0.01 to 0.1</td>
<td>33</td>
<td>28 (85)</td>
<td>5 (15)</td>
</tr>
<tr>
<td>More than 0.1 to 1</td>
<td>48</td>
<td>33 (69)</td>
<td>15 (31)</td>
</tr>
<tr>
<td>More than 1 to 10</td>
<td>42</td>
<td>42 (100)</td>
<td></td>
</tr>
<tr>
<td>More than 10</td>
<td>7</td>
<td>7 (100)</td>
<td></td>
</tr>
<tr>
<td>Extraprostatic Spread:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confined</td>
<td>101</td>
<td>77 (76)</td>
<td>24 (24)</td>
</tr>
<tr>
<td>Not confined</td>
<td>40</td>
<td>5 (13)</td>
<td>35 (87)</td>
</tr>
<tr>
<td>Extracapsular extension</td>
<td>27</td>
<td>5 (19)</td>
<td>22 (81)</td>
</tr>
<tr>
<td>Extracapsular extension plus</td>
<td>13</td>
<td>13 (100)</td>
<td></td>
</tr>
<tr>
<td>seminal vesicle invasion</td>
<td></td>
<td></td>
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</tbody>
</table>

Ploidy correlated significantly with volume (chi-square 48.7, p < 0.0001), grade (chi-square = 50.3, p < 0.0001) and extraprostatic spread (chi-square 49.0, p < 0.0001). p values were determined by chi-square analysis.

patients (12%) the largest tumor (0.46 to 12.1 cc) was diploid but a smaller nondiploid lesion (0.02 to 2.44 cc) was also present. Representative tumor maps of radical prostatectomy specimens from each clinical stage are shown in figure 1, along with the DNA ploidy histogram of each separate cancer and of the control cells on the same sections.

Grade. Ploidy results correlated closely with the grade (Gleason sum) of the cancer (chi-square = 50.3, p < 0.0001, table 2). All 10 well differentiated cancers (Gleason sum 3 or 4) were diploid. All 84% of the Gleason sum 5 tumors. In contrast, 85% of the tumors with a Gleason sum of 7 or more contained a nondiploid cell population. Intermediate grade disease (Gleason sum 6) was evenly split, with 49% diploid and 51% nondiploid, and 24% of the tumors with a primary or secondary Gleason grade of 4 or 5 were diploid. Nondiploid tumors of a given grade were less likely than diploid tumors to exhibit aggressive pathological features. For example, there were 36 Gleason sum 6 cancers of peripheral zone origin. Of the 19 nondiploid tumors 12 (63%) extended outside of the prostate. In contrast, all 17 diploid neoplasms were confined to the prostate (p < 0.001). The correlation between grade and ploidy was highly significant for peripheral zone disease (chi-square 32.9, p < 0.0001) but it was much weaker for transition zone cancer (chi-square 6.5, p = not significant) where there were few cancers with a Gleason sum of 6 or more.

Tumor volume. There was a highly significant correlation between tumor volume and ploidy status (chi-square 48.7, p < 0.0001, table 2). All cancer foci of 0.01 cc or smaller and 85% of those greater than 0.01 to 0.1 cc were diploid. Tumors larger than 1 cc were predominantly (80%) nondiploid. Nevertheless, some small cancers were nondiploid, 1 as small as 0.02 cc and 2 others 0.03 cc (fig. 2), and some large tumors were diploid, especially in the transition zone. Within any given range of tumor volumes, nondiploid tumors were more likely to extend outside of the prostate. Of the tumors larger than 0.1 to 1.0 cc 33 were diploid and all were confined to the prostate. Only 15 lesions were nondiploid and 5 (33%) of these extended outside of the prostate (p < 0.01).

Extraprostatic spread. There was a strong correlation of ploidy with extraprostatic spread of prostate cancer (chi-square 49.0, p < 0.0001). Of 40 tumors that could be seen on the whole mount sections of the prostate to extend directly through the capsule of the prostate or to invade the muscular wall of the seminal vesicle 87% were nondiploid. Of 101 tumors confined to the prostate pathologically 77 (76%) were diploid (table 2). Extracapsular extension and seminal vesicle invasion were associated with nondiploid cancer. Of 27 tumors that penetrated through the capsule into the periprostatic fat or soft tissue but did not invade the seminal vesicles 22 (81%) were nondiploid. All 13 cancers that invaded the seminal vesicles were nondiploid and all 5 patients with lymph node metastases in our series had a nondiploid primary tumor. One of these patients had a large 12 cc diploid cancer in the transition zone that was confined to the prostate and a smaller 2.44 cc nondiploid cancer in the peripheral zone that completely penetrated the capsule.

