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An Update on the Role of Ploidy in Prostate Carcinoma

Jill M. Peters-Gee, MD*

One of the characteristic features of prostate carcinoma is its marked variation in biologic behavior. DNA quantitation has been studied in prostate carcinoma using a variety of techniques. Evaluation of tumor ploidy suggests that this may be the best predictor of the biologic behavior of prostate cancer in individual patients. This comprehensive review addresses the current studies, stage by stage, to clarify the clinical significance of these findings. (Henry Ford Hosp Med J 1992;40:99-102)

Prostate adenocarcinoma exhibits marked biologic variability (1). Response to treatment, time to progression, and ultimate survival all vary, independent of stage or grade (2). Histologic grade and pathologic stage are the chief parameters currently used to determine individual therapy (3). While useful in poorly and well-differentiated tumors, these parameters have less prognostic significance in moderately differentiated tumors which comprise the largest group of patients with prostate carcinoma. The ability to predict prognosis accurately in individual patients continues to elude practicing urologists. For this reason, we continue to look for a reliable means of predicting prognosis in patients with prostate carcinoma.

It is well known that chromosomal aberrations are associated with neoplastic transformation (4). Chromosomal changes, which are often nonspecific, may result in measurable increases in the DNA content of nuclei (5). The normal human somatic cell contains 46 chromosomes (23 pairs) and is referred to as diploid. A cell with an identifiable deviation from 46 normal chromosomes is described as aneuploid and may include deletions, translocations, or duplications of an entire chromosome or portion of a chromosome.

Nuclear DNA content or ploidy, as well as changes in cell cycle kinetics, have been found to correlate with the biologic behavior of other tumors (6). One of the challenges in prostate carcinoma is to find either morphologic or biologic changes that can be useful predictors of disease progression. This information, if available at the time of diagnosis, can be used clinically to assist in therapeutic planning and to assess the need for adjuvant therapy posttreatment. Much of the information used currently (i.e., lymph node status, seminal vesicle or capsular penetration by tumor) is obtained with excisional therapy and pathologic staging (7). While it is becoming increasingly accepted that ploidy is a valuable prognostic determinate in prostate cancer, its role in patient management has yet to be defined. Ploidy determination may be useful in selecting patients not suitable for conservative therapy. Further clinical decisions must take into account many other factors such as patient age and tumor grade and stage. These decisions are made on an individual basis, taking into account all we know about the biology of prostate cancer.

Techniques Available to Measure Ploidy

Nuclear DNA quantitation and analysis of cell cycle kinetics have given us insight into the biology of prostate carcinoma. Information about nuclear DNA changes can be obtained by tumor cell chromosomal analysis, computer-assisted image analysis, or flow cytometry (6). Each of these techniques has technical limitations and advantages.

Chromosomal analysis

Specific chromosomal changes can be measured using cytogenetic analysis of tumor cells. Such studies require that the tumor be disaggregated either enzymatically or mechanically. The resulting suspension is exposed to a mitotic inhibitor and the cells are swelled in a hypotonic solution, fixed and spread on glass slides. Specific staining techniques allow characteristic bands to be identified in the metaphase chromosomes (8). While chromosomal analysis is applicable to leukemias, in solid tumors such analysis is difficult with interpretable chromosome spreads obtained in only 10% to 20% of cases (9). Thus, technical problems preclude the use of chromosomal analysis of prostate carcinoma on a routine basis.

Computer-assisted image analysis

Identification of individual chromosomes is possible only during metaphase. Nuclear DNA quantitation can be determined on interphase cells, independent of the proliferative activity of the tumor (10). Quantitation to detect measurable increases in nuclear DNA can be performed by either flow cytometry or slide cytophotometry. Slide cytophotometry involves computer-assisted image analysis of individual cells identified histologically as tumor or control cells. This high-resolution technique quantitates DNA content of feulgen-stained nuclei. Because only tumor cells are analyzed, fewer cells (200-300) are

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needed for analysis. The DNA histograms generated are compared to normal diploid cells to determine the presence of abnormal DNA content or aneuploidy (11). Computer-assisted image analysis, which allows DNA quantitation on small tissue samples, is useful in prostate biopsies where often a small amount of tumor is admixed with normal cells (12). Technically, it is easy to prepare tissue for image analysis. Any pathology laboratory can prepare the slides and analysis can be done immediately, or the slides may be sent for commercial DNA analysis.