Even within subsets of tumors of a given grade and volume, extracapsular cancers were far more likely to be nondiploid (table 3). None of 72 diploid tumors of less than 1 cc extended outside of the prostate, compared to 5 of 20 nondiploid cancers (25%, chi-square 21.9, p < 0.0001). The smallest nondiploid cancer with extracapsular extension was 0.4 cc while the smallest diploid cancer with extracapsular extension was 1.29 cc, 3 times larger. There were 31 Gleason sum 6 cancers of 1 cc or smaller and 5 of these extended outside of the prostate. All 5 tumors were nondiploid. In 29 patients there were at least 1 diploid and 1 nondiploid cancers. All of these diploid cancers were confined to the prostate, while 17 of the nondiploid cancers (50%) extended outside of the prostate (chi-square 31, p < 0.0001).

Of the transition zone cancers 5 (14%) extended beyond the prostate into the anterior fibromuscular stroma (none had seminal vesicle invasion) but only 2 of these were nondiploid (table 4). Of 105 peripheral zone cancers 35 (33%) extended beyond the prostate and 33 (94%) of these were nondiploid (p < 0.0001). Thus, apparent extension of a transition zone cancer into the anterior fibromuscular stroma, where there is no discrete capsule, may not be as indicative of biological aggressiveness as complete capsular penetration by a peripheral zone cancer.

Zone of origin. The relationship between volume and ploidy was substantially different in the transition and peripheral zones (table 4). All small nondiploid cancers arose in the peripheral zone. All 23 transition zone cancers of 1 cc or less were diploid, while 29% of the 69 peripheral zone cancers of this size were nondiploid. Only 38% of 13 transition zone cancers larger than 1 cc were nondiploid compared to 94% of 36 peripheral zone cancers this size. The markedly different relationship of ploidy status to tumor volume for transition zone and peripheral zone cancer is apparent from figure 2. This relationship was similar in stage A (fig. 2, A) and stage B (fig. 2, B) tumors. When the data from stages A and B are combined, the appearance of a nondiploid population was noted at a much smaller volume in peripheral zone (mean 3.6 cc, median 2.24 cc, range 0.02 to 19.22 cc) compared with transition zone (mean 6.50 cc, median 7.30 cc, range 1.17 to 13.0 cc) disease (p < 0.001, t test).

DISCUSSION

The biological behavior of a prostate cancer is best characterized by the stage and grade of the tumor, yet there is considerable variation in the course of cancers within any given
STAGE A

Fig. 2. A, stage A prostate cancer. Diploid and nondiploid transition zone (TZ) and peripheral zone (PZ) cancer foci are plotted by tumor volume on log scale with primary Gleason grade indicated. Most transition zone cancers were diploid even though some were large. All nondiploid transition zone cancers were large. In contrast, while most small peripheral zone cancers were diploid, some as small as 0.02 cc were nondiploid. B, stage B prostate cancer. Transition zone cancers were almost always diploid regardless of grade, while peripheral zone cancers were predominantly nondiploid. Again, some small peripheral zone cancers (0.03 cc) were nondiploid.

Table 3. Frequency of extraprostatic spread (extracapsular extension, seminal vesicle invasion or lymph node metastases) among diploid and nondiploid cancers within each volume range

<table>
<thead>
<tr>
<th>Tumor Vol. (cc)</th>
<th>Diploid, Extraprostatic Spread (%)</th>
<th>Non-Diploid, Extraprostatic Spread (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 or less</td>
<td>0/11 (0)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>More than 0.01 to 0.1</td>
<td>0/28 (0)</td>
<td>0/28 (0)</td>
</tr>
<tr>
<td>More than 0.1 to 1</td>
<td>0/33 (0)</td>
<td>0/33 (0)</td>
</tr>
<tr>
<td>More than 1 to 10</td>
<td>4/8 (50)</td>
<td>25/54 (47)</td>
</tr>
<tr>
<td>More than 10</td>
<td>1/2 (50)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>No./Total (%)</td>
<td>5/82 (6)</td>
<td>35/59 (56)</td>
</tr>
</tbody>
</table>

Chi-square 70.21, p < 0.0001.

Table 4. DNA ploidy status of cancers of transition zone and peripheral zone origin, correlating ploidy status with grade, tumor volume and extraprostatic spread

<table>
<thead>
<tr>
<th>Transition Zone</th>
<th>Peripheral Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diploid (%)</td>
<td>Nondiploid (%)</td>
</tr>
<tr>
<td>Grade:</td>
<td></td>
</tr>
<tr>
<td>3 to 4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>More than 0.1 to 1</td>
<td></td>
</tr>
<tr>
<td>More than 1 to 10</td>
<td></td>
</tr>
<tr>
<td>More than 10</td>
<td></td>
</tr>
<tr>
<td>P values</td>
<td></td>
</tr>
<tr>
<td>Extraprostatic spread:</td>
<td></td>
</tr>
<tr>
<td>Confined</td>
<td></td>
</tr>
<tr>
<td>Not confined:</td>
<td></td>
</tr>
<tr>
<td>Extracapsular extension only</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td></td>
</tr>
<tr>
<td>P values</td>
<td></td>
</tr>
</tbody>
</table>

P values determined by chi-square analysis.

stage and grade. More objective indicators of prognosis are needed so that treatment recommendations can be appropriate to the threat of the cancer. Investigations of DNA ploidy status as a prognostic factor have yielded conflicting results. Some studies have shown that ploidy correlates with prognosis and adds significant information to that available from stage and grade alone. In other studies ploidy did not add independent information once grade and other features were considered.

In our studies ploidy results generally correlated with the grade of the tumor (table 2) but there were instances of discordance so that grade alone could not be used to predict ploidy results. All well differentiated tumors (Gleason sum 5 or less) were diploid but only a few were included in our series. Most poorly differentiated tumors (Gleason sum 7 or more) were nondiploid but 24% of the tumors with a primary or secondary component of Gleason grade 4 or 5 cancer were diploid. Tumors of intermediate grade (Gleason sum 5 to 7), which make up the majority of clinically localized prostate cancer, were nearly evenly divided between diploid and nondiploid. While the relative prognostic significance of grade versus ploidy remains controversial, several studies report a worse prognosis for nondiploid cancers when controlled for stage and grade. When we controlled for the grade of the tumor in our series, nondiploid tumors were much more likely to extend outside of the prostate. It seems likely that DNA ploidy and Gleason grade reflect independent, although often concordant, parameters. The former reflects nuclear properties and the latter architectural features.

Ploidy results also correlated strongly with tumor volume (table 2). Nevertheless, a surprising proportion of small tumors were nondiploid, including 15% of those 0.01 to 0.1 cc and 31% of those 0.1 to 1.0 cc in volume. A 0.02 cc tumor was nondiploid (grade 2 plus 3) in a patient with a much larger (3.01 cc) diploid transition zone cancer of the same grade as well as a separate nondiploid peripheral zone cancer (0.61 cc). In another patient a nondiploid tumor of 0.03 cc in volume was found along with 3 other diploid cancers (0.03, 0.09 and 0.26 cc). Overall, there were 8 patients in whom the prostate harbored nondiploid cancer along with a larger index cancer that was diploid. Furthermore, among tumors of any given volume, nondiploid tumors were significantly more likely to extend outside of the prostate.

The powerful association of nondiploidy with tumor volume may reflect a growth advantage of the nondiploid cell population within the tumor. If nondiploid cells proliferate more rapidly, most large cancers would be nondiploid while most small cancers would be diploid. Tumor volume, then, would be a consequence of the progression through the multiple steps necessary for malignant expression rather than a cause of such progression. The validity of this hypothesis would not detract
somewhat useful in the assessment of prostate cancer, since larger cancers would likely be nondiploid. At any given time only a few small nondiploid cancers would be found, since their faster growth rate would not allow them to remain small for long. This concept helps to explain our observation that some small cancers (0.01 to 0.1 cm) were nondiploid (table 2). Thus, the ploidy status of a tumor would be a way to distinguish between latent cancers and small clinical cancers.