Flow cytometry

Flow cytometry is a low-resolution technique by which the nuclear DNA of cells in suspension can be quantitated. Cells of solid tumors must be disaggregated prior to flow cytometry. A narrow fluid stream containing the cells in suspension passes through a laser beam. As each cell intersects the beam, light is scattered. Detectors transform the light scattering into electrical pulses which are measured and recorded by computer. The intensity of the scattering is a function of the size, shape, and structure of the cell. Even though the measurements obtained are quantitative, unless all cells are visually examined by cell sorting, one cannot be certain which cell generated the signal. Prostate cancer samples usually contain a mixture of tumor, stromal, inflammatory, and hyperplastic cells. This lack of specificity is offset by the large number of cells that can be examined and the rapidity with which it can be performed. Most flow cytometers can measure 5,000 to 10,000 cells/second. The measurement is objective with no user bias introduced, in that all cells are analyzed. Not only can flow cytometry quantitate nuclear DNA, but other parameters such as cell size, volume, and nuclear roundness can be measured (13).

Flow cytometry is a rapid and objective means of quantitating DNA. Because all cells are measured, aneuploid tumor cells may be diluted if only a few are admixed with normal glandular cells and stroma. Thus, studies by flow cytometry may underestimate the ploidy in tumor cells.

Clinical Applications

DNA ploidy was first found to be correlated with prostate cancer outcome in early studies using microspectrophotometry (14). Subsequent studies using computer-assisted image analysis confirmed these early studies (15-18). Ploidy was found to correlate with tumor grade; well-differentiated tumors are primarily diploid, and poorly differentiated tumors are primarily aneuploid (19,20). Early studies suggested that response to hormonal therapy was improved in diploid patients when compared to an aneuploid group (14). Ronstrum et al (21) determined ploidy by using flow cytometry on 500 patients with either benign prostatic hypertrophy (BPH) or prostate carcinoma. Aneuploidy was found in 73% of prostate carcinomas compared to only 8% of BPH samples (21). These studies suggest that ploidy may be a useful prognostic indicator for prostate carcinoma.

The ability to quantitate DNA on paraffin-embedded archival specimens allows retrospective studies to be performed on patients with known outcomes, using both image analysis and flow cytometry (22). These studies are discussed according to stage to allow for easier comparison of results and clinical application.

Localized prostate carcinoma

Approximately 9% of stage A1 and 36% of stage A2 tumors will progress. Using flow cytometry, McIntire et al (23) demonstrated that 67% of aneuploid stage A2 tumors progressed while none of diploid stage A1 tumors progressed. In addition, Epstein et al (24) found that nuclear roundness was a significant predictor of prognosis in untreated stage A1 or A2 patients.

Many studies have looked at stage B tumors. Montgomery et al (25) analyzed with flow cytometry the tumors of 283 patients removed by radical prostatectomy. DNA quantitation revealed 68% diploid, 28% tetraploid, and 4% aneuploid. Overall, 20% progressed during a mean follow-up of 9.4 years. All of the aneuploid tumors progressed. Using image analysis on patients treated with I^{125} implantation, we have shown comparable results; 11% of stage A or B patients were aneuploid and 89% diploid. Progression to stage D2 disease occurred in 27% of patients, 80% of whom were aneuploid and 20% diploid. The difference is highly significant ($P < 0.0001$) (26). These studies indicate that the small percentage of stage A or B patients having aneuploid tumors accounts for most of the disease progression.