The powerful association of nondiploidy with extraprostatic spread of cancer in our series supports the prognostic significance of ploidy determined by nuclear image analysis. Of 40 cancers that extended outside of the prostate 87% were nondiploid, while 76% of those confined to the prostate were diploid. Although tumor volume has been shown to correlate strongly with extraprostatic spread, 4, 5, 6 ploidy correlated with extension even within a given range of tumor volumes. Small diploid cancers were uniformly confined to the prostate while many small nondiploid cancers extended through the capsule.

While these results show a strong correlation between ploidy and the pathological features of prostate cancer, they do not indicate whether the ploidy status of a cancer is an inherent feature of a tumor from its inception or gradually changes with time as the cancer progresses. At our laboratory we performed a series of experiments using the mouse prostate reconstitution model of prostate cancer to investigate changes in ploidy with time. 10 These tumors were initially diploid but a nondiploid cell population emerged immediately before the rapid, logarithmic growth phase of the cancer and the acquisition of a more malignant phenotype. Such longitudinal studies are difficult to perform in patients. In the only study reported in the literature, Adolfssson and Tribulak performed serial fine needle aspiration in untreated cancers, and found progressive dedifferentiation and acquisition of a more aneuploid cell population in 24% of prostate cancers during a 2-year period. 22 Thus, it seems reasonable to interpret the presence of a nondiploid cell population in some small cancers as an indication that these tumors have acquired aggressive malignant features and can be expected to grow and invade more rapidly than diploid cancer.

DNA tetraploidy is common in prostate cancer 12, 23 and some studies suggest that tetraploid cancers have a more favorable prognosis than non-tetraploid aneuploid disease. While we did not present the details of our analysis in this study, we could find no significant differences in the pathological features of tetraploid and aneuploid prostate cancers. Both were distinctly different from diploid tumors. In the animal model the emergence of a tetraploid (4N) population, seen first, is followed by an 8N population as the tetraploid cells begin to cycle through G2/M, then by the appearance of aneuploid cells. A similar pattern may be present in human cancers but our study was not designed to identify it.

Our ploidy results lend support to previous morphometric observations suggesting a fundamental difference between cancer of transition zone and nontransition zone origin. 16, 18, 20 Transition zone cancer tends to remain well differentiated even at large volumes and to remain confined to the prostate either because of mechanical factors 24 or because of inherent inability to invade across anatomical boundaries. 16 In this study we have shown that transition zone cancer also tends to remain diploid even at large volumes. The difference between the smallest nondiploid transition zone cancer (1.17 cc) and peripheral zone cancer (0.02 cc) was nearly 2 orders of magnitude in our series. The similarity of our results in stages A and B cancer (fig. 2) indicates that the method of diagnosis of prostate cancer determines the biological features of the tumor less than the zonal location of the tumor. Ploidy abnormalities have been reported in high grade prostatic intraepithelial neoplasia, 25 frequently found in association with peripheral zone but not transition zone cancer. 19 The substantially different relationship between tumor volume and grade, and between volume and ploidy in cancer of transition zone and peripheral zone origin, even among tumors of the same grade, argues that the factors that control progression of these tumors differ substantially. Further investigations of the molecular mechanisms that underlie differences between cancers that arise in these zones are underway in our laboratory.

While the internal consistency of our results supports the accuracy of ploidy determinations by image analysis, our results differ substantially from those reported for flow cytometry. 11, 12 For example, in a review of the flow cytometry literature Badalament et al calculated that 93% of 490 tumors confined to the prostate were diploid, compared to 76% in our series. Only 27% of clinically localized cancers not confined to the prostate were nondiploid by flow cytometry, compared to 87% in our series (table 2). Furthermore, 88% of Gleason sum 6 and 72% of Gleason sum 7 or greater clinically localized prostate cancers were diploid by flow cytometry, compared to 49% and 15% in our series, respectively (table 2).