Several investigators have studied tumors removed by radical prostatectomy to determine if ploidy is useful in predicting advanced pathologic stage from capsular invasion, lymph node metastasis, or seminal vesicle invasion (27-29). Ritchie et al (29) followed 109 patients for a mean of 60.7 months after radical prostatectomy. Tumor grade was the most important determinant of time to disease recurrence. Ploidy did not correlate either with grade or anatomical extent of disease. Only six patients were aneuploid and none had recurrence; however, only three were followed for more than three years. Lee et al (30) assessed 88 radical prostatectomy patients similarly. In this series aneuploidy, Gleason grade, and seminal vesicle involvement all correlated significantly with disease recurrence. Aneuploidy was found in 58% of patients (68% with seminal vesicle involvement compared to 38% without seminal vesicle involvement).

A subsequent study revealed a strong association between DNA ploidy and serum prostate-specific antigen (PSA) levels preoperatively (31). All patients with an aneuploid or tetraploid tumor had elevated PSA, and all patients with a PSA less than 4.0 ng/mL were diploid. These data suggest that if ploidy is in fact a useful predictor of the biologic behavior of prostate cancer in an individual patient, PSA may be a useful predictor of ploidy in localized disease. However, PSA is related to tumor volume, and ploidy may also be related to the volume of tumor present. This question was addressed by Jones et al (32) who studied 57 patients who had undergone radical prostatectomy. They compared ploidy (determined by flow cytometry) to tumor volume, lymph node status, and histologic grade. All aneuploid tumors,

which were found in 46% of patients, were greater than 4 mL in volume with only one exception. Because there was an overlap in behavior of diploid and aneuploid tumors, Jones et al (32) concluded that ploidy could not be used as an independent predictor to direct preoperative treatment. Thus, while many studies support the use of ploidy as a prognostic determinant, its clinical usefulness is still debated.

Stage C prostate carcinoma

Ploidy has been reported to be a useful predictor of disease progression in stage C tumors. Lee et al (30) found that patients with stage C diploid tumors had an 85% chance of remaining disease-free for 5 years, compared to only 9% with aneuploid DNA. In a larger series of 146 patients with stage C tumors treated by radical prostatectomy, Nativ et al (33) found that the median time to progression was 3.5 years in the aneuploid group compared to 7.4 years in the diploid or tetraploid patients. Stage C patients with low-grade diploid tumors have a progression-free survival of 92% at 10 years, compared to 57% for patients with low-grade nondiploid tumors. However, other investigators have not been able to confirm these findings (34,35).

Stage D prostate carcinoma

Patients with stage D1 prostate carcinoma are a clinical challenge. The role and timing of adjuvant therapy is still debated (36). Using image analysis in this group of patients, we have found a significant difference in time to progression of disease in aneuploid patients compared to diploid patients (median time 37.2 months versus 76.9 months, respectively) (36). This study using image analysis assessed ploidy in the lymph node metastases. Stephenson et al (37), using flow cytometry to assess ploidy in lymph node metastases, found a median survival of 8.8 years for diploid patients compared to 5 years for the aneuploid group.

Using flow cytometry on the primary tumor, Winkler et al (38) demonstrated 13% aneuploid tumors. Only 15% of the DNA diploid tumors progressed locally or systemically compared to 75% of tetraploid or aneuploid tumors (38). These studies indicate that ploidy is a significant predictor of progression and/or survival. How this will impact clinical decision-making is not yet clear.

Summary

Analysis of tumor ploidy may be a significant prognostic determinant providing insight into the biologic behavior of the prostate cancer in individual patients. Available data demonstrate marked variability in tumor ploidy, which may be due to differing techniques of DNA quantitation and variability in the definition of aneuploidy. Before clinical decisions can be made based on ploidy, one must know the predictive value, sensitivity, and specificity of the technique being used. There is much debate concerning the heterogeneity of prostate cancer. It is possible that ploidy varies throughout the tumor. Sampling error may be part of the reason variability exists in ploidy measurements found in different studies. Prospective studies and continued improvement in techniques for DNA analysis are necessary before precise recommendations can be made concerning clinical use of the data. Research centers utilizing ploidy determination in clinical decision-making should clarify this issue.

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