There are several plausible explanations for these discrepancies in ploidy results. Prostate cancer is a multifocal and heterogeneous tumor. 16, 27 As we have shown, the index or ploidy value of the largest tumor may be different from that of any tumor present in the same gland. Studies that used flow cytometry sampled only 1 cancer per prostate, although the pathological stage and grade were determined on the basis of all disease within the radical prostatectomy specimen. 11, 12, 27 In addition to the heterogeneity among multifocal tumors within a prostate, there is often heterogeneity of grade within a given cancer and there is evidence to suggest variation of ploidy values in different areas of the same cancer as well. Most solid tumors contain a population of cells with a diploid DNA content and we found a diploid component within many of the nondiploid prostate lesions in our study (figs. 1 and 2). When ploidy is determined in a biopsy specimen 12, 22, 23 or a small sample excised from radical prostatectomy specimen, 12, 26 the nondiploid cell population may be missed.

There is also an inherent bias toward diploid values when nuclear DNA content is determined by flow cytometry, since the sample analyzed does not consist solely of malignant cells but is a poorly quantified mixture of malignant cells with benign stromal or epithelial cells, depending on the proportion of the cells present in the area of tissue sampled. The normal diploid cells might variably dilute the effects of the malignant cells in the flow cytometry spectrum. Since ploidy assignment depends upon the per cent of nondiploid cells present, the effect could be to designate more cancers as diploid. Image analysis allows the examiner systematically to exclude obviously benign cells from the analysis and to assess the status of the malignant cells themselves.

Nevertheless, flow cytometric studies do give results that correlate significantly with prognosis. 11, 13, 14, 26, 27 Even though nondiploid tumors have a worse prognosis than diploid cancers, ploidy abnormalities are probably underestimated by flow cytometry in prostate cancer. Yet flow cytometricists have not been able to explain why some patients with a diploid cancer have a poor prognosis. 12, 13, 23 Whether this discrepancy in flow cytometric results stems from sampling errors, dilutional artifacts, or too broad a definition of diploidy or progression from a diploid to a nondiploid state remains unclear.

There are also potential problems with ploidy determinations in tissue sections using image analysis. Since the examiner selects the cells for analysis, the most abnormal cells may be chosen. Ploidy values are determined from the amount of DNA per nucleus but nuclei are often not intact in tissue section as they are in smears or cell suspensions. 14 Sections of 4 μm would leave proportionally less DNA per malignant nucleus than identical sections of the smaller benign nuclei used as controls, and would bias the result toward a diploid value. Finally, our definition of nondiploid value, similar to that in other reports, may be too strict, assigning some nondiploid cancers a diploid result. Nevertheless, the coefficients of variation in our series...
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similar to those in flow cytometry studies. Nuclear image
this is now a well established technique for accurately
measuring DNA ploidy in benign and malignant tissues. The
number of tissue sections was essential to our goals, since we wanted
to examine each separate focus of cancer in the whole mount
sections so that ploidy results could be correlated with the
morphometric features and grade of each tumor, no matter how
small. Epstein et al showed a reasonably good correlation
between DNA ploidy results from 4 mm sections and smears of
the same prostate cancer using the same system (CAS 200) as
that used in our study,14 although they did report discordant
results in about 10% of the cases. Most importantly we recently
demonstrated a significant correlation between these ploidy
results and the prognosis of our patients.28

The clinical significance of the small foci of prostate cancer
examined in our studies is not clear. Most investigators have
ignored their presence. These small foci appear similar histologically
to the small cancers found at autopsy. To
our knowledge the DNA ploidy status of such small cancers has
not been reported. Our hypothesis was that small incidental
foci of cancer would be diploid and we were surprised to find
that some were not. Since all of our specimens were from
patients with clinically detected prostate cancer, we do not
know whether some small cancers found incidentally at autopsy
would also show an abnormal ploidy pattern.

We conclude that nondiploid prostate cancers are likely to
exhibit adverse pathological features and that ploidy is an
independent predictor of extraprostatic spread. Some small
cancers are nondiploid and they arise in the peripheral zone of
the prostate. Ploidy and volume relationships provide further
support for a difference in malignant potential between cancers
of peripheral and transition zone origin. In the future, ploidy
results will prove most useful in the management of prostate
cancer patients if ploidy can be accurately determined from
biopsy specimens, so that it can be assessed before treatment.
Since a biopsy obtains only a sample of this multifocal and
heterogeneous cancer, the value of ploidy results in biopsy
specimens will depend upon the degree to which the biopsy
sample is representative of the cancer present in the prostate.

